8

Laboratory procedures and methods for characterisation of faecal sludge

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OBJECTIVES

The objectives of this chapter are to:

- To provide methods for protecting health and safety during collection, handling, transportation, storage, and disposal of faecal sludge.
- To provide information required to adapt and develop standard methods for faecal sludge characterisation, including quality assurance and quality control (QA/QC) strategies and selection of appropriate methods.
- To provide an overview of existing methods for faecal sludge analysis being used in partner laboratories.

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8.1 INTRODUCTION

Faecal sludge management is a relatively new and rapidly developing field. There is a need for increased scientific knowledge and understanding of faecal sludge characteristics. To help fill this gap, standard methods that can be used in scientific research and monitoring of treatment plants are needed. However, the characterisation of faecal sludge is challenging due to the high variability and wide range of characteristics, as presented in Chapter 1, which requires rigorous sampling methods, quality assurance and quality control (QA/QC) measures, and well-defined procedures to reduce the uncertainties in data generation and analysis. When using existing standard methods developed for other sample matrices, these methods need to be validated and adapted for the specific type of faecal sludge being analysed. Presented in this chapter are examples of how laboratories are adapting existing standard methods and manufacturers' test kits for this difficult matrix, and also developing new methods.

When adapting existing methods for faecal sludge, appropriate methods for more liquid matrices (*e.g.* from wastewater) or more solid matrices (*e.g.* from fresh faeces, soil and food science) need to be selected. As explained in Chapter 2, four types of faecal sludge are defined in this book as liquid (< 5% TS), slurry (5-15% TS), semi-solid (15-25% TS), and solid (> 25% TS). Due to the range of TS concentrations, mass concentrations in this book are expressed as both weight/volume and weight/weight. Unfortunately, it is not possible to make a hard and fast rule about when to analyse volumetrically (weight/volume) or gravimetrically (weight/weight), and in practice this has to be determined by how accurately volumes of faecal sludge can be measured. In general, it is practical to analyse liquid and slurry samples volumetrically and semi-solid and solid samples gravimetrically, but in reality, analysis needs to be done consistently, and samples can span the entire range of concentrations. Whenever possible, it is therefore recommended to report the density of samples, as this can be used to convert between concentrations, and TS analysis can be done in parallel to report the results in weight/weight of a dry sample mass. Due to the wide range of TS concentrations, faecal sludge samples may require different preparation steps before characterisation, compared with the sample preparation described in existing standard methods for other matrices. Sludge with higher TS concentrations may require additional preparation techniques such as mixing, blending, diluting, centrifuging and filtering. For example, the method for characterising the pH of faecal sludge involves sample preparation techniques from the soil standard methods for high TS samples, techniques from food science for semi-solid samples, and techniques from wastewater methods for slurry and liquid samples. For liquid sample matrices such as treatment plant effluent, established standard methods for wastewater analysis should be applicable.

Common concerns when adapting existing methods for faecal sludge include homogenisation, dilutions, sample size, and filtration. Homogenisation needs special attention in order to obtain representative samples, and as discussed in Chapter 3, sampling plans need to be carefully designed and executed to produce repeatable results. Filter pore size needs to be adjusted to address issues with clogging. Special pre-treatment steps such as treatment with activated carbon may be required when employing colorimetric methods to ensure the inherent colour of the sample matrix does not influence the measurement. Faecal sludge in general has higher organic loads than wastewater or digested sludge, hence more dilutions will be required in the sample preparation stage. In other cases, the analytical measurements or the size of the sample are modified in order to adapt the method to faecal sludge. Settleability and dewaterability methods are also modified to reflect operational differences at faecal sludge treatment plants compared with wastewater treatment plants, for example, allowing for different settling or mixing times to match actual process conditions. For analysis of helminths, the UKZN PRG laboratory found that the USEPA Ascaris method was not giving adequate quantitative results for faecal sludge, as it is often mixed with sand, soil and other materials, and the silica particles can be confused as helminth eggs under the microscope. To address this, the UKZN PRG developed a method that includes dissolving the silica particles and additional sieving - UKZN PRG helminth method (Method 8.8.1.4).

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Methods for fresh faeces also need adaptation for use with faecal sludge, as illustrated by the example of faecal sludge simulants in Chapter 7.

The methods presented in this chapter are the first step towards the development of standard methods for analysis of faecal sludge. Additional method development and adaptation is still required by laboratories around the world. The TS method is the most complete method that is presented, as TS is one of the most basic and fundamental characteristics to report for faecal sludge and is often the source of many errors. The chapter also includes a method for sand content, which is important to consider with faecal sludge, for example when interpreting VS to TS ratios. Other categories of methods are not comprehensive, and are based on the experience of the partner laboratories. For example, COD is the only method presented for the analysis of organic content, as experience indicates that it is a representative measurement and it is robust to the variability and levels of organic loads in faecal sludge. Other methods to consider include biochemical oxygen demand (BOD), volatile fatty acids (VFA), total organic carbon (TOC), and biomethane potential (BMP), which will be included in future editions of the book. A general overview of metal analysis is provided, but only one example of a digestion. For nutrient analysis, options of manufacturers' kit-based methods, spectrophotometers, and titrations are presented but not for all parameters. Methods for characterising crude fat and fibre are included, but further methods for measuring other fibre fractions and crude protein are not yet included in this book. Throughout this chapter, information is provided about the standard methods being adapted, the extent of adaptation and validation that has taken place for faecal sludge, examples of implementation, and links to further resources.

Method development and adaption for new sample matrices is a standardised experimental procedure. For example, in the Standard Method for Method Development and Evaluation (1040), Rice *et al*. (2017) present the steps of single operator characteristics, analysis of unknown samples, and method ruggedness. The first step is to evaluate the systematic error or bias and the precision of the method for the specific sample matrix. The systematic error is the inherent fluctuation in a method. For example, if the same person was analysing replicates of the same sample, the variability within the results would be used to calculate the systematic error. To evaluate this, faecal sludge samples need to be prepared across the range of TS that will be evaluated, and then spiked with known concentrations of a standard of the constituent to be measured, across the concentration range of the method. Then, each of the samples is measured 10 times to calculate the systematic error and precision. The second step requires analysing blind samples that were prepared independently from the laboratory conducting the analysis. This means that the laboratory doing the analysis does not know the concentration in advance, but the known concentration is used after analysis to evaluate the accuracy of the method. The third step is making minor changes to the method, such as mixing time, sample size, temperature, or pH, and based on the results, the ruggedness of the method is calculated. Important quality assurance and quality control (QA/QC) measures to have in place for method development are covered in Section 8.3. In addition, important components of method development and experimental procedure are covered throughout this book, including setting up a laboratory (Chapter 2), how to develop and execute a sampling plan (chapters 3 and 5), and experimental design (Chapter 4).

For the future, as more laboratories develop new methods and adapt existing methods for the analysis of faecal sludge, collaborative testing can take place, where blind samples are analysed in parallel in multiple laboratories, as a step further to the establishment of standard methods for faecal sludge analysis. This can also help to develop and standardise new lower-cost methods developed specifically for faecal sludge, such as measurement of sludge colour using image analysis (Method 8.6.8, Ward *et al*., 2021), *in situ* characterisation of rheological properties in pit latrines using a portable penetrometer (Chapter 3), and development of field laboratory methods for characterisation of faecal sludge in resource-limited settings (Chapter 2).

Presented in this chapter is a general overview of factors to consider when conducting the laboratory

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analysis. The reader is referred to additional references throughout the text for more in-depth knowledge. Section 8.2 provides an overview of the health and safety and occupational safety measures for risk prevention, Section 8.3 summarises quality assurance measures, Section 8.4 provides an overview of the included methods, and guidelines for how to select methods for implementation based on criteria such as available budget, required accuracy, sample preparation and testing time, and laboratory capacity, Section 8.5 provides information about packaging and shipping faecal sludge samples, Section 8.6 contains the methods for chemical and physicochemical characteristics, Section 8.7 contains the methods for physical and mechanical characteristics, and Section 8.8 contains the methods for biological characteristics.

8.2 HEALTH AND SAFETY (H&S)

Specific concerns with setting up laboratories for faecal sludge analysis are covered in Section 2.5. For a further discussion of EHS (environment, health, and safety) management systems, including laboratory safety, chemical handling and management, emergency planning, evaluating hazards and risks, working with equipment, and management of waste, please refer to the free online guidelines, Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards (National Research Council, 2011). Care must be taken when handling faecal sludge, and it should always be considered a hazardous biological agent (HBA) due the content of pathogens. Pathogens pose risks to human health that require strict health and safety procedures when working with faecal sludge in the laboratory and the field. People involved at every step of the sampling and analytical process need to take precautions. These precautions include required vaccinations that will depend on regionally prevalent diseases and the extent of exposure to faecal sludge (*e.g.* cholera, tetanus, polio, typhoid fever, hepatitis). As discussed in Section 2.5.1, the laboratory workflow includes the transmission chain, and preventative and protective measures. Pathways for human contamination need to be carefully considered in advance, and appropriate procedures put in place to manage them. Pathways of contamination include the following (European Agency for Safety and Health at Work (EU-OSHA):

- Reservoir: the source of the infective agent (*e.g.* faecal sludge sample)
- Portal of exit: the biological agent leaves the reservoir and/or is transmitted to another reservoir
- Mode of transmission: direct (*e.g.* inhalation), semi-direct (*e.g.* transmitted through dirty hands); or indirect (*e.g.* transmitted on a contaminated surface)
- Portal of entry: respiratory tract (*e.g.* inhalation), digestive tract (*e.g.* ingestion), skin (*e.g.* existing or new injury), or mucus (*e.g.* splashing into eyes)
- Potential hosts: employees or workers who have been exposed via contamination pathways.

In addition to pathogens, general occupational health and safety are an essential part of faecal sludge laboratory practices. The right to health and safety at work is stipulated in the constitution of the World Health Organisation (WHO) and is supported by a number of international and local organisations and regulations. 'Occupational risk factors' include chemical, physical, biological or other agents that may cause harm to an exposed person in the workplace. Examples of categories of occupational risk factors are carcinogens (*e.g.* 150 known probable human carcinogens (IARC, 2012)), airborne particulates, noise, ergonomic stressors, risk factors for injuries, and exposure to hazardous biological agents. For more information, the reader is referred to international organisations and guidelines for occupational health and safety listed below[1].

8.2.1 Monitoring and responsibilities

Setting up the work flow in a laboratory conducting faecal sludge analysis is covered in Section 2.5.1,

[1] a) Wolf J., Prüss-Ustün A., Ivanov I., Mugdal S., Corvalán C., Bos R., Neira M. and World Health Organization (2018). Preventing disease through a healthier and safer workplace.
b) World Bank (1984). Occupational health and safety guidelines. Office of Environmental Affairs, Washington DC.
c) Occupational Health and Safety Administration (OSHA), https://www.osha.gov.
d) European Agency for Safety and Health at Work (EU-OSHA), http://osha.europa.eu
e) World Bank (2019). Health, Safety and Dignity of Sanitation Workers an Initial Assessment.

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including receiving samples. In order to achieve an environment where accidents and hazards are minimised, there needs to be a clear chain of command and defined responsibilities. This includes laboratory management (Section 2.5.3) and health and safety practices (Section 2.5.2). The laboratory manager has the overall responsibility for health and safety and delegates specific health and safety responsibilities to all persons working in the laboratory. The laboratory manager also has the responsibilities to issue annual and *ad hoc* reports on the laboratory activities and procedures, raise concerns relating to health and safety, and promote and ensure compliance with all health and safety procedures. Line managers are accountable for workplace health and safety and the welfare of employees in their chain of command while working in the laboratory. They are responsible for establishing safety goals and objectives under their responsibility, consulting and motivating their staff to adopt good health and safety practices, and providing the necessary risk controls and maintenance procedures to ensure a safe and healthy workplace. Everyone working in the laboratory is required to make every effort to ensure the health and safety of themselves and their co-workers, and that their acts or omissions do not affect the health and safety of other workers. They must also have undergone the appropriate safety training, and have an intimate understanding of their own workplace in order to be in a position to identify, report, and minimise risks. They should always be prepared to communicate with their line managers as the first point of contact in the workplace, and with community representatives when sampling in the field. It is especially important that everyone working in a laboratory immediately reports any health and safety issues to management as they arise in the workplace. To ensure a safe environment and compliance, there should be no repercussions for employees who identify risks or hazards, and this should in fact be rewarded. In addition, a health and safety committee should be formed that is responsible for organising general health and safety committee meetings and keeping up to date with the newest regulations and guidelines. Members of the committee should be clearly informed regarding their responsibilities, including investigation of incidents, monthly laboratory inspections and reports that are communicated with the laboratory manager, line managers and/or all persons working in the laboratory.

To mitigate the risks of working with faecal sludge, a number of steps for assessing the degree of risk associated with biological and other types of hazards should be followed, such as: identifying the hazards, identifying who may be affected and how they could be harmed, evaluating the risks and taking precautions, and documenting, implementing and regularly updating these steps. Based on this process, standard operating procedures and risk assessments must be developed to minimise the risks and mitigate the hazards.

8.2.2 Standard operating procedures (SOPs)

All laboratory operations should follow standard operating procedures (SOPs), including what to do in the case of accident, exposure, and spills of faecal sludge, for example information on flushing with eye baths, emergency showers, and how to properly contain spills. SOPs are written instructions describing in detail the steps to be performed, and also include procedures for sampling, transportation, analysis, use of equipment, quality assurance and quality control (QA/QC), calibration, and documentation of the entire sample chain of command. SOPs should be written by laboratory personnel who are the most knowledgeable on experimental processes, and should be regularly reviewed by the laboratory manager and the health and safety committee. The SOPs should cross-reference all other related SOPs and expand upon them where necessary. When developing a SOP, the following should be considered:

- Type, quantity, and nature of the hazardous materials used
- Location of use, including fume hood or other containment devices
- Process details
- Available safety equipment, including personal protective equipment (PPE)
- Waste collection, storage, and disposal requirements
- Decontamination procedures.

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Table 8.1 sets out examples of procedures where health and safety SOPs should be developed to reduce potential risks through the entire chain of command - from faecal sludge sampling, through sample collection and transportation, to sample storage and disposal.

Table 8.1 Examples of SOPs for health and safety in activities dealing with faecal sludge.

No.	Activity	Potential risks	Potential controls	Related section
1	Travel to field location	Defective roads causing vehicle accidents	- Ensure vehicles are roadworthy and insured; ensure driver has appropriate licence. - Ensure mobile phones are fully charged. - Carry emergency numbers and First Aid kit to field.	Section 8.2.3.4
2	Sampling in community	Bullying and intimidation by members of community	- Municipality consent and site induction is essential. Communicate with municipality and selected liaison officers from the community. - Arrange introductions to caretaker and/or householder in charge of facility before sampling begins, keep updated on activities.	Section 8.2.3.4
3	Sample collection and transportation	Illnesses due to contact with faecal sludge	- Wear PPE, including safety boots, overalls, elbow length and sharps-resistant gloves, dust masks and goggles. Vaccinations against Hepatitis A and B, tetanus, and typhoid must be current. Use anthelmintic medicines (if necessary) and have stool samples tested 3 times a year for helminths.	Section 8.2.3.1 Section 8.2.3.4

		Sample spill	- Ensure sample containers are securely closed before removing from the facility; secure container or box with a secondary containment at the back of the vehicle.
			- In the case of spillages or splashes:
			  - in environment: clean with disinfectant and paper towels,
			  - on person: rinse affected area with water, dry with paper towel, sanitise affected area with sanitiser, *e.g.* 70% ethanol.	

4	Storage of samples at laboratory	Biological samples stored in an unsafe manner resulting in contamination risk to environment and/or personnel	- Label samples appropriately and record sample number and other details on data sheet. - Store samples in a designated location, under appropriate storage conditions at 4 °C, and maintain a database of all the samples.	Section 8.4.1
6	Cleaning/ washing of PPE	PPE stored or disposed of in an unsafe manner resulting in contamination risk to environment and/or personnel	- Wash or spray sharps-resistant gloves used in the field with ethanol, place in a plastic bag for transportation, and wash well using anti-bacterial detergent. - Before leaving the field, place overalls and safety boots into plastic bags, then wash on the same day. Never clean PPE at home. - Place gloves and dust masks into a separate plastic bag and dispose of in allocated areas for contaminated waste in the laboratory. - Wash and disinfect goggles in the laboratory. - All PPE used during laboratory work with faecal sludge must be either safely disposed of, or disinfected and stored in designated areas.	Section 8.2.3.1
7	Disposal of samples	Health and environmental risk	- The correct PPE must be worn. - Discard small amounts of sample in a drain connected to a sewer.	Section 8.4.3

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8.2.3 Handling of faecal sludge

This section gives the health and safety guidelines to take into consideration during field sampling and laboratory analysis, including preparation, sample collection and transportation. The provided guidelines can vary depending on the specific local context and regulations and each laboratory may adopt and modify them accordingly. Chapter 3 contains a detailed description of different techniques and tools for faecal sludge sampling, how to calculate the needed volume and number of samples, and the importance of PPE and health and safety during sampling.

8.2.3.1 Personal protective equipment

To ensure safe laboratory practices are followed, there are many references that are freely available to develop SOPs and laboratory training, including 'Promoting Chemical Laboratory Safety and Security in Developing Countries and Security' (National Research Council, 2020). The hierarchy of health and safety levels of protection are discussed in Section 2.5.2 and include elimination, substitution, engineering controls, administrative controls, and personal protective equipment (PPE). In general, good PPE practices to prevent infection or contamination include:

- *Good housekeeping*
  Any equipment taken out of the laboratory into a 'clean' environment should be handled only with clean gloves and disinfected with 70% ethanol. Clean all contaminated equipment and surfaces thoroughly with soap and water, and then disinfect with 70% ethanol for 30 sec. Dispose of faecal sludge samples and chemicals properly. If possible, autoclave contaminated materials prior to disposal or reuse (*e.g.* samples, glassware, tools). Dispose of materials in properly allocated and labelled waste bins with lids (*e.g.* biohazard). Adequately wash hands with soap and water for 20-30 sec, followed by hand sanitiser. Small cuts or wounds on the skin must be adequately covered with a protective barrier prior to handling of faecal sludge.
- *Laboratory coat and/or safety overalls*
  Laboratory coats and overalls provide a basic level of protection from accidental chemical and sample spills, and from spreading contamination in 'clean' environments. Coats and overalls need to be fully buttoned at all times. Laboratory coats should not be worn outside of the laboratory, and sampling PPE (*e.g.* used for sampling in the field) should be removed upon return from the field. Jackets and overalls must never be worn in clean areas outside the laboratory (*e.g.* office space). It is also recommended to cover exposed skin with long trousers.
- *Safety shoes*
  Closed, toed shoes and/or rubber boots are recommended when working in a faecal sludge laboratory. Shoes should be non-slip, non-porous and preferably have an impact-resistant front (*e.g.* toes and insoles protected with metal).
- *Eye protection*
  Eye protection should be worn at all times while working with faecal sludge, especially during activities involving risk of splashing such as sample collection, preparation and analysis. Safety goggles protect the eyes and areas immediately surrounding the eyes, while face shields protect more of the face. Safety goggles and face shields can be worn over prescription glasses.
- *Gloves*
  Disposable gloves must be worn at all times when handling faecal sludge. Depending on the type of work, select gloves that provide resistance to abrasion, tearing, punctures, heat and chemicals. In a faecal sludge laboratory, all surfaces, equipment and consumables should be regarded as contaminated. Do not pick up the telephone or touch the door knob, or other common surfaces, while wearing gloves. Do not handle faecal sludge samples directly with gloved hands, as faecal sludge can contain sharp items that could tear or rip gloves. Use appropriate tools such as a spatula or forceps.
- *Masks*
  Respiratory protection minimises the risk of infection through aerosols, inhalation, ingestion and splashing of faecal sludge. FFP2 disposable particulate/filtering masks provide the minimum level of protection from sample splashes, aerosol inhalation and odours. If working with large samples or sampling in the field, FFP3 masks or

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half respirator masks are recommended. If respirators are provided in the laboratory, disinfect and inspect them before use and check for proper fit. FFP2 disposable particulate/filtering masks or FFP3 half respirator masks are recommended for sampling faecal sludge in the field (see Chapter 3).

8.2.3.2 Vaccinations / inoculations

Inoculations provide protection against infectious diseases that are associated with the types of samples received and analysed in the laboratory. For work related with any faecal sludge activities, examples of required vaccines are: Tetanus, Polio, Typhoid fever, Hepatitis A and B. Additional inoculations may be required depending on the prevalence of local diseases and the extent of exposure.

8.2.3.3 'Clean' and 'dirty' work areas

It is important to keep clean and dirty work areas throughout sample collection and processing to ensure protection from pathogens. This includes designated areas for putting on PPE before and after field sampling, and also areas of laboratories where samples are processed during sample preparation and disposal (see Chapter 2). Any activities that are not related to handling of faecal sludge should not be carried out in the 'dirty' areas to prevent exposure to pathogens. This includes normal office work and data analysis. During sampling and analysis, the containers, boxes, tools and equipment used for faecal sludge handling should be placed on washable surfaces. Any 'clean' items that are frequently removed from the laboratory, *e.g.* cameras and data sheets, should be stored in allocated areas on a workbench, and be handled with clean gloves.

8.2.3.4 Sampling

Protocols for the collection and transport of samples are covered in detail in Chapter 3 including the necessary equipment, proper recording of processes, chain of custody, transport, and a field sampling kit. Specific SOPs for sampling are included in Table 8.1. Additional concerns for health and safety include:

- Ensure the sampling vehicle has a First Aid kit and all the passengers have valid identification, permission, and documentation of medical insurance.
- Ensure mobile phones are fully charged and check network coverage.
- Be sure to inform the line manager of the planned itinerary and estimated time of return to the laboratory.
- Prepare sufficient drinking water and weather protection (*e.g.* hats, sunscreen, and waterproof clothing).
- Avoid working alone in the field.
- After sampling, contain the tools tightly in a container or bag to prevent contamination.
- Tightly seal the sampling containers and store them in a large, solid cool box during transportation to avoid spills.
- Brush shoes and spray shoes (including soles) and protective clothing with 70% ethanol after sample handling is complete.
- Dispose of used gloves in an allocated waste bag after sample handling and cleaning of equipment is complete. Reusable gloves (*e.g.* sharp-resistant) must be placed in a separate bag for disinfection in the laboratory.
- Wash hands for 20-30 sec with soap and water, followed by hand sanitiser.
- Upon arrival at the laboratory, clean the sampling tools and PPE in the designated area.
- Leave the washed and disinfected tools and sampling containers in the wash area until dry, then store safely.
- Send PPE clothing to the laundry facility.

8.3 QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC)

Once samples have been collected and transferred to the laboratory, protocols need to be in place to ensure representative, comparative, and repeatable results when carrying out analytical methods. The goal of quality assurance and quality control (QA/QC) is to ensure precise and accurate test results within acceptable limits. The purpose of quality assurance specifically is to give relevant, reliable and timely test results that are correctly interpreted. Quality assurance includes the laboratory procedures that are carried out to produce defensible results with reliable precision and accuracy, and to ensure that the laboratory functions efficiently and effectively. Each laboratory should have an operation manual, with a defined set

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of procedures that includes all the necessary aspects to demonstrate the laboratory's competence and to ensure and document the quality of its analytical data (Rice *et al.,* 2017). Quality assurance is a total process whereby the overall quality of laboratory results can be guaranteed (WHO, 2011). This includes, but is not limited to, sample collection and processing, good laboratory practice and management skills (*e.g.* roles and responsibilities of staff, training of staff, tracking of samples, SOPs, structures for clear reporting and documenting, maintenance of equipment, calibration curves, protocols for determining method detection limits (MDLs), and sample disposal). Quality assurance also includes quality control. Quality control is primarily concerned with the control of errors in the performance of sampling and analysis, and verification of the accuracy and precision of test results (*e.g.* standards, blanks, duplicates, and standard reference materials). Quality control should be practical, achievable and affordable. The broad aim of quality control is that results are both accurate and precise, which ensures that results from one laboratory are comparable with the results from any other laboratory in the world, if the same method is followed. This can even then be optionally verified through sample exchange. For further information on implementing a quality assurance programme, and how to apply for accreditation, see ISO 9001 and ISO/IEC 17025:2017.

8.3.1 Training

It is important that all staff are adequately trained for the tasks they have to perform. Training must be documented so that management and other personnel can verify that staff are competent to conduct the duties required of them. Laboratory training, a competency assessment, SOPs and a hazard identification risk assessment (HIRA) are required before any person is permitted to access the laboratory or use laboratory equipment for the analysis of faecal sludge.

8.3.2 Standard operating procedures

Standard operating procedures (SOPs) were defined in Section 8.2.2. They provide the core of most of the day-to-day operations of any quality assurance programme. SOPs are based on internal experience, manuals and published references. In practice, SOPs should clearly present the procedural steps in a way that avoids potential differences in interpretation, thereby avoiding subtle changes in the way methods are performed or equipment is used. Such differences can impact the overall quality of the results. An SOP should be clear, concise and contain all the relevant information to perform the procedure it describes. In addition, it should include the methods and frequency of calibration, MDLs, maintenance and quality control, remedial action to be taken in the event of malfunction or loss of control, and information on how to properly dispose of samples and required laboratory consumables (*e.g.* including biowaste, and handling of toxic chemicals). SOPs must be regularly revisited and updated by experienced laboratory technicians.

8.3.3 Laboratory facilities

Resources are required for regular laboratory work as well as for the additional workload associated with quality assurance. It is essential resources of space, staff, equipment and supplies are sufficient for the volume of work. The environment in which the work is conducted must be controlled to eliminate interferences. In practice, anything that restricts the efficient running of the laboratory would be a cause for concern. Further details on setting up a faecal sludge laboratory are provided in Section 2.5.

Basic laboratory equipment and glassware as defined by the American Chemical Society (ACS) (2020) include a balance, beakers, beaker tongs, Bunsen burner, burette, clay triangle, crucible, crucible tongs, Erlenmeyer flask, evaporating dish, forceps, funnel, graduated cylinders, mortar and pestle, pipette bulb, ring stand, scoopula, striker, stirring rod, test tube, test tube clamp, test tube rack, thermometer, utility clamp, volumetric flasks, volumetric pipettes, wash bottle, watch glass, and wire gauze. Their website presents pictures of the equipment, and a brief description. Further required equipment and analytical machines will depend on the specific procedures being conducted in the laboratory.

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8.3.4 Sample chain of command

Protocols for the collection and transport of samples are covered in detail in Chapter 3 including the necessary equipment, proper recording of the process, chain of custody, transport, and a field sampling kit. Upon delivery of faecal sludge samples to the laboratory, another important activity is how the samples are received, organised and stored for analysis. These activities are an essential part of the chain of command and need to be done in a systematic manner. Improper sample storage can compromise the analytical results and incomplete labelling can lead to unidentifiable sources of the samples, incomplete data sets, and increase the potential need to repeat the sampling and analysis. All incoming samples should be systematically labelled according to a standard system that has the sample number, project name, date, and name of the sampler clearly labelled on the sample container. Assign different IDs (a coding system) to the samples and write on the container with permanent marker. If undertaking a large sampling campaign, assign a number to each container and keep a separate ID (coding key). After labelling, place the samples in storage (see Section 8.4.1). If storing the samples in a cold room with many other samples, consider developing a sample register for improved sample management. This information must also be stored electronically (a database) using a sample coding key (ID) system that enables the samples to be tracked to their source. Data from paper copies should be transferred to the electronic sample database as soon as possible. The database must be updated regularly and stored in a specifically allocated directory. Basic characteristics may be recorded at this time, for example a photograph, and the mass and type of the faecal sludge. If dealing with samples of individual fresh faeces, use the Bristol Stool Chart for form (Lewis and Heaton, 1997), and for more details refer to Chapter 7. The procedures for receiving and logging faecal sludge samples can vary depending on the specific local context and regulations, and each laboratory should adopt and modify them accordingly.

8.3.4.1 Laboratory photographs and notebooks

Photographs are useful to capture at this stage, and can be a part of the labelling and sample management system. This is useful to understand the texture and appearance of the sample during data analysis and discussions, or to help recover the sample's ID at a later stage in cases when they have not been recorded properly. Two people are recommended for this task – one to handle the samples and the other to handle the camera, in order to prevent contamination of the equipment. The sample handler should open the lids of the sample containers and make sure that the sample inside and all the details on the containers, such as sample name, dates and times are visible for the photos. The person holding the camera should take the photos making sure that all the details are captured. If working with a large number of samples, photographing can be done in a manageable batch order - *e.g.* five to ten samples at a time. A data sheet can be used to record details such as amount of sample, type and condition of sample (*e.g.* visibly degraded, covered with maggots, liquid or solid, and specific odours). After completing the photography, the sample handler should close all the sample containers and return them to storage. Save the updated database (with photos) in a designated electronic directory. Use 70% ethanol spray to disinfect the camera and surfaces as a preventative measure. A sample collection also frequently includes questionnaire data. It is important that this information is all stored electronically in the same format for data analysis. For a detailed description of sample collection with questionnaires and photographs, and possible methods for data analysis, see Ward *et al*. (2021).

It is very important to keep a detailed notebook documenting every step taken while carrying out laboratory methods. Further information on keeping laboratory notebooks ('lab books'), and a general reference for other ethical guidelines when conducting research, can be found in Marcrina (2005) and Barker (2005). Lab books are important for transparency and ethical reporting of results, and also for troubleshooting laboratory procedures. For example, if there are two different balances in the laboratory, when weighing samples, it should always be recorded which balance is used. If one is discovered to be malfunctioning, then it is clear which samples need to be re-evaluated. A general rule is that it is best to err on the side of too much detail. Other general rules include: lab books must be bound (pages are not

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removable), pages should be numbered and dated in chronological order, pages cannot be removed, information must be recorded in ink, if an error is made a line should be drawn through the text followed by the correct information (absolutely no white-out or correction fluid), blank pages or spaces must not be left (if left intentionally, draw a line through the space), entries cannot be modified (additional information can be added as a new entry in chronological order), and any attachments should be permanently placed in the lab book (*e.g.* a print-out of a calibration curve attached with staples). In addition, lab books are the property of the laboratory and should be tracked, recorded, and archived. Laboratory books cannot be removed from the laboratory, although photocopies or carbon copies can be made for this purpose.

8.3.4.2 Equipment maintenance and calibration

The laboratory must regularly service and maintain all the equipment, which is monitored by a competent and trained laboratory manager or experienced laboratory technicians. Frequent checks on the reliability of equipment must also be performed. To reduce the use of malfunctioning equipment, calibration and maintenance records of all instruments must be stored in a filing system, allowing for the operational status of all the apparatus to be monitored. Analytical equipment should be serviced at regular intervals, according to the manufacturer's instructions and recommendations in standard methods. The frequency and complexity of routine servicing is dependent on the instrument and use. Along with this, the laboratory should perform daily inspections and weekly maintenance of all the equipment and develop a monthly maintenance plan. Each laboratory should develop their own equipment maintenance plan based on frequency of use, age of equipment, user experience, service plans, maintenance checks, and availability of funds. Further guidelines on equipment maintenance and calibration are provided in ISO/IEC 17025:2017, particularly for accredited laboratory systems (IEC, 2017).

As described in Prichard and Barwick (2003), instrument calibration is a part of the majority of laboratory analysis. As it is such an important step in an analytical method, it is essential to have a good understanding of how to set up a calibration experiment and evaluate the results. Calibration entails making a set of standards of known concentration, measuring the instrument response to the standards, and then establishing the relationship between the instrument response and concentrations of the analyte. Detailed information is provided by the LGC group, and the report can be downloaded free of charge (Prichard and Barwick, 2003). Included is information on how to determine the number of standards and range of concentrations that should be used, including a blank. Whether or not the standards are made up in the sample matrix or a solvent will depend on the type of analysis and the analytical machine. The accuracy of some methods can be improved by using an internal standard in standards and samples. In general, seven standards should be used, and they should be evenly spaced across the range of concentrations to be measured with the majority of concentrations falling in the middle range. The standards should be analysed in random order, and the results must be plotted. The statistical analysis of the results is critical, and can be a common source of inaccurate results. It is important to know when to fit the curve through zero, and how to check for bias or leverage due to outliers. The residual standard deviation is used as an estimate of the uncertainty in the predicted concentration values. It is also important to reduce the uncertainty of concentrations through quality control measures such as the use of proper glassware, and an adequate grade and purity of chemicals (Prichard and Barwick, 2003).

The instrument lower-level detection limit (IDL) needs to be quantified by using replicate measurements of aliquots from one standard. The lower limit of detection (LOD) is the lowest concentration that can be statistically detected from a blank. However, the method detection limit (MDL) also needs to be calculated, and to take into account uncertainties that can be introduced during analysis at each step of the method (*e.g.* dilutions, digestions, sub-sampling, sample matrix, type of instrument, and laboratory skill). The MDL is the minimum value that can be reported with 95% confidence that the measured value is above zero. The MDL should be calculated with replicate measurements of aliquots from the actual sample matrix, with low level spikes

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2-10 times the expected MDL, together with blanks (minimum 7 of each). This analysis is performed in triplicate on three separate days, and should be repeated annually. The lowest level of MDL that can be reported is calculated as the mean determined concentration plus three times the standard deviation of a set of method blanks.

Any value less than the MDL is reported as non-detectable (ND). There is more variability in the MDL, so it is normally expected to be higher than the instrument detection limit. For more information on calculating the MDL, see USEPA 40 CFR Part 136 Appendix B, and tutorials that can be found by searching for 'method detection limit' at the USEPA website[2] (USEPA, 2017).

For most environmental samples, the actual level of quantification (LOQ) will be 5-10 times the MDL (Rice *et al.,* 2017). However, this is for environmental samples that can be considered to be more 'clean' than faecal sludge, so it is very important to calculate the method LOQ for each specific method and sample matrix. The LOQ will depend on defined levels of precision and accuracy, and can be reliably achieved during routine operations. Calibration curves must include a standard with the LOQ reported by laboratories. A common definition is 10 times the reagent water blank signal (Rice *et al.,* 2017). The LOD, LOQ and MDL are all important parameters to consider when comparing results between laboratories.

8.3.4.3 Reporting of results

At the end of the analysis, the final product of the laboratory is the reported data to be used for data analysis, either by clients, other institutional departments, regulators, or for research publications. Quality assurance ensures that the data is suitable for use in an assessment. This includes the final stages of reporting and interpreting the results. The data should be examined at many stages in the quality assurance system and no data should be reported if it is out of range of the methods. Reports must be prepared according to an agreed procedure, and they must accurately reflect the findings of the study. They should include reference to all the calibration and quality control data, any problems or limitations encountered during the study, and all the calculations or correction factors. Data should be reported in standard units, as described in the SOP. Results should also include the method uncertainty, and correct representation of significant digits (Method 1050B). Whenever possible, the open sharing of raw data is strongly encouraged, to ensure transparency of the results and to increase the overall knowledge of characteristics of faecal sludge.

8.3.4.4 Checking compliance

In order to maintain the quality assurance system, it is necessary to periodically check each area of the laboratory for compliance with the quality assurance system. The audit must be independent, hence the need for a quality assurance officer who reports directly to the highest level of management, or an external auditor. Laboratories should routinely monitor and assess the quality of the testing process in the pre-analytical, analytical and post-analytical phases from sampling and transporting to reporting of the obtained faecal sludge data.

The pre-analytical phase encompasses the following procedures:

- Laboratory training, laboratory safety, number of trained personnel available
- Sample collection, labelling, transport, processing before testing, and storage
- Number, types and sources of samples tested
- Chemical reagent storage conditions, selection of test kits and regular monitoring of the expiry dates
- Regular recording of all information, proper data storage systems.

The analytical phase encompasses the laboratory analysis using laboratory methods:

- Written standard operating procedure manual
- Testing performance, and performance and preventive maintenance of equipment
- Reagent preparation and correct use

[2] https://www.epa.gov/cwa-methods

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- Inclusion of internal and/or external quality control
- Quality control monitoring procedure.

The post-analytical phase encompasses all the steps that occur following the analysis:

- Maintaining records for traceability of the documented results, calibrations, standards, calculations and feedback
- Data entry and storage (computer or hard copy)
- Data interpretation and reporting
- Reviewing and addressing queries.

8.3.5 Quality control

Quality control consists of the operational techniques (internal and external) used by the laboratory staff for continuous assessment of accuracy and precision. Internal quality control focuses on the individual method and tests its performance against the precision and accuracy of the given SOPs. Quality control external to the laboratory is a way of establishing the accuracy of analytical methods and procedures and the representativeness and repeatability of sampling by comparing the results obtained in one laboratory with the results obtained by others conducting the same analysis on the same material. In the future, for methods of faecal sludge analysis to become standardised, reference laboratories will have to send out sets of samples with known concentrations of variables to a group of participating laboratories. Each participating laboratory will then analyse the samples for the specified variables and report the results to the reference laboratory. Examples of quality control measures in general to ensure accuracy and precision are provided in Table 8.2, and the required minimum quality control steps are included in each of the methods.

Precision and accuracy play an important role in quality control of experimental analysis and measuring errors. Although they are often used interchangeably, they have different meanings and are independent from each other. Accuracy refers to how close a measurement is to the true value, while precision refers to how close measurements using the same method and equipment are to each other. Two important elements of precision are repeatability and reproducibility. Repeatability is the variation observed when the same person repeats the same method using the same equipment. Reproducibility is the variation observed when different people repeat the same method using the same equipment. A measurement can be very accurate but not precise, or very precise but not accurate. The best quality control is achieved when an analysis is both accurate and precise. This is further illustrated in Figure 8.1, with the example of a dartboard, where the bull's eye is the true value and darts hitting the dartboard close to it are accurate. A illustrates neither accuracy nor precision; B illustrates precision (close repetitions) but not accuracy; C illustrates accuracy but not precision, and D illustrates both accuracy and precision (Byron Inouye, n.d.).

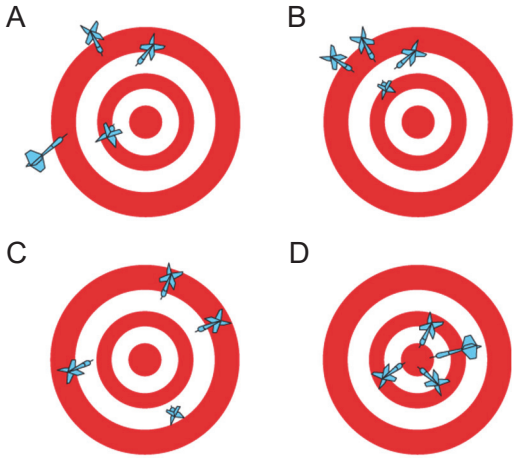

Figure 8.1. Dartboards showing different accuracy and precision scenarios. A: neither accuracy, nor precision; B: precision (close repetitions) but not accuracy; C: accuracy but not precision; and D: both accuracy and precision (required result of QC measures). Source: Byron Inouye, 2020.

Different measures for quality control are presented in Table 8.2. In practice, different methods to measure parameters will be selected based on the required precision, sensitivities and cost. Once a method has been validated and embedded into routine use in the laboratory with an SOP, it is necessary to ensure that it continues to produce satisfactory results.

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Table 8.2 Example of quality control steps and measures for faecal sludge analysis to ensure precision and accuracy.

Quality control measure	Description	Main purpose	Minimum frequency
Field replicates	Multiple samples that are collected at the same time and place, and analysed separately.	Identify whether the sample collection is reproducible.	Depending on analytical goal.
Laboratory replicates	One sample that is split into subsamples in the laboratory, and each one is then analysed separately.	Identify whether the laboratory analysis is reproducible.	After every 10 samples, should be within 10% of each other.
Pipette volume	Weighing a known volume of pipetted water.	Identify the accuracy of the pipette and technician pipetting.	Before starting a new experiment and after every 100 samples.
Standard curve	Standard concentrations purchased from high quality laboratories, and/or self-prepared, used for device calibration and for evaluating accuracy of analytical devices.	Determine accuracy of instrument.	Frequency is dependent on analytical method, but recommended to include at least one standard or set of standards in every series of analyses.
Continuing calibration verification	An individual standard that is analysed between every batch of 10-15 samples.	Identify whether the analysis is accurate and ensure instrument is not drifting.	After every 10-15 sample analyses.
Calibration blank (machine blank)	A blank (often deionised water) is used to ensure that analytical devices are not over or under-estimating or drifting.	Identify background contamination and drifts of the analytical device.	Before every series of analyses.
Method blank	A method blank is deionised water processed in the same fashion as samples and used to 'zero' the instrument.	Detect any contamination during the course of analysis and sample preparation.	After every 15-20 samples.
Spiked samples	Adding a known amount of the analysed compound to a sample to test the accuracy of sample concentration measurements.	Determine the accuracy of the method for different sample matrices.	Depends on objective of study.
Reference standards	Standards in a sample matrix purchased from reference laboratories (*e.g.* National Institute of Standards and Technology (NIST), UK Bioresearch Centre (UKBRC)) to evaluate the percentage recovery during sample preparation and analysis (*e.g.* acid digestion for metals).	Identify the percentage recovery of the specific compound that is tested for throughout the entire method (sample preparation and analysis).	Depends on objective of study.
Internal standard	An analyte that is similar to the compound of interest spiked into standards (*e.g.* for use with GC-MS, LC-MS, HPLC).	Monitor retention times and calculate concentrations.	Depends on type of analysis.
External analysis	Sending out a sample for external analysis to verify the accuracy of laboratory results.	Estimate analysis precision and accuracy.	Depends on objective of study.

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A validity check standard sample should be included in a batch of samples to monitor precision, standard deviation and accuracy. Precision and accuracy checks allow for quality control of data quality, including calculations and records, standard solutions, reagents, equipment and quality control materials. Some controls such as duplicates ensure precision (reproducibility), whereas other controls ensure accuracy (*e.g.* standards and blanks). If any of the quality control procedures indicates that a problem exists, corrective action must be taken immediately. Quality control measures should also be tracked over time, and reported with the data when published. A discussion of how to statistically interpret results of blanks can be found in Statistics in Analytical Chemistry: Part 40 - Blanks (Coleman and Vanatta, 2010).

8.4 SELECTION OF THE APPROPRIATE METHOD FOR THE PURPOSE OF CHARACTERISATION

In this book, faecal sludge is classified into four categories based on the total solids (TS) content: liquid (TS < 5%), slurry (TS 5-15%), semi-solid (TS 16-25%), and solid (TS > 25%), as defined in Chapter 2. This is important, as the type of faecal sludge determines the selection of appropriate sample preparation, analytical methods and/or steps that are taken within the methods. For example, semi-solid and solid types of sludge may require greater dilution and/or centrifugation in addition to filtration for particular methods, while the liquid sludge may not require dilution and may require only filtration to remove suspended particles. Specific concerns for sample preparation are detailed in each of the methods. The category of sludge is also relevant in regard to how results are reported, either as a mass/volume concentration (mg/L) or as a mass/mass concentration (g/g). In general, semi-solid to solid samples are reported as mass concentration, and liquid and slurry samples as concentration. However, it is not possible to make a hard and fast rule, and this will also depend on the range of samples being analysed. Sample density and TS concentrations can be used to convert results between concentrations and mass fractions (Section 2.2).

Selection of the most suitable method for characterisation depends on the purpose, required level of accuracy, cost of analysis (often a limiting factor), access to analytical machines, and the laboratory capacity required to undertake specific analyses, as discussed in Chapter 2. In general, the level of accuracy, cost of analysis, and access to analytical machines are related to each other, with more expensive laboratory equipment and analytical machines leading to increased accuracy. However, the highest level of accuracy is not always necessary. For example, nitrate test strips might be appropriate to get a rough idea of concentrations when working in the field, or in the laboratory when determining a required dilution to fall within the spectrophotometer method limits. If analysing ammonia, the choice of using a test kit based on the phenate method *versus* a titration will depend on factors such as the available budget for purchasing test kits, the costs of different manufacturers' test kits *versus* the cost of chemicals, access to a spectrophotometer, and access to titration equipment or an automated titration system. For analysis of heavy metals, the appropriate method will depend on whether total metals or available metals (*e.g.* plant available) are of interest, or for dewatering if settling or filtration (*e.g.* drying beds) are of interest. Therefore, an assessment needs to be undertaken before the analysis is conducted, to weigh these factors when selecting the most appropriate method for the specific context.

As discussed in chapters 2, 3 and 5, the number of samples is also a factor contributing to cost. Frequently, budgets limit the possibility of collecting a number of samples required for 95-99% confidence, and decisions on how to ensure representativeness need to be taken carefully. Another way to reduce costs is to carefully define the objectives and then only analyse metrics that answer the specific objectives. This might sound obvious, but *E. coli* should not necessarily be selected as a parameter of interest based solely on having the laboratory capacity. For example, when setting up experiments to scale up inline dosing of conditioners for enhanced dewatering performance as described in Chapter 4, TSS and CST are the metrics of interest, and the additional cost of *E. coli* analysis would not be justified. In addition, the total

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number of parameters that are analysed contributes to increasing levels of data analysis.

The methods provided in this chapter are summarised in Table 8.3, including estimates of time needed for sample preparation and analysis.

Table 8.3 Summary of methods presented in this chapter, by the properties being analysed: chemical and physico-chemical (Section 8.6), physical (Section 8.7) and biological (Section 8.8).

No.	Method	Preparation time	Analysis time
8.6	CHEMICIAL AND PHYSICO-CHEMICAL PROPERTIES		
8.6.1	*Solids and moisture content*		
8.6.1.1	Total solids and moisture content - volumetric and gravimetric method by oven drying	< 1 hr	> 24 hr
8.6.1.2	Volatile and fixed solids - ignition method	< 5 min	< 2 hr
8.6.1.3	Total suspended solids and total dissolved solids - oven-drying method	< 1 hr	> 24 hr
8.6.1.4	Volatile suspended solids - ignition method	< 5 min	< 2 hr
8.6.1.5	Total solids and moisture content - thermal balance (moisture analyser) method	< 30 min	< 30 min
8.6.1.6	Sand content	< 30 min	< 3 hr
8.6.2	*Chemical oxygen demand (COD)*		
8.6.2.1	Chemical oxygen demand - closed reflux spectrophotometric method	< 30 min	< 30 min
8.6.2.2	Chemical oxygen demand - closed reflux titrimetric method	> 24 hr	< 3 hr
8.6.3	*Fat and fibre*		
8.6.3.1	Crude fat - Soxhlet extraction method	> 24 hr	> 24 hr
8.6.3.2	Crude fibre - filtration method	> 24 hr	> 6 hr
8.6.4	*Nitrogen*		
8.6.4.1	Total nitrogen - spectrophotometric method	< 30 min	< 30 min
8.6.4.2	Ammonium - colorimetric (test strip method)	< 15 min	< 30 min
8.6.4.3	Ammonium - phenate spectrophotometric method	< 30 min	< 30 min
8.6.4.4	Ammonia - distillation and titration method	< 1 hr	< 1 hr
8.6.4.5	Nitrite - colorimetric (test strip method)	< 15 min	< 30 min
8.6.4.6	Nitrite - spectrophotometric method	< 30 min	< 30 min
8.6.4.7	Nitrate - colorimetric (test strip) method	< 15 min	< 30 min
8.6.4.8	Nitrate - cadmium reduction spectrophotometric method	< 30 min	< 30 min
8.6.4.9	Total Kjeldahl nitrogen - distillation and titration method	> 30 min	> 3 hr
8.6.5	*Phosphorus*		
8.6.5.1	Total phosphorus and orthophosphate - spectrophotometric method	< 30 min	< 30 min
8.6.5.2	Orthophosphate - colorimetric (test strip) method	< 15 min	< 30 min
8.6.6	*pH and electrical conductivity*		
8.6.6.1	pH - electrode method	> 10 min	< 15 min
8.6.6.2	Electrical conductivity - electrode method	> 10 min	< 15 min
8.6.7	*Elemental analysis*		
8.6.7.1	Metals - overview	Dependent on selected method	Dependent on selected method
8.6.7.2	Metals - acid digestion for environmentally available metals	< 2 hrs	< 30 min
8.6.7.3	Ultimate analysis - total carbon, hydrogen, nitrogen, oxygen, and sulphur	Dependent on selected method	Dependent on selected method
8.6.7.4	Chlorine - colorimetric (test strip) method	< 15 min	< 30 min
8.6.7.5	Chlorine - spectrophotometric method	< 30 min	< 30 min

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8.6.7.6	Chloride - colorimetric (test strip) method	< 15 min	< 30 min
8.6.7.7	Chloride - spectrophotometric method	< 30 min	< 30 min
8.6.8	*Colour and turbidity*		
8.6.8.1	Colour - visual comparison method	< 30 min	< 15 min
8.6.8.2	Turbidity - nephelometric method	5 min	5 min
8.6.9	*Settleability and dewaterability*		
8.6.9.1	Jar test	30 min	< 60 min
8.6.9.2	Capillary suction time	5 min	< 15 min
8.6.9.3	Water activity	< 10 min	< 15 min
8.6.9.4	Sludge volume index	< 15 min	< 3 hr
8.7	PHYSICAL PROPERTIES		
8.7.1	*Physical and mechanical*		
8.7.1.1	Density - mass and volume measurement method	< 15 min	< 15 min
8.7.1.2	Density - volume displacement method	< 15 min	< 30 min
8.7.1.3	Particle size - laser light scattering method	5 min	< 15 min
8.7.1.4	Rheological properties - rheometer method	< 10 min	< 30 min
8.7.1.5	Liquid limits - cone penetrometer method	10 min	< 15 min
8.7.1.6	Plastic limits - thread-rolling method	30 min	< 60 min
8.7.1.7	Compressibility and stickiness - texture analyser method	< 15 min	< 15 min
8.7.2	*Physical and thermal*		
8.7.2.1	Thermal conductivity - thermal conductivity analyser method	< 10 min	< 15 min
8.7.2.2	Calorific value - bomb calorimeter method	> 24 hr	< 15 min
8.8	BIOLOGICAL PROPERTIES		
8.8.1	*Pathogens*		
8.8.1.1	*E. coli* and total coliforms - colony forming unit method	> 30 min	< 60 min
8.8.1.2	*E. coli*, faecal coliforms, and total coliforms - most probable number method	< 30 min	< 30 min
8.8.1.3	Bacteriophage - plaque assay method	< 3 hr	< 2 hr
8.8.1.4	Helminths - microscopy method	< 30 min	< 30 min

8.4.1 Faecal sludge storage and preservation

As discussed in Section 3.12, proper preservation helps ensure that no significant changes in composition occur before the analyses are made. It is best to analyse samples immediately upon arrival at the laboratory. However, time and capacity constraints do not always allow this. Therefore, short-term storage is frequently required (*e.g.* up to a few days at 4 °C), and sometimes longer-term storage is required. Some properties are more affected by sample storage conditions and durations than others. Whenever possible, longer-term storage with the addition of preservatives should be avoided, as adding preservatives can also change the composition of the sample and can affect the properties. In this case, it is recommended to only use preservative in a sub-sample of the original sample. Examples of preservation methods depending on the intended analysis include pH control, chemical addition, the use of amber and opaque bottles, filtration prior to storage, drying, dry-freezing, refrigeration (4 °C), and freezing (-20 °C). However, all methods of preservation may be inadequate when applied to suspended matter. Preservative should not be added if volatile, semi-volatile or microbial contaminants are to be analysed, unless specified in the standard operational procedure. For the analysis of stable compounds, such as the total metal analysis of properly stored and dried samples, longer-term storage is allowable. Information on specific preservation techniques is included in each method, and more information can be found in Method 1060C (Rice *et al.,* 2017).

8.4.2 Faecal sludge sample preparation for analysis

Before analysis, samples are usually homogenised, diluted and, if necessary, filtered or centrifuged. The purpose of homogenisation is to ensure that the

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sample is thoroughly-mixed and that the analysis is representative of the sample. Further processing may be necessary in order to ensure that the sample is able to be analysed using a specific method or instrument. This can include drying, grinding, sieving, diluting, filtering, and/or centrifuging. The sample preparation methods vary depending on the type of faecal sludge (solid, semi-solid, slurry or liquid), and also on the type of analysis. For example, blending and dilution are appropriate for preparation for chemical analysis such as COD and ammonia but not for other methods. For rheological and viscosity analysis, the samples must be analysed as received because blending or mixing will change the structure of the sample and affect the results of the analysis. Similarly, dilution should not be applied to samples that are going to be analysed for total or volatile solids content. Information on specific preparation techniques is included in each method. It is important to consider the different processing requirements for all of the intended methods, as this will dictate the required sample volume and laboratory preparation pathways. An example of this is provided in Figure 3.23, for a series of preparations with the same sample that includes blending/not blending, centrifugation, and further dilutions.

8.2.4.1 Homogenisation of samples

Homogenisation is especially important for faecal sludge, as it is typically a very heterogeneous and complex matrix, and inadequate homogenisation can be a significant source of error in the final results. Homogenisation techniques include blending, shaking, vortexing, mixing, stirring, and grinding. Prior to homogenisation, remove any large non-faecal materials, such as stones, plastics, textiles, hair, maggots, and rubbish.

For liquid and slurry sludge (TS < 5%, TS 5-15%, respectively), mix the sample by gently inverting the container until the settled particles are in suspension, or apply rapid stirring with a ladle so the sample can be distributed prior to any settling. If slurry samples are too thick to homogenise in this fashion, use the method for semi-solid and solid sludge. Whether or not samples should be blended is specified in each method, and will depend on whether or not destroying the sludge structure would interfere with the subsequent analysis. For semi-solid and solid sludge (TS > 15%), mix the entire sample using a stainless steel rod (or another appropriate tool), until visibly homogenised. Blending before dilution may not be suitable for these types of samples because of their high TS content. For dried resource recovery products, such as dried sludge, samples can be air-dried and then ground using a mortar and pestle or mechanical grinder to homogenise.

8.2.4.2 Dilution of samples

Depending on the type of analysis and the type of faecal sludge, dilution may be necessary so that the sample concentration is within the quantification range for the specific method. For example, the COD concentration in undiluted faecal sludge is frequently relatively high and requires a series of dilutions. Care needs to be taken with dilution, as it can be a significant source of error in the final results, especially with highly heterogeneous faecal sludge. The level of uncertainty of volumetric measurements from glassware and pipettes needs to be taken into account to know what can be accurately measured. For example, the level of uncertainty for a 1 mL pipette is ± 0.01 mL, for a 10 mL volumetric flask ± 0.01 mL, and for a 10 mL graduated cylinder ± 0.1 mL. Pipetting small volumes into large volumes for dilution needs to be avoided, and in general, a series of dilutions should be used for more than two orders of magnitude. For example, for a 1:1,000 dilution, pipette 1 mL into 9 mLs for a total of 10 mL diluted sample volume, and repeat this three times; and never pipette 1 mL into 999 mL. Serial dilutions are defined as repeating the same dilution step over and over, and hence represent a geometric series (*e.g.* 1/10, 1/100, 1/1,000, or 1/3, 1/9, 1/27). It is of utmost importance that at each step in the dilution, the sample is thoroughly mixed before making the next dilution. Depending on the method, this could be stirring, shaking, vortexing, or even mixing in a blender; exact protocols need to be developed for each method and local context.

For liquid and slurry types of faecal sludge (TS < 15%), sub-samples for dilution are measured out volumetrically. Mix the sample as described in the 'homogenisation' section and measure out an appropriate volume for dilution into a volumetric

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flask, and then add distilled water to a specific volume. A serial dilution will be necessary depending on the required concentration range, and could be followed by filtration or centrifugation to separate the suspended solids depending on the specific method. For semi-solid and solid sludge (TS > 15%), tare a beaker on an analytical balance and then weigh out the sample into the beaker. Calculate the volume of required dilution water, and weigh it out in a separate tared container. Add a volume of the dilution water to the sample, and mix it well, then transfer to the blender. Reserve some of the volume of dilution water to rinse the beaker that held the sample and also transfer the wash water to the blender. For example, this could mean weighing out between 1.8 g and 2.0 g into a 50 mL beaker, and then rinsing it in in series to obtain the required volume of 200 mL. Blend the diluted sample at the highest setting until homogenised, then pour the diluted sample into a bottle for analysis or storage. It is not necessary to remove all of the solids from the blender as the sample has been thoroughly mixed.

The results should be represented as either a volumetric concentration (mg/L) or as a mass fraction by total dry solids (g/g TS). In general, it is not recommended to report results as a wet basis mass fraction (g/g wet sample), due to the high variability in percentage solids or moisture in faecal sludge samples. This means that density (mass per volume) and TS (mass of TS per volume) should also be measured for each sample for accurate reporting and to allow for comparison between results. The definition of the dilution factor that is used in the methods is:

$$\text{Dilution factor} = \frac{\text{Final adjusted volume of dilution}}{\text{Original volume of sample aliquot to be diluted}}$$

For example, if 10 mL is the final dilution volume and 1 mL was the original sample volume, then the dilution factor would be 10.

8.2.4.3 Filtration

Filtration of samples could include sieving of dried ground samples prior to metals digestion, or filtration of liquid samples to remove suspended solids. For example, as described in Method 8.6.1.3 for total suspended solids, acceptable glass fibre filters will range in pore size from 0.45 µm to 2.0 µm depending on the thickness of sludge and clogging of the filters. In addition to pore size, clogging is addressed through the process of sample dilution for semi-solid to solid samples, as explained in the method description. The type of filter material will also depend on the intended method, and is explained in each section; for example, glass fibre filters are specific to methods that use a 550 °C muffle furnace, and paper filters can be used in nutrient methods.

8.2.4.4 Centrifugation

Centrifugation can be used instead of, or in addition to, filtration to separate suspended particles from the sample. For liquid faecal sludge (TS < 5%), mix and blend the sample as described in the 'homogenisation' section and transfer an aliquot of the sample into a clean glass beaker. Withdraw sample aliquots of equal volume into one or more centrifuge tubes. Place an even number of centrifuge tubes diagonally opposite each other to balance the centrifuge. If there are an odd number of centrifuge tubes filled with sample, fill an additional tube with the same volume of water to balance the centrifuge. Centrifuge the tubes at a specific g-force and time. Decant the filtrate (supernatant) into a clean glass beaker and discard the pellet. Centrifuge the samples until the required volume for sample analysis is obtained. Centrifugation can also be used for separation of the suspended solids in slurry, semi-solid, and solid samples (TS > 5%); however, centrifugation will be more effective if the samples are diluted beforehand. In cases where the sludge is very difficult to filter, centrifugation can be used prior to filtration to remove most of the suspended material.

8.4.3 Sample and chemical disposal

Waste minimisation and pollution prevention in the laboratory is the preferred approach to managing laboratory waste. However, when the remaining material is disposed of, it is important to ensure the protection of public and environmental health, and disposal needs to be included in each SOP. For more

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detailed information on methods of disposal, see National Research Council (2011).

Faecal sludge samples and laboratory equipment should be contained and sterilised to eliminate all pathogens. All contaminated equipment should be disinfected before washing, storage, or disposal. If possible, it is recommended to autoclave materials contaminated with faecal sludge before discarding them. After sterilisation, waste can be handled safely and disposed of in the local waste collection. Incinerated or pasteurised samples can also be contained and disposed of in the general waste. If autoclaving is not possible, small volumes of faecal sludge samples (*e.g.* 1-5 L) should be flushed down a drain that is connected to the sewer, and the whole area then properly sterilised. If this is not possible, store the waste samples in a sealed container in a cold room or refrigerator at 4 °C. The person responsible for the samples (*e.g.* the student, researcher, or laboratory personnel) then needs to arrange for the waste samples to be discarded at a local wastewater or faecal sludge treatment plant. If a faecal sludge treatment plant is not available, the samples may be discarded in the laboratory toilet facilities. This, however, should be done cautiously due to potential blockages and contamination. The toilet facilities should be well washed and disinfected after the sample disposal.

It is important to document in SOPs which acids, bases, salts, and solutions can be poured down the drain with dilution, and which chemicals may not be disposed of in this fashion under absolutely any conditions (*e.g.* heavy metals, pesticides, oils, nitrite). A management plan needs to be in place for disposal of toxic compounds.

Containers need to be labelled for separate collection of waste that is safe to be managed with the local waste collection, *e.g.* biohazard and sharps, and waste chemicals. Material such as broken glassware, sharp objects and fine powders that could harm workers during collection also needs to be separately collected. For more information on developing waste handling procedures, refer to Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards (National Research Council, 2011).

8.5 SHIPPING AND RECEIVING OF FAECAL SLUDGE SAMPLES AND EQUIPMENT

In some cases, faecal sludge samples may be shipped locally or internationally; for example, when the required analysis is not locally available, when samples are collected from remote areas for comparison among multiple laboratories of the same samples, or for research studies. In this case, the sending and receiving parties need to fulfil specific regulatory requirements and documentation based on the local context and requirements of the shipping company. These may include import-export permits for biological samples issued by the relevant authorities (*e.g.* see the Department of Health example in Figure 8.2), and a material transfer agreement (MTA) signed by the sender and receiver organisations. Further information and details on specific local regulations can be obtained by local courier companies. Sufficient time between application for all the documents and the shipping date must be planned, including customs regulations. For example, in some countries this process may take up to 3 months.

The sample packaging method depends on the type of faecal sludge samples to be shipped. For example, oven- dried and/or autoclaved samples are relatively easy to ship due to their reduced risk of leakage and contamination whereas more liquid samples need special care. Liquid samples need to be contained in order to minimise the risk of leakage. Samples should be packed in sealed, airtight containers with headspace, wrapped in double containment (*e.g.* watertight plastic bags) along with absorbent material in case of leakage, and shipped with ice packs (for very short transport times) or dry ice. Below in Figure 8.3 are examples of packaging, but specific guidelines must be obtained from each courier company, and further information on packaging instructions can be found through the International Air Transport Association (IATA). Samples should be shipped as soon as possible after collection to ensure that they are well preserved. In addition, the shipping time should be as short as possible to avoid biodegradation processes in the samples and accumulation of biogas in the shipping containment.

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DEPARTMENT OF HEALTH
Private Bag X828 PRETORIA 0001
Inquiries: Mr JR Mokonoto Tel.: (012) 312-0395 Fax: (012) 312-0287

APPLICATION FOR AN IMPORT PERMIT FOR BIOLOGICAL SUBSTANCES

Person applying for an import permit:			
NAME			
RANK/POSITION			
Organisation:			
NAME			
ADDRESS			
TEL. NO.		FAX. NO.	
Specific substance(s) for which an import permit is required:			
SUBSTANCE		QUANTITY	
Period during which import will take place			
Contact person and organisation supplying the substance(s):			
NAME: PERSON			
NAME: ORGANISATION			
ADDRESS			
TEL. NO.		FAX. NO.	
Purpose(s) for which substance(s) is(are) to be used. Although detail is not required, the specific purpose(s) must be clearly stated:			

SIGNATURE OF APPLICANT:.....DATE:.....

Figure 8.2 Example of an export permit application in South Africa (source: Department of Health, Pretoria, South Africa).

For primary containment, samples should be shipped in rigid plastic bottles or containers sealed with a watertight, screw-on cap (see Figure 8.3 A). Samples should be packed directly from the refrigerator to maintain temperature. Absorbent material should be packed around the primary containers before sealing the plastic bags. For secondary containment, primary containment containers are packed into watertight, sealed, strong plastic bag/s (see Figure 8.3 B and C). Absorbent material must be packed on the outside of the secondary containment. There must be a sufficient amount of absorbent material inside of the package to ensure absorbance of the sample volume in the event of leakage. Examples of absorbent materials are sponges, vermiculite, paper towels, or wooden chips. Sealed plastic bags with the sample containers must be packed inside a rigid container to ensure that samples are fixed during shipping (see Figure 8.3 E). A cardboard or a rigid outer packing (*e.g.* a hard-plastic cooler box) is recommended (see Figure 8.3 F). It may be additionally packed into a larger box if required by shipping regulations. Include paperwork inside the shipping box explaining the contents of the shipment and the regulations it falls under.

Cooling packs, ice or dry ice may be used in the shipping container (see Figure 8.3 D). Samples should be kept at 4 °C for the entire duration of shipping. General cooling rules for samples are:

- The use of ice packs is recommended, *e.g.* gel packs or hard ice packs. Water produced from melting ice and condensation should be well contained within the packaging to prevent leakages.
- Dry ice can be used; however, ensure that there is a safe escape of the carbon dioxide gas from the container. There are specific limits for dry ice quantities and this needs to be confirmed with the shipping company.
- No use of liquid nitrogen.

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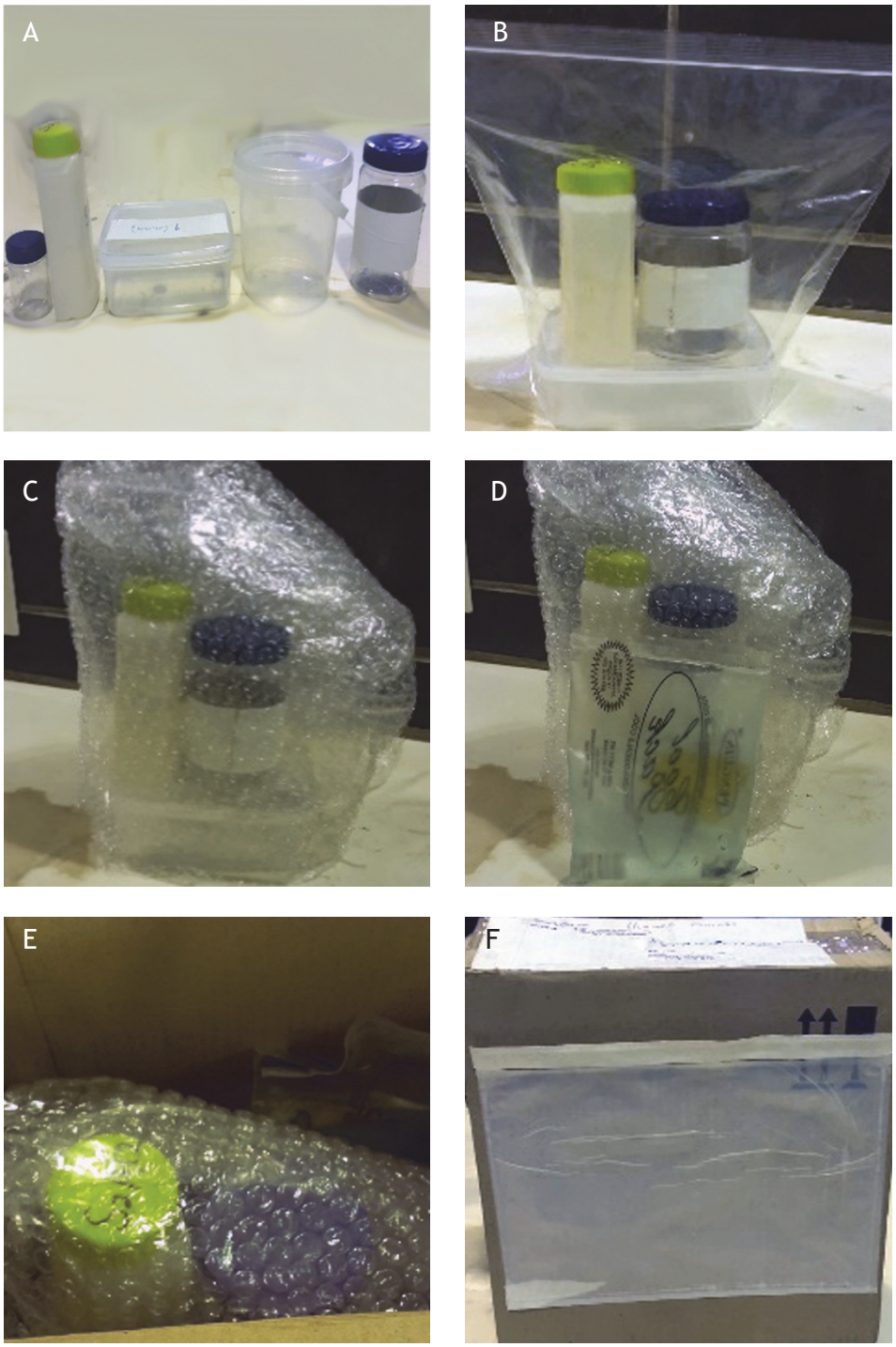

Figure 8.3 Examples of packaging in the laboratory (photos: UKZN PRG).

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8.6 CHEMICAL AND PHYSICO-CHEMICAL PROPERTIES

8.6.1 Solids and moisture content

Total solids is a term applied to the material left in a vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids are comprised of total suspended solids (TSS), total dissolved solids (TDS), fixed solids (FS) (ash) and volatile solids (VS). Total solids are determined for all types of faecal sludge – liquid, slurry, semi-solid and solid. The same methods are used to determine TS and moisture content; the total mass of a sample before the analysis is the sum of its TS and moisture content. Sand content (measured as silica as an indicator of soil content in faecal sludge) is the concentration of sand in the TS of an unfiltered faecal sludge sample. Sand can influence faecal sludge treatment processes (*e.g.* dewatering), increase abrasion of mechanical equipment, and affect the quality of faecal sludge treatment end products.

8.6.1.1 Total solids and moisture content – volumetric and gravimetric methods by oven drying[3]

8.6.1.1.1 Introduction

Total solids (and/or moisture content) is one of the most commonly used faecal sludge parameters, and is used for almost every design or management decision. For example, for making decisions on treatment design, settling, or emptying. A known volume (the volumetric method) and/or weight (the gravimetric method) of a thoroughly-mixed sample is evaporated to a constant weight in a crucible (porcelain or silica) or an aluminium weighing boat, in a drying oven at 103-105 °C; the remaining solids are cooled down to room temperature in a desiccator to avoid absorption of air moisture and then re-weighed. The residual material remaining in the crucible are TS, and can consist of organic and inorganic material, and dissolved, suspended or volatile matter.

The gravimetric method is recommended for semi-solid and solid types of sludge, as it is often difficult to measure volumes accurately for sludge with higher TS concentrations. For more liquid types of sludge, either the gravimetric or volumetric method can be used. However, these are general recommendations, and a final decision of which method to use needs to be assessed for each application individually. Conversion between volumetric and gravimetric measurements can be done if the density is known (Chapter 2). Density of faecal sludge can easily be measured by weighing a known volume of sludge (Method 8.7.1.1). When doing such conversions, it is always recommended to measure the actual density of the specific samples, and this becomes even more important with samples at the higher range of % TS.

Solid and semi-solid sludge types can form a water-trapping crust if the initial rate of drying is too high. This can be avoided by placing the samples in the drying oven at a lower temperature, and gradually increasing the temperature of the oven until the prescribed temperature of 103-105 °C is reached.

8.6.1.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Always conduct the TS analysis in a room with sufficient airflow and an exhaust system.
- Wear gloves suitable for withstanding high temperatures when placing and removing crucibles from the oven.
- Use appropriate mechanical tools, such as metal tongs, to remove crucibles and trays after drying in the oven to avoid direct contact with hot surfaces.

[3] The volumetric method is based on Method 2540B of the Standard Methods for the Examination of Water and Wastewater. The gravimetric method is based on ASTM E1756−08 Method A and on Method 2540G of the Standard Methods for the Examination of Water and Wastewater. Both methods should be cited as: Rice *et al.* (2017) as described in Velkushanova *et al*. (2021).

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8.6.1.1.3 Apparatus and instruments

- Porcelain crucibles or aluminium weighing boats
- Desiccator with dry desiccant
- Drying oven
- Analytical balance with four decimal places
- Spatula
- Stainless steel tray (optional, to move crucibles in and out of the oven)
- Heat-resistant gloves
- Pencil
- Thermometer (for quality control procedure)
- Set of standard calibration weights (for quality control procedure)

8.6.1.1.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- The analytical balance and oven must be checked and calibrated weekly.
- Check the temperature throughout the oven area by placing a calibrated thermometer on each shelf. After 30 min, check the temperature at each level against the oven setting. Using the same method, also check for temperature differences between the front and back of the oven. Adjust the oven setting if necessary. If temperatures are uneven on the shelves, check the insulation.
- To calibrate the analytical balance, place a standard calibration weight on the balance and weigh. Adjust the balance manually if necessary. Do this with the whole range of weights from the calibration set. Make sure to include a standard weight of a mass similar to the mass of the expected sample + crucible.
- Make sure the desiccant in the desiccator is not saturated, otherwise samples can absorb water while cooling down in the desiccator. Routinely dry the desiccant in the oven at 105 °C (or at a different temperature, depending on the manufacturer's instructions), prior to the colour indicating that the desiccant is nearly saturated.
- Always keep the lid of the desiccator on and use a lubricant on the rim to ensure airtight sealing. Do not overload the desiccator.
- The volume or mass of the wet sample used should be chosen so that the drying will yield a residue between 2.5 and 200 mg of the dried sample (in general approximately 30 mL for the volumetric method, or 10-20 g for the gravimetric method, but this will depend on the type of sludge).
- For solid, semi-solid and slurry samples: limit the sample to no more than 10-20 g faecal sludge, otherwise the sample will take too long to dry and can form a moisture-trapping crust on top. If crust formation is occurring, the samples should be placed in the oven at a lower temperature initially, gradually increasing the temperature until 103-105 °C is reached.
- For liquid samples, the volume of the sample can be higher because the TS content is much lower. The proportion of the weight of the sample to the weight of the porcelain or aluminium crucible should be also taken into account, so that weight differences in the sample can be measured accurately.
- Make sure that the samples are fully cooled in a desiccator to ambient temperature prior to weighing.
- Sludges that contain highly mineralised water with a significant concentration of calcium, magnesium, chloride and sulphate can be hygroscopic and require prolonged drying, complete desiccation and rapid re-weighing.
- Exclude larger, inconsistent or floating particles from the sample if it is determined that their inclusion can affect the final result (*e.g.* hair, stones, glass, and maggots).
- Disperse visible floating oil and grease with a blender or stainless steel mixing rod before withdrawing a sample portion for analysis.

8.6.1.1.5 Sample preservation

Samples should be analysed as soon as possible. If samples cannot be analysed immediately, they should be stored in a refrigerator at 4 °C for no longer than 7 days and, if TSS or VSS analysis is conducted, no longer than 48 hours. Before starting analysis, let the samples return to ambient temperature. Do not freeze the samples.

8.6.1.1.6 Sample preparation

- Uniformly mix all the samples using a stainless steel rod (or other appropriate tool) in order to have thoroughly mixed representative samples. If

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desired, samples can also be blended (see Section 8.4.2).

- Measure out an appropriate sample volume/mass (indicatively 30 mL for the volumetric method, or 10-20 g for the gravimetric method) which will yield a residue between 2.5 and 200 mg of dried sample, by using a volume measuring cylinder or analytical balance. With very dilute faecal sludge samples, a pipette can be used. For other sludge types, clogging of the pipette will occur, and therefore using a graduated cylinder to measure volume is recommended.

8.6.1.1.7 Analysis protocol

Preparation of equipment

- Pre-heat the oven to 103-105 °C.
- If analysing multiple samples or replicates at the same time, number the bottom of the crucible with a pencil and record in a laboratory notebook which sample and replicate is in which number crucible to distinguish between crucibles. If using aluminium weighing boats, the replicates can also be marked by scratching the number on the weighing boat with a sharp item.
- Place the clean crucible in the oven at a temperature of 103-105 °C for 1 hr prior to use (to remove any moisture). After drying, place the crucible in the desiccator and allow it to cool down to room temperature. Keep the crucible in the desiccator until the next step.
- Note: if measuring volatile solids after the TS, prepare the crucible in a furnace at 550 °C for 15 min prior to use to remove any potential residual organic material from previous measurements. Only porcelain crucibles should be used (see Method 8.6.1.2).

Procedure

- Remove the crucible from the desiccator and weigh it using the analytical balance. Record the weight of the dry, empty crucible (W_1).
  - For the gravimetric method (semi-solid to solid sludge):
    - Weigh out 10-20 g mass of the sample to the weighed crucible using a spatula.
    - Record the wet mass + mass of the crucible (W_2).
  - For the volumetric method (liquid to slurry sludge):
    - Measure 30 mL of the sample volume using a measuring cylinder and record the exact volume of the sample (V_{sample}).
    - Transfer to the weighed crucible. Rinse the cylinder with small volumes of distilled water to dislodge heavy particles. Make sure that all the particles are transferred to the crucible. Add the washings to the crucible but note, calculations must be based on the sample volume and exclude the volume of the washings.
- Oven-dry the sample at 103-105 °C for at least 24 hr or until a constant weight is achieved (which could take longer). To do this, cool and weigh the sample as described below, place the sample back in the drying oven for 1 hr and cool and weigh again. Repeat the steps of drying, cooling and weighing until a constant weight is obtained, or until the weight change is less than 0.5 mg, or 4% of the previous measurement. The length of drying time needs to be evaluated for each specific type of sample, and revisited periodically.
- Take the sample out of the oven and place it in the desiccator to reach room temperature.
- Weigh the dry mass of sample + crucible using an analytical balance and record the weight (W_3).

8.6.1.1.8 Calculation

Liquid and slurry samples (volumetric method):

Total Solids in wet sample (mg/L) =

$$\frac{(W_3\ (g)-W_1(g)) \times 1{,}000{,}000}{V_{sample}\ (mL)}$$

Total Solids in wet sample (g/L) =

$$\frac{(W_3(g)-W_1(g)) \times 1{,}000}{V_{sample}\ (mL)}$$

Semi-solid and solid samples (gravimetric method):

Total Solids in wet sample (g/g) =

$$\frac{(W_3(g)-W_1\ (g))}{(W_2(g)-W_1\ (g))}$$

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Moisture content in wet sample (g/g) =

$$\frac{(W_2\ (g)-W_3(g))}{(W_2\ (g)-W_1(g))}$$

Moisture content (%) =

$$\frac{(W_2\ (g)-W_3(g))}{(W_2\ (g)-W_1(g))} \times 100(\%)$$

Where:

W_1 = Crucible mass (g)

W_2 = Wet sample mass + crucible mass before drying (g)

W_3 = Dry sample mass + crucible mass after drying (g)

V_{sample} = Volume of sample used (mL)

For an explanation of the conversion of these units into %TS, refer to Chapter 2, Section 2.2.

8.6.1.1.9 Data set example

Described in Engund *et al*. (2019) and Strande *et al*. (2018) are the collection of 60 faecal sludge samples in Hanoi, Vietnam, and 180 samples in Kampala, Uganda. Solids analysis for TS, TSS, VS, VSS, and fixed solids were carried out and reported as concentrations. The complete raw data set is available using the link below[4].

A faecal sludge sample was collected from a ventilated improved pit latrine in Durban, South Africa. It was analysed gravimetrically in six replicates using Method 8.6.1.1. The average COD (g/g wet sample) was 0.23. The results for TS and moisture content are presented in Table 8.4 (source: unpublished data UKZN PRG).

Table 8.4 Mass of samples before and after the analysis and analysis results for the gravimetric method.

Sample no.	Crucible mass (g) (W_1)	Sample mass (g)	Sample + crucible (g) (W_2)	Residue + crucible mass after drying (g) (W_3)	Moisture (g/g wet sample)	Total solids (g/g wet sample)
1-a	64.7232	19.9688	84.6920	69.4310	0.7642	0.2358
1-b	48.0356	20.0035	68.0391	52.7174	0.7660	0.2340
1-c	38.6685	20.0007	58.6692	43.2768	0.7696	0.2304
1-d	36.5180	20.0119	56.5299	41.2682	0.7626	0.2374
1-e	41.1442	20.0934	61.2376	45.8654	0.7650	0.2350
1-f	34.8260	20.0226	54.8486	39.5203	0.7655	0.2345
Average					0.7655	0.2345
SD					0.0023	0.0023

8.6.1.2 Volatile and fixed solids – ignition method[5]

8.6.1.2.1 Introduction

The dry sample residue from Method 8.6.1.1 is ignited at 550 °C for 30 min or until constant weight. The remaining ash represents the fixed (inorganic) solids, while the weight lost on ignition represents the volatile solids (organic matter) in faecal sludge. For more details, see Chapter 2.

8.6.1.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.

[4] https://doi.org/10.25678/0000tt.

[5] This method follows Method 2540E of the Standard Methods for the Examination of Water and Wastewater, and should be cited as: Rice *et al.*, (2017)

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- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Always conduct the volatile solids analysis in a room with sufficient airflow and preferably with an exhaust system.
- Never remove crucibles or trays by directly touching objects in the furnace, even if heat resistant gloves are worn. Use appropriate metal tools (such as stainless steel tongs) to place and remove crucibles and trays from the furnace to avoid direct contact with hot surfaces. Always wear heat-resistant gloves suitable for withstanding high temperatures.

8.6.1.2.3 Apparatus and instruments

- Porcelain crucibles
- Desiccator with dry desiccant
- Muffle furnace that can reach temperatures of 550 °C
- Analytical balance with four decimal places
- Stainless steel tray (optional, to move crucibles in and out of the furnace)
- Stainless steel tongs (to move crucibles in and out of the furnace)
- Heat-resistant gloves
- Pencil

8.6.1.2.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- The analytical balance and furnace must be checked and calibrated weekly.
- Check the temperature throughout the furnace area by placing a calibrated thermocouple on each shelf or reading the temperature with a laser thermometer.
- After 30 min, check the temperature at each level against the furnace setting. Using the same method, also check for temperature differences between the front and back of the furnace. Adjust the furnace setting if necessary. If temperatures are uneven on the shelves, check the insulation.
- To calibrate the analytical balance, place a standard calibration weight on the balance and weigh. Adjust the balance manually if necessary. Make sure to use a standard weight of a mass similar to the mass of the expected sample + crucible.
- Limit the sample to no more than 200 mg of residue after ignition at 550 °C (initial faecal sludge mass before TS analysis 10-20 g).
- Sludges that contain highly mineralised water with a significant concentration of calcium, magnesium, chloride and sulphate can be hygroscopic and require prolonged drying, proper desiccation and rapid re-weighing.

8.6.1.2.5 Sample preservation

Samples should be analysed as soon as possible. If samples cannot be analysed immediately, they should be stored in a refrigerator at 4 °C for no longer than 7 days and, if TSS or VSS analysis is conducted, no longer than 48 hours. Before starting the analysis, let the samples return to room temperature (20 °C). Do not freeze the samples.

8.6.1.2.6 Sample preparation

Dry the samples to constant weight to remove moisture content, following Method 8.6.1.1.

8.6.1.2.7 Analysis protocol

Preparation of equipment

- Pre-heat the furnace to 550 °C temperature before inserting the sample.
- Before conducting TS analysis (Method 8.6.1.1), position clean, dry crucibles in the furnace at 550 °C for 1 hr to remove any potential organic matter.

Procedure

- Ignite the residue from the TS in a muffle furnace at a temperature of 550 °C for 20 min. Note: for some solid and semi-solid faecal sludge samples, 20 min might not be enough, as they might need more time to combust all the volatile matter. For each type of sludge, check that constant weight is achieved after 20 min. To do this, cool and weigh the sample as described below, place the sample back in the furnace for 10 min and cool and weigh again. Repeat the steps of drying, cooling and weighing until a constant weight is obtained, or until weight change is less than 4% of the previous

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measurement. The length of combustion time needs to be evaluated for each specific type of sample, and revisited periodically.

- Transfer the crucibles to the stainless tray and let them cool partially until cool enough to transfer to a desiccator.
- Transfer to the desiccator for final cooling. Do not overload the desiccator.
- Weigh the crucible on the analytical balance as soon as it has cooled to ambient temperature and record the weight (W_4).

8.6.1.2.8 Calculation

Liquid and slurry samples (volumetric method):

Volatile solids in wet sample (g/L) =

$$\frac{(W_3\ (g)-W_4\ (g)) \times 1{,}000}{V_{sample}\ (mL)}$$

Fixed solids in wet sample (g/L) =

$$\frac{(W_4(g)-W_1(g)) \times 1{,}000}{V_{sample}\ (mL)}$$

Where:

W_1 = Crucible mass (g)
W_2 = Crucible mass + wet sample mass (g)
W_3 = Crucible mass + sample after drying (g)
W_4 = Crucible mass + sample after incinerating (g)
(W_3-W_1) = Sample mass after drying (g)
(W_4-W_1) = Sample mass after incinerating (g)
V_{sample} = Sample volume used (mL)

Slurry, semi-solid and solid samples (gravimetric method):

Volatile solids in wet sample (g/g) =

$$\frac{(W_3\ (g)-W_4(g))}{(W_2(g)-\ W_1\ (g))}$$

Volatile solids in dry sample (g/g) =

$$\frac{(W_3\ (g)-W_4\ (g))}{(W_3(g)-W_1(g))}$$

Volatile solids (%TS) =

$$\frac{(W_3(g)-W_4(g))}{(W_3(g)-W_1(g))} = \frac{VS\ (\frac{g}{g})}{TS\ (\frac{g}{g})} \times 100\ (\%)$$

Fixed solids in wet sample (g/g) =

$$\frac{(W_4\ (g)-W_1\ (g))}{(W_2\ (g)-W_1\ (g))}$$

Fixed solids in dry sample (g/g) =

$$\frac{(W_4(g)-W_1(g))}{(W_3(g)-W_1(g))}$$

Fixed solids (%TS) =

$$\frac{(W_4(g)-W_1(g))}{(W_3(g)-W_1(g))} = \frac{\text{Fixed solids}\ (\frac{g}{g})}{TS\ (\frac{g}{g})} \times 100\ (\%)$$

Note: for values of W_1 to W_3 and how to calculate them, see Method 8.6.1.1.

8.6.1.2.9 Data set example

A faecal sludge sample was collected from a ventilated improved pit latrine in Durban, South Africa. It was analysed in six replicates using Method 8.6.1.2. The average initial samples mass was 5 g dry weight - the same dry samples from Section 8.6.1.1.9 were used for ignition. The average VS (g/g dry sample) was 0.56. The results for VS and fixed solids are presented in Table 8.5 (source: UKZN PRG).

Table 8.5 Mass of samples before and after the analysis for the ignition method.

Sample no.	Volatile solids (g/g dry sample)	Fixed solids (g/g wet sample)	Fixed solids (g/g dry sample)
1-a	0.5574	0.1044	0.4426
1-b	0.5673	0.1013	0.4327
1-c	0.5896	0.0946	0.4104
1-d	0.5499	0.1069	0.4501
1-e	0.5571	0.1041	0.4429
1-f	0.5599	0.1032	0.4401
Average	0.5635	0.1024	0.4365
SD	0.0140	0.0042	0.0140

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8.6.1.3 Total suspended solids and total dissolved solids – oven drying method[6]

8.6.1.3.1 Introduction

The TSS method is used to determine the efficiency of treatment technologies, such as settling tanks and biological filters. The measured volume of a thoroughly-mixed sample is vacuum-filtered through a dried, pre-weighed glass fibre filter. The filters and residue are then dried to a constant weight at 103-105 °C. The increase in weight of the filter represents the total suspended solids. Total dissolved solids are the TS minus the TSS.

For faecal sludge, clogging of the filters is a common problem. For this reason, this method is only suitable for liquid and slurry samples. If clogging occurs, the method can be adapted by dilution of the sample and/or choosing a larger pore size (maximum up to 2.0 µm), but needs to be carefully documented.

8.6.1.3.2 Safety precaution

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Always conduct the total suspended solids analysis in a room with sufficient airflow and an exhaust system.
- Wear gloves suitable for withstanding high temperatures when removing crucibles from the oven.
- Use appropriate mechanical tools, such as metal tongs, to remove crucibles and trays after drying in the oven to avoid direct contact with hot surfaces.

8.6.1.3.3 Apparatus and instruments

- Analytical balance with four decimal places
- Büchner funnel with a rubber bung and fitting conical filtration flask
- Vacuum pump with a rubber tubing
- Glass fiber filters (GF/C grade) ranging in size from 0.45 µm to 2.0 µm depending on the thickness of the sludge and clogging of the filters. It is important to use GF/C grade to withstand 550 °C and that the filter diameter matches the Büchner funnel diameter.
- Desiccator with dry desiccant
- Aluminium weighing boats or porcelain crucible
- Drying oven
- Graduated cylinder
- Forceps
- Pencil
- Stainless steel tray (optional, to move the crucibles in and out of the oven)
- Heat-resistant gloves
- Thermometer (for the quality control procedure)
- Set of standard calibration weights (for the quality control procedure)

8.6.1.3.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- The analytical balance and oven must be checked and calibrated weekly.
- Check the temperature throughout the oven area by placing a calibrated thermometer on each shelf. After 30 min, check the temperature at each level against the oven setting. Using the same method, also check for temperature differences between the front and back of the oven. Adjust the oven setting if necessary. If temperatures are uneven on the shelves, check the insulation.
- To calibrate the analytical balance, place a standard calibration weight on the balance and weigh. Adjust the balance manually if necessary. Do this with the whole range of weights from the calibration set. Make sure to at least use a standard weight of a mass similar to the mass of the expected sample + crucible.
- Make sure the desiccant in the desiccator is not saturated, otherwise samples can absorb water

[6] This method is adapted from Method 2540D of the Standard Methods for the Examination of Water and Wastewater, and should be cited as: Rice *et al.* (2017) as adapted in Velkushanova *et al*. (2021).

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while cooling down in the desiccator. Routinely dry the desiccant in the oven at 105 °C (or at a different temperature, depending on the manufacturer's instructions), prior to the colour indicating that the desiccant is nearly saturated.

- Always keep the lid of the desiccator on and use a lubricant on the rim to ensure airtight sealing. Do not overload the desiccator.
- The volume or mass of the wet sample used should be chosen so that the drying will yield a residue between 2.5 and 200 mg of the dried sample (in general around 30 mL for the volumetric method, or 10-20 g for the gravimetric method, but this will depend on the type of sludge).
- For solid, semi-solid and slurry samples: limit the sample to no more than 10-20 g faecal sludge, otherwise the sample will take too long to dry and can form a moisture-trapping crust on top. If crust formation is occurring, samples should be placed in the oven at a lower temperature initially and the temperature gradually increased until 103-105 °C is reached.
- For liquid samples, the volume of the sample can be higher as the TS content is much lower. The proportion of the weight of the sample to the weight of the porcelain or aluminium crucible should also be taken into account, so that weight differences in the sample can be measured accurately.
- Make sure samples are fully cooled in a desiccator to ambient temperature prior to weighing.
- Sludges that contain highly mineralised water with a significant concentration of calcium, magnesium, chloride and/or sulphate can be hygroscopic and require prolonged drying, proper desiccation, and rapid re-weighing.
- Exclude larger, inconsistent or floating particles from the sample if it is determined that their inclusion can affect the final result (*e.g.* hair, stones, glass and maggots).
- Glass fibre filters are delicate, especially when wet, and care should be taken not to rip or damage them during filtration and handling. If a filter is damaged during filtration, particles might not be captured or pieces of the filter could be washed away, which will lead to measurement errors. Filters need to be prepared as described in Section 8.6.1.3.7.

8.6.1.3.5 Sample preservation

Samples should be analysed as soon as possible. If samples cannot be analysed immediately, they should be stored in a refrigerator at 4 °C for no longer than 48 hours. Before starting the analysis, let the samples return to ambient temperature. Do not freeze the samples.

8.6.1.3.6 Sample preparation

- Thoroughly mix all the samples using a stainless steel rod (or other appropriate tool) in order to have representative samples. For liquid samples, invert the closed sample bottle with the sample about 3 times.
- When measuring total dissolved solids, in addition to following this method, TS should be measured (following Method 8.6.1.1 or Method 8.6.1.5).

8.6.1.3.7 Analysis protocol

Equipment preparation

- Pre-heat the oven to 103-105 °C.
- Rinse the Büchner funnel with distilled water.
- Place the Büchner funnel with the rubber bung (stopper) on top of the filtration flask to seal the apparatus.
- Attach the filtration flask to a vacuum pump.
- If analysing multiple samples or replicates at the same time, mark each crucible/aluminium weighing boat with a unique identification number/letter. Number the crucible with a pencil or scratch the number into the aluminium weighing boat and note down which sample and replicate is in which number crucible to be able to distinguish between samples later.
- Pre-wash the glass fibre filter: place a filter onto the funnel (rough side up), apply the vacuum, and rinse three times with an aliquot of distilled water.
- Place the washed filter in a crucible or aluminium weighing boat and place in the oven at a temperature of 103-105 °C for 1 hr, prior to use (to remove any moisture). Afterwards, place the crucible with the filter in the desiccator and allow it to cool to room temperature. Always keep the rough side of the filter up.
- Note: if measuring volatile suspended solids after the total suspended solids, prepare the filter + crucible at 550 °C for ≥15 min in a muffle furnace instead of in the oven prior to use to remove any

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potential residual organic material from previous measurements. Only porcelain crucibles should be used (see Method 8.6.1.2).

Procedure

- Weigh the filter + crucible or aluminium weighing boat on a balance and record its mass (W_1).
- Place the filter into a Büchner funnel, with the rough side up.
- Measure out a 30 mL sample volume using a graduated cylinder. Note: choose the sample volume to yield between 2.5 and 200 mg residue. For slurry sludge, measure 20 mL sample using a graduated cylinder. (Use less sample volume if the dried residue is more than 200 mg or use a smaller pore size if the dried residue is lower than 2.5 mg).
- Wet the filter with distilled water to seal the edges of the filter to the surface of the funnel.
- Turn on the vacuum pump.
- Pour the sample onto the filter, keeping the sample in the middle of the paper.
- Wash the graduated cylinder with distilled water until thoroughly rinsed (at least 2-cylinder volumes). Ensure all the particles are washed onto the filter.
- Pour rinse water onto the filter. For liquid and slurry samples >5 % TS, wash with at least two successive volumes of 10 mL distilled water and pour the rinse into the filter. Allow complete drainage between washings, and continue suction until all the traces of water are removed.
- If the sample is clogging the filter during filtration, dilute the sample using an appropriate dilution factor (*e.g.* 1:5 or 1:10) and filter the diluted sample. Note: the dilution factor needs to be reported and accounted for when calculating the total suspended solids concentration.
- If clogging still occurs even with the dilutions (*i.e.* if filtration takes >10 min to complete), then the next size larger pore size filter should be used. It is very important to document this and report it in the methods. In general, the smallest pore size possible in the range 0.45 µm to 2.0 µm should be used.
- When filtration is complete, remove the filter with forceps gently along the edge of the filter paper and then lift slowly (or first with a spatula and then forceps).
- Remove the paper with a pair of forceps, taking care not to tear the paper.
- Carefully place the filter in its marked crucible or aluminium weighing boat, rough side (containing the sample) facing up.
- Place in the oven at 103-105 °C for at least 2 hr, until constant weight is achieved. To do this, cool and weigh the sample as described below, place the sample back in the drying oven for 1 hr and then cool and weigh again. Repeat the steps of drying, cooling and weighing until a constant weight is obtained, or until weight change is less than 0.5 mg. The length of drying time needs to be evaluated for each specific type of sample, and revisited periodically.
- Remove from the oven, place in the desiccator and cool to room temperature.
- Weigh the crucible or weighing boat with the filter on the analytical balance and record the mass (W_2).

8.6.1.3.8 Calculation

Total suspended solids (g/L) =

$$\frac{(W_2(g)-W_1(g))}{V_{sample}(L)} \text{ (× DF if using dilution factor)}$$

W_1 = Weight of filter + crucible/aluminium weighing boat before drying (103-105 °C) (g)
W_2 = Weight of residue + filter + crucible/aluminium weighing boat after drying (103-105 °C) (g)
V_{sample} = Volume of sample used (L)
DF = Dilution factor

Total dissolved solids (g/L) =

Total solids (g/L) − Total suspended solids (g/L)

8.6.1.3.9 Data set example

A faecal sludge sample was collected from a ventilated improved pit latrine in Durban, South Africa. It was analysed in six replicates using Method 8.6.1.3. The average TSS (g/L) was 0.37. The results for TSS are presented in Table 8.6 (source: unpublished data UKZN PRG).

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Table 8.6 Total suspended solids obtained by oven drying methods.

Sample no.	Filter paper mass (g) (W_1)	Residue + filter mass after drying (g) (W_2)	Sample volume (L)	Total suspended solids (g/L)
1-a	0.4146	0.4289	0.0300	0.4767
1-b	0.4186	0.4313	0.0300	0.4233
1-c	0.4289	0.4446	0.0300	0.4298
1-d	0.4287	0.4427	0.0300	0.4292
1-e	0.4137	0.4264	0.0300	0.4233
1-f	0.4268	0.4276	0.0300	0.0267
Average				0.3682
SD				0.1685

8.6.1.4 Volatile suspended solids – ignition method[7]

8.6.1.4.1 Introduction

The dry sample residue from Method 8.6.1.3 is ignited at 550 °C to constant weight. The remaining ash represents the fixed (inorganic) suspended solids, while the weight lost on ignition represents the volatile suspended solids (organic matter).

8.6.1.4.2 Safety precaution

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Always conduct the VS analysis in a room with sufficient airflow and preferably with an exhaust system.
- Never remove crucibles or trays by directly touching objects in the furnace, even if heat resistant gloves are worn. Use appropriate metal tools (such as stainless steel tongs) to place and remove crucibles and trays from the oven to avoid direct contact with hot surfaces. Always wear heat resistant gloves suitable for withstanding high temperatures.

8.6.1.4.3 Apparatus and instruments

- Muffle furnace that can reach temperatures of 550 °C
- Desiccator with dry desiccant
- Analytical balance with four decimal places
- Porcelain crucibles
- Heat resistant gloves
- Stainless steel trays (optional, to move crucibles in and out of the furnace)
- Tongs (to move crucibles in and out of the furnace)
- Pencil

8.6.1.4.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- The analytical balance and furnace must be checked and calibrated weekly.
- Check the temperature throughout the furnace area by placing a calibrated thermocouple on each shelf or read the temperature with a laser thermometer.
- After 30 min, check the temperature at each level against the furnace setting. Using the same method, also check for temperature differences between the front and back of the furnace. Adjust the furnace setting if necessary. If temperatures are uneven on the shelves, check the insulation.
- To calibrate the analytical balance, place a standard calibration weight on the balance and weigh. Adjust the balance manually if necessary. Make sure to use a standard weight of a mass similar to the mass of the expected sample + crucible.
- Limit the sample to no more than 200 mg of residue after ignition at 550 °C (the initial faecal sludge mass before TS analysis 10-20 g).
- Sludges that contain highly mineralised water with a significant concentration of calcium,

[7] This method follows methods 2540D and E of the Standard Methods for the Examination of Water and Wastewater, and should be cited as: Rice *et al.*, (2017).

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magnesium, chloride and sulphate can be hygroscopic and require prolonged drying, proper desiccation, and rapid re-weighing.

8.6.1.4.5 Sample preservation

Samples should be analysed as soon as possible. If samples cannot be analysed immediately, they should be stored in a refrigerator at 4 °C for no longer than 48 hr. Before starting analysis, let the samples return to ambient temperature. Do not freeze the samples.

8.6.1.4.6 Sample preparation

Filter and dry the samples to constant weight, following Method 8.6.1.3.

8.6.1.4.7 Analysis protocol

Preparation of equipment

- Pre-heat the furnace to 550 °C temperature before inserting the samples.
- Before conducting analysis, position clean, dry crucibles in the furnace at 550 °C for 1 hr to remove any potential organic matter (see Section 8.6.1.3.7).

Procedure

- Ignite the crucibles containing glass fibre filters + residue from the total suspended solids measurement in a muffle furnace at a temperature of 550 °C for 20 min. Note: for some solid and semi-solid faecal sludge samples, 20 min might not be enough, as they might need more time to combust all the volatile matter. For each type of sludge, check that a constant weight is achieved after 20 min. To do this, cool and weigh the sample as described below, place the sample back in the furnace for 10 min, and cool and weigh again. Repeat the steps of drying, cooling and weighing until a constant weight is obtained, or until the weight change is less than 4 % of the previous measurement. The length of combustion time needs to be evaluated for each specific type of sample and revisited periodically.
- Transfer the crucibles to the stainless tray and let them cool partially until cool enough to transfer to the desiccator.
- Transfer to a desiccator for final cooling. Do not overload the desiccator.
- Weigh the crucible on the analytical balance as soon as it has cooled to ambient temperature and record the weight (W_3).

8.6.1.4.8 Calculation

Volatile suspended solids in wet sample (g/L) =

$$\frac{(W_2\ (g)-W_3\ (g))}{V_{sample}\ (L)}\ (\times \text{DF, if used})$$

W_2 = Weight of residue + filter + crucible after drying (103-105 °C) (g)

W_3 = Weight of residue + filter + crucible after ignition in furnace at 550 °C (g).

V_{sample} = Volume of sample used (L)

DF = Dilution factor. Note: if a dilution was used during the determination of total suspended solids (Method 8.6.1.3), this should be taken into account in the calculation.

8.6.1.4.9 Data set example

A faecal sludge sample was collected from a ventilated improved pit latrine in Durban, South Africa. It was analysed in six replicates using Method 8.6.1.4. The average VSS (g/L) was 0.03. The results for VSS are presented in Table 8.7 (source: unpublished data UKZN PRG).

Table 8.7 Volatile suspended solids obtained by oven drying and ignition methods.

Sample no.	Residue + filter mass after drying (g) (W_2)	Sample volume (L)	Residue + filter mass after incineration (g) (W_3)	Volatile suspended solids (g/L)
1-a	0.4289	0.0300	0.4150	0.0313
1-b	0.4313	0.0300	0.4190	0.0274
1-c	0.4446	0.0300	0.4300	0.0318
1-d	0.4427	0.0300	0.4264	0.0355
1-e	0.4264	0.0300	0.4140	0.0279
1-f	0.4276	0.0300	0.4268	0.0018
Average				0.0260
SD				0.0122

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8.6.1.5 Total solids and moisture content – thermal balance (moisture analyser) method[8]

8.6.1.5.1 Introduction

A moisture analyser is designed to determine relative moisture content in small samples of various substances, by measuring the change of weight due to water evaporation during convective drying. This method is applicable for all types of sludge – liquid, slurry, semi-solid and solid; however, samples with a higher moisture content will have a longer drying and measurement time. Method 8.6.1.1 and Method 8.6.1.5 are equally suitable to determine TS and moisture content, and should be selected depending on the availability of equipment.

8.6.1.5.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Always conduct the TS analysis in a room with sufficient airflow and an exhaust system.
- Do not place any flammable substances on or near the moisture analyser.

8.6.1.5.3 Apparatus and instruments

- Aluminium weighing boats
- Thermal balance (moisture analyser)

8.6.1.5.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Before using a moisture analyser, make sure the instrument was left on for a sufficient period of time (see Section 8.6.1.5.7).
- Minimise external environmental influences such as air draught, vibrations or direct sunlight.
- Ensure the analyser is levelled. This is essential for testing liquid samples, which must be at uniform level in the sample container.
- Exclude larger, inconsistent or floating particles from the sample if it is determined that their inclusion could affect the final result (*e.g.* hair, stones, glass and maggots).
- Disperse visible floating oil and grease with a blender or stainless steel mixing rod before withdrawing a sample portion for analysis.

8.6.1.5.5 Sample preservation

Samples should be analysed as soon as possible. If samples cannot be analysed immediately, they should be stored in a refrigerator at 4 °C for no longer than 7 days. Before analysis, let the samples return to ambient temperature. Do not freeze the samples.

8.6.1.5.6 Sample preparation

Thoroughly mix all the samples using a stainless steel rod (or other appropriate tool) in order to have representative samples. If desired, samples can also be blended (see Section 8.4.2).

8.6.1.5.7 Analysis protocol

Equipment preparation

- Switch the instrument on. Wait until the analyser completes its self-examination and finishes heating up. To deliver accurate results and enable the moisture analyser to reach the required operating temperature, it must be switched on for at least 20-30 minutes every time before use. The program must be set to end when the sample mass changes less than 0.05% of mass per minute.
- Check that the temperature is 105 °C for moisture analysis.

Procedure

- Press 'Start Program' and follow prompts on the display screen; this can vary per model and brand.
- Open the lid of the moisture analyser, place the clean and empty weighing boat on the weighing cradle.
- Close the cover gently and tare the boat weight; the LCD screen should now show weight as '0'

[8] This method follows Method B of the ASTM Standard Test Method for Determination of Total Solids in Biomass (E1756-08) and should be cited as: ASTM (2015).

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and a flashing icon to indicate that the machine is ready for loading the sample.
- Lift the lid of the moisture analyser and then evenly spread 1-3 g of the wet sample on the weighing boat.
- Close the cover gently.
- The halogen light will start to heat the sample until it reaches a steady reading. Note: this process usually takes between 2-15 min, depending on the sample weight and its moisture content.
- Record the moisture reading (before lifting the lid); this is the end of the drying procedure.
- Press 'Stop' and lift the lid to end the current testing.

8.6.1.6 Sand content[9]

8.6.1.6.1 Introduction

The residual ash from Method 8.6.1.2 is washed with 0.1 M HCl and combusted at 550 ºC. The leftover residual is silica, and is typically reported as the 'sand' content as an indicator of soil content in faecal sludge.

8.6.1.6.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.3.
- Always conduct the volatile solids analysis in a room with sufficient airflow and preferably with an exhaust system.
- Never remove crucibles or trays by directly touching objects in the furnace, even if heat-resistant gloves are worn. Use appropriate metal tools (such as stainless steel tongs) to place and remove crucibles and trays from the oven to avoid direct contact with hot surfaces. Always wear heat-resistant gloves suitable for withstanding high temperatures.

8.6.1.6.3 Required chemicals

- Distilled water
- 0.1 M HCl solution

8.6.1.6.4 Required apparatus and instruments

- Analytical balance
- Porcelain crucibles
- Ashless filter paper (*e.g.* Whatman grade 44)
- Heat-resistant gloves
- Stainless steel tray (optional, to move the crucibles in and out of the oven)
- Stainless steel tongs (to move the crucibles in and out of the furnace)
- Forceps
- Desiccator with dry desiccant
- Furnace (operating at 550 °C)
- Vacuum filtration setup
- Pencil
- Thermometer (for quality control procedure)
- Set of standard calibration weights (for the quality control procedure)

8.6.1.6.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- The analytical balance and oven must be checked and calibrated weekly.
- Check the temperature throughout the furnace area by placing a calibrated thermocouple on each shelf or reading the temperature with a laser thermometer.
- After 30 min, check the temperature at each level against the oven setting. Using the same method, also check for temperature differences between the front and back of the oven. Adjust the oven setting if necessary. If the temperatures are uneven on the shelves, check the insulation.
- To calibrate the analytical balance, place a standard calibration weight on the balance and weigh. Adjust the balance manually if necessary. Make sure to use a standard weight of a mass similar to the mass of the expected sample + crucible.

[9] This method is from the German Association for Water Wastewater and Waste guidelines, and should be cited as: Method DWA-M 383 for the determination of sand content (DWA, 2008).

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- The filters used for sand content analysis must be ashless.
- Make sure that the desiccant in the desiccator is not saturated, otherwise the samples may absorb water while cooling down in the desiccator. Routinely dry the desiccant in the oven at 105 °C (or at a different temperature, depending on the manufacturer's instructions), prior to the colour indicating that the desiccant is nearly saturated.
- Always keep the lid of the desiccator on and use a lubricant on the rim to ensure airtight sealing. Do not overload the desiccator.
- Make sure the samples are fully cooled in a desiccator to ambient temperature prior to weighing.

8.6.1.6.6 Sample preservation

Samples have to be 100 % dry and should be stored in a desiccator after volatile solids analysis (Method 8.6.1.2) until the start of the sand content analysis.

8.6.1.6.7 Sample preparation

Sand content analysis is performed on the 100% dry ash remaining from the volatile solids analysis (Method 8.6.1.2).

8.6.1.6.8 Analysis protocol

- Preheat the furnace to 550 °C.
- Place the filter on the filter apparatus and rinse it with 3 portions of approximately 20 mL of demineralised water.
- Place one crucible with one filter paper in the furnace at 550 °C for 15 min.
- Take the porcelain crucible with the filter paper out of the oven (using appropriate heat-resistant tools) and place them in the desiccator until they reach ambient temperature.
- Take the porcelain crucible and filter paper out of the desiccator and weigh them (W_1).
- Place the weighed filter paper in the (vacuum) filtration apparatus.
- Transfer all of the residue from the crucible left after the volatile solids analysis (*i.e.* fixed solids, Method 8.6.1.2) onto the paper filter inside the filtering apparatus. Ensure that all the residue is transferred onto the filter paper. If needed, rinse the crucible with the 0.1 M HCl solution to remove the last bits of the ash and pour onto the filter paper. Wash the residue on the paper filter with the 0.1 M HCl solution. Keep rinsing until the mass of residue stays constant and the filtrate is clear. If the filtrate is clear from the beginning, rinse at least three times with an aliquot of HCl solution.
- Place the paper filter inside the weighed porcelain crucible using tweezers.
- Place the porcelain crucible into the furnace operating at 550 °C for two hours.
- Remove the porcelain crucible from the furnace. Wait for it to partially cool and place it in the desiccator. Wait until the porcelain crucible has reached ambient temperature.
- Remove the porcelain crucible from the desiccator. Place it on the analytical balance and note the weight (W_2).

8.6.1.6.9 Calculations

Liquid and slurry samples (volumetric method):

Sand content $\left(\frac{g}{L}\right) =$

$$\frac{(W2\ (g) - W1\ (g))}{\text{Sample volume from TS analysis (mL)}} \times 1{,}000\ \left(\frac{mL}{L}\right)$$

Sand content (%TS) =

$$\frac{\text{Sand content}\ \left(\frac{g}{L}\right)}{\text{TS}\ \left(\frac{g}{L}\right)} \times 100\ (\%)$$

Sand content (% ash) =

$$\frac{\text{Sand content}\ \left(\frac{g}{L}\right)}{\text{Ash}\ \left(\frac{g}{L}\right)} \times 100\ (\%)$$

Slurry, semi-solid, and solid samples (gravimetric method):

Sand content of wet weight $\left(\frac{g}{g}\right) =$

$$\frac{(W2\ (g) - W1\ (g))}{\text{Wet sample weight from TS analysis (g)}}$$

Sand content of dry weight $\left(\frac{g}{g}\right) =$

$$\frac{(W2\ (g) - W1\ (g))}{\text{Weight of dry solids from TS analysis (g)}}$$

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Sand content (%TS) =

$$\frac{\text{Sand content } (\frac{g}{g})}{\text{TS } (\frac{g}{g})} \times 100\ (\%)$$

W1 = Weight of dried filter + crucible
W2 = Weight of filter + crucible + residue

8.6.1.6.10 Data set example

In Gold *et al.* (2017), 73 faecal sludge samples from septic tanks, lined pit latrines, unlined pit latrines and johkasou[10] tanks in Uganda, Vietnam and Japan, and 18 samples from wastewater treatment plants in Switzerland, were analysed for sand content following Method 8.6.1.6. The results were used to determine influence on dewaterability. They report a 75% confidence interval (where 75% of the data is expected to lay) ranging from 7-9% sand of TS for lined johkasou septic tanks from Japan, 9-33% sand of TS for wastewater sludges from Switzerland, to a maximum of 45-69% sand of TS from unlined pit latrines in Uganda. When higher %TS of dewatered sludge are due to high sand content, it can have a negative impact on resource recovery.

8.6.2 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is defined as the amount of oxygen that is required for the chemical oxidation of organic matter using a strong chemical oxidant. For faecal sludge, it is used as a proxy for the organic matter content of a sample. For example, it is measured to characterise sludge prior to treatment, or during monitoring of treatment processes. COD is one of multiple parameters to measure organic content of a sample. Other parameters include biochemical oxygen demand (BOD), volatile fatty acids (VFA), total organic carbon (TOC), and biomethane potential (BMP), which are not yet included in this book. Common perception in the sector, is that COD is more accurate than BOD for faecal sludge analysis, due to the high variability and concentrations of organic matter. More information on these methods can be found in Rice *et al.* (2017).

8.6.2.1 Chemical oxygen demand – closed reflux spectrophotometric method[11]

8.6.2.1.1 Introduction

Chemical oxygen demand (COD) is a proxy measurement of the amount of organic matter in water or wastewater. The principle of the COD test is that organic compounds are oxidised to carbon dioxide with a strong oxidising agent such as dichromate under acidic conditions. The amount of oxidising agent required to completely oxidise the organic matter in the sample is compared to the equivalence of oxygen. COD can be determined by an open reflux method, a closed reflux titrimetric method (Method 8.6.2.2) or a closed reflux colorimetric method. The method described here is the closed reflux colorimetric method which uses a spectrophotometer to measure absorbance. The principle is that when a sample is digested, the dichromate ion oxidises COD in the sample and the chromium is reduced from Cr^{6+} to Cr^{3+}. These chromium species are coloured and absorb in the visible region of the spectrum. Dichromate ion ($Cr_2O_7^{2-}$) is extremely absorbent in the 400-nm region, whereas the chromic ion (Cr^{3+}) is extremely absorbent in the 600-nm region.

Commercial test kits based on standard methods for measuring COD are available, with pre-packaged individual aliquots of the necessary chemical in pillows (dry chemicals) and vials (liquid chemicals). Commonly used COD test kits from manufacturers such as Hach and Merck employ the closed reflux colorimetric method.

The example provided here is the Merck COD spectrophotometric test[D] (Merck, 2020d) for samples with concentrations of 15-300 mg COD/L, and it is based on the manufacturer's protocol for water and wastewater using the standard method 5220 (Rice *et al.,* 2017). For faecal sludge, samples must be diluted

[10] Johkasou is the Japanese word for on-site wastewater treatment.
[11] This method should be cited as: Method 5220 D (Rice *et al.,* 2017), and if test kits are used, also as per the manufacturer's directions, including any modifications.

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to prevent false high readings associated with turbid solutions.

8.6.2.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.2.1.3 Required chemicals

- Distilled water

8.6.2.1.4 Required apparatus and instruments

- Spectrophotometer that operates in the region of 420-600 nm (*e.g.* Merck, Hatch, and Hanna)
- Heating block capable of heating to 150 °C, with fitting digestion tubes
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipettes and pipette tips (2 mL or 5 mL)
- Reaction cells with reagents (supplied by the manufacturer)
- Volumetric flask (1 L)
- Glass beakers (50 or 100 mL)
- Glass storage bottle
- Laboratory tissue.

8.6.2.1.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with COD standard solution with every test batch (or on a daily or weekly basis, depending on the testing load).
- For spectrophotometric measurements, the cuvette must be clean. Before analysis, wipe with a clean, dry paper tissue.
- Measurement of turbid solutions yields false high readings. For faecal sludge, samples should be diluted with an appropriate dilution factor through serial dilutions based on the type of sludge for accurate measurements.
- To ensure accuracy of measurement, blank samples must be prepared and analysed together with the samples.
- Chlorides react with silver ions to form a silver chloride precipitate that inhibits the catalytic activity of silver, and this must be avoided.
- To prevent chloride interference, samples suspected to have high chloride concentration must be analysed for chloride (Method 8.7.5.6) prior to COD analysis. For chloride concentration > 2,000 mgCl^-/L, samples must be diluted with distilled water before COD analysis.
- The measurement value remains stable for up to 60 min after the end of the reaction time; thus, the spectrophotometric measurement should be conducted within that timeframe.

8.6.2.1.6 Sample preservation

- Samples should be analysed as soon as possible. If samples cannot be analysed immediately, they should be stored in a refrigerator at 4 °C for no longer than 24 hr. If samples cannot be analysed within that time period, they should be acidified to pH ≤ 2 by adding concentrated sulphuric acid.
- For samples preserved with sulphuric acid, pH must be adjusted to 7 with sodium hydroxide solution of a known normality (5.0 N NaOH is recommended) before analysis.
- Samples must be thawed to room temperature before analysis is performed.
- Homogenise the samples prior to COD analysis.

8.6.2.1.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Samples containing concentrations of COD beyond the range of the test kit must be diluted appropriately with distilled water, following serial dilutions.

For slurry to solid samples with an estimated range of 15-300 mg COD/L:

- Weight out between 1.8 g and 2.0 g thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender to a total of 250 g, as described in Section 8.4.2.

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- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- For samples with an estimated range of 500 to 10,000 mg COD/L, accurately weigh 0.1 g of homogenised faecal sludge sample into a 50 ml beaker and follow the steps described above.
- Measure the COD concentration according to the analysis protocol.
- If required, TS analysis should be performed on the samples so that the results of the COD measurement can be expressed as mg COD/gTS.

8.6.2.1.8 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration, since calibration procedure differs between instruments. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary.

- Prepare a series of at least four different concentrations of a standard solution, making sure to include the lowest and highest concentrations of the kit testing range. It is typical to do serial dilutions or dilutions with a uniform interval including the lowest and highest concentrations.
- Determine the COD concentration of the standard solutions.
- Multiply the answer by the appropriate dilution factor and report the results in mg COD/L.
- Prepare a calibration curve by plotting the instrument response against the standard concentrations.

8.6.2.1.9 Procedure

This method is for a measuring range of 15-300 mg/L COD.

- Heat up the heating block or digester to 148 °C.
- Pipette 2 mL of the sample and 2 mL of the blank into separate reaction cells.
- Tightly screw the screw cap on the cells.
- Mix the content of the cells vigorously.
- Heat the reaction cells at 148 °C in the preheated heating block for 2 hr.
- Remove the hot cells from the heating block and allow to cool in a test tube rack. Do not cool with cold water.
- Wait 10 min, swirl the cells, and return to the rack for complete cooling to room temperature (cooling time at least 30 min).
- Wipe the reaction cells with a laboratory tissue to clean it (*e.g.* remove water spots, fingerprints)
- Measure the COD concentration in the spectrophotometer.

8.6.2.1.10 Calculation

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A \left(\frac{mg}{L}\right) \text{ x DF}$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

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8.6.2.1.11 Data set example

COD is measured in most characterisation studies for faecal sludge and it is essential for the design of faecal sludge treatment plants. In a study to quantify and characterise faecal sludge from urban areas in Douala, Cameroon, Maffo *et al.,* (2019) used the COD spectrophotometric test kit to measure the COD of seven faecal sludge composite samples collected within a day from trucks at a dumping site in Douala. The COD concentrations measured ranged from of 28,900-73,150 mg/L with an average of 39,925 mg/L and a standard deviation of 15,034 mg/L.

Similarly, Semiyaga *et al*. (2016) used the COD spectrophotometric method in the characterisation of faecal sludge from lined and unlined pit latrines. For 22 faecal sludge samples from lined pit latrines, the average COD was 65,521 ± 43,960. The 10 unlined pit latrines sampled had an average COD of 132,326 ± 43,786 mg/L. Variability in COD concentration was evident in the 3 settlements where samples were taken, with an average COD of 107,137 ± 32,542 mg/L, 75,120 ± 28,778 mg/L, and 20,794 ± 8,456 mg/L for the Bwaise II, Kibuye and Kamwokya settlements, respectively.

Gold *et al*. (2017) also determined the COD concentration of faecal sludge and wastewater samples from different types of containments in Uganda, Vietnam, Japan and Switzerland in a cross-country analysis of FS dewatering. The results of COD for septic tanks in Uganda, septic tanks in Vietnam, johkasou systems (onsite technologies) in Japan and digested wastewater sludge in Switzerland were in the range of 7.2-21.5 g/L, 15.3-31.5 g/L, 10.1-16.5 g/L and 17.0-22.1 g/L, respectively. Using percentage TS (% TS) after centrifugation as a metric of dewaterability, it was observed that COD correlated to a decreased dewaterability (decreased final % TS), a relationship that has also been observed in wastewater.

8.6.2.2 Chemical oxygen demand – closed reflux titrimetric method[12]

8.6.2.2.1 Introduction

A sample is digested at 150 ºC for 2 hours in a concentrated dichromate solution, using silver sulphate as a catalyst and mercuric sulphate as a masking agent to prevent chloride interference. The dichromate will be partially reduced by the oxidisable material present in the sample. The excess dichromate is then titrated with ammonium iron (II) sulphate and the COD value calculated from the amount of dichromate used.

The half reaction for the reduction of dichromate is:

$$Cr_2O_7^{2-} + 14\ H^+ + 6e^- \rightarrow 2\ Cr^{3-} + 7\ H_2O$$

The remaining dichromate is titrated with a standard ammonium iron (II) sulphate solution:

$$Cr_2O_7^{2-} + 6\ Fe^{2+} + 14\ H^+ \rightarrow 6\ Fe^{3+} + 7\ H_2O + 2\ Cr^{3+}$$

The equivalence point is indicated by a sharp colour change from blue-green to reddish brown as the ferroin indicator undergoes reduction from iron (III) to the iron (II) complex.

8.6.2.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- In general, alternatives to methods that use toxic metals should be investigated, the smallest possible volumes should be used, and extreme care needs to be taken in the safe handling and disposal of used chemicals.
- Handle concentrated acid with care.

[12] This method is adapted from Method 5220C of the Standard Methods for the Examination of Water and Wastewater and should be cited as: Rice *et al.* (2017), as described in Velkushanova *et al.* (2021).

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- Use eye and hand protection when preparing acid or handling colour reagents. Ensure when handling concentrated acid that an acid-proof lab coat and acid-proof gloves are used. For more detailed information on selecting the correct type of glove, consult the glove comparison chart (Berkeley Environment, Health & Safety).
- Prepare and keep the reagents in a fume hood.
- Dispose waste containing mercury according to H&S procedures.

8.6.2.2.3 Required chemicals

Standard potassium dichromate (K_2Cr_6O) - digestion solution: 0.13 M

- Potassium dichromate ($K_2Cr_6O_7$)
- 32% concentrated sulphuric acid (H_2SO_4)
- Mercuric sulphate ($HgSO_4$)
- Distilled water.

Sulphuric acid (H_2SO_4)/silver sulphate reagent (Ag_2SO_4) - COD reagent

- Silver sulphate crystals or powder
- Concentrated sulphuric acid (H_2SO_4)

Ferroin indicator

- 1,10-phenentroline monohydrate
- Ferrous sulphate (FeSO4·7H2O)

Ferrous ammonium sulphate (FAS) ($Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$): 0.10 M

- Ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$)
- 98% concentrated sulphuric acid
- Distilled water

Potassium hydrogen phthalate (KHP) - standard solution

- Potassium hydrogen phthalate (KHP)
- Distilled water

8.6.2.2.4 Required apparatus and instruments

- Heating block capable of heating to 150 ºC or microwave digester
- Digestion tubes (use borosilicate glass tubes if using a block digester or Teflon-coated digestion vessels if using a microwave digester) (the number depends on the number of samples, replicates and blanks)
- Titration setup with burette in a metal clamp (or automatic titration unit)
- 100 mL Erlenmeyer flasks (the number depending on the number of samples)
- 5 mL pipette and pipette tips
- Blender
- Analytical balance
- Crucible
- 2 x 1 L volumetric flasks
- 2.5 L volumetric flask
- 10 mL volumetric flask
- Drying oven
- Pestle and mortar

8.6.2.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Check the accuracy of the measurement procedure by using a standard solution of known COD. This calibration should be done with every set of samples. Dilute and reanalyse any samples exceeding the highest standard on the calibration curve.
- Prepare and analyse blanks with every set of samples.
- In samples with a high level of chloride (>500 mgCl^-/L), the chloride can undergo oxidation, resulting in an incorrectly high measured value for COD. This error is overcome by the addition of mercuric sulphate to samples before digesting. The chloride ions are then eliminated from the reaction by forming a soluble mercuric chloride complex. This, however, should not be common practice because the sample might become more toxic.
- The sample volume should be based on the volume of the digestion tubes. The method described below is based on 50 mL digestion tubes, but other sizes are available (the digestion tubes must match the heating block/microwave digester used). For smaller or larger tubes, the sample and reagent volumes should be adjusted to fit the tube size appropriately, but the same ratios as presented in this method should be used. For more information on selecting the correct sample

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and reagent volumes, consult Table 5220:I in Rice *et al*. (2017).

- Rinse the glassware with 20% H_2SO_4 to remove any organic residues from previous use.

8.6.2.2.6 Sample preservation

- The samples should be analysed as soon as possible. If they cannot be analysed immediately, they should be stored in a refrigerator at 4 °C for no longer than 24 hr. If the samples cannot be analysed within that time period, they should be acidified to pH ≤ 2 by adding concentrated H_2SO_4.
- The KHP standard solution is stable if stored in a refrigerator at 4 °C and can be stored for a period of 3 months; however, with any visual signs of biological growth it needs to be immediately discarded.

8.6.2.2.7 Sample preparation

This method is valid within a concentration range of 40-3,600 mgCOD/L

For liquid, slurry, semi-solid or solid samples:

- Samples containing concentrations of COD beyond the concentration range must be diluted appropriately with distilled water, following serial dilutions. The sample needs to be homogenised thoroughly (with a blender or stirring rod) before diluting.

For slurry to solid faecal sludge:

- Accurately weigh 0.1 g homogenised faecal sludge sample into a beaker with distilled water.
- Dilute the sample gravimetrically and transfer to a blender to a total of 250 g, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- If required, TS analysis should be performed on the samples so that the results of the COD measurement can be expressed as mgCOD/gTS.

8.6.2.2.8 Analysis protocol

Preparation of reagents, chemicals and standard solutions

- Standard potassium dichromate (K_2Cr_6O)
  Digestion solution: 0.13 M for faecal sludge samples. Molarity of the digestion solution is adapted here from Rice *et al.* (2017) to fit the COD range commonly seen in faecal sludge. When analysing faecal sludge with a COD ranging between 40-400 mg/L, using a digestion solution of 0.017 M (following Rice *et al*.) is recommended.
  - Dry $K_2Cr_6O_7$ in the oven (150 °C) for 2 hr and let it cool to ambient temperature in a desiccator. Afterwards, weigh 39.93 g of dried $K_2Cr_6O_7$ and transfer into a 1 L volumetric flask, and add approximately 500 mL distilled water.
  - To this volumetric flask, add 167 mL of 32% concentrated sulphuric acid (H_2SO_4) and 13.3 g mercuric sulphate ($HgSO_4$). This reaction produces heat!
  - Completely dissolve the mercuric sulphate by swirling the volumetric flask or by using a magnetic stirrer. Allow the flask contents to cool to room temperature and then dilute the mixture with distilled water to the mark of the volumetric flask.
- Sulphuric acid $HgSO_4$/silver sulphate reagent Ag_2SO_4 (COD reagent)
  - Add 26 g silver sulphate crystals or powder to the 2.5 L volumetric flask of concentrated sulphuric acid while mixing using a magnetic stirrer.
  - Leave for 2 days to dissolve and mix well before use.
- Ferroin indicator
  - Dissolve 1.485 g 1,10-phenentroline monohydrate and 0.695 g ferrous sulphate ($FeSO_4 \cdot 7H_2O$) in distilled water and dilute to 100 mL in a 100 mL volumetric flask.
- Ferrous ammonium sulphate (FAS) ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$): 0.10 M
  - Dissolve 39.2 g $Fe(NH_4)_2(SO4)_2 \cdot 6H_2O$ in distilled water.
  - Add 20 mL of 98% concentrated sulphuric acid and dilute to 1 L in a volumetric flask.

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Standardise daily (or before every set of analysis) by titration against the standard potassium dichromate digestion solution.

Calibration

- Prepare a potassium hydrogen phthalate (KHP) stock solution. Gently grind KHP with pestle and mortar. Dry the KHP at 110 °C until a constant weight is obtained. Weigh 340 mg of dried KHP into a 1 L volumetric flask and dissolve the content with distilled water up to the mark. This solution has a theoretical COD of 400 mg COD/L. The stock solution is diluted to make up four points of standard solutions.
- For samples with high expected COD values, dissolve 425 mg KHP into a 1 L volumetric flask. This KHP solution has a theoretical COD of 500 mg COD/L.
- Test the standard solutions following the COD procedure.
- Prepare a calibration curve by plotting instrument response against standard concentration and calculating the linear regression line. Compute the sample concentration by adjusting the sample response with the offset of the standard curve.

Standardisation

Use a standard $K_2Cr_2O_7$ digestion solution (daily, or before every analysis set) to correct any variation in the concentration of the FAS, following the COD measurement procedure below. The molarity of the FAS is calculated by the following equation:

Molarity of FAS =

$$\frac{\text{Volume } K_2Cr_2O_7 \text{ solution titrated (mL)}}{\text{Volume FAS used in titration (mL)}} \times 0.10$$

Procedure

Equipment preparation

- If using a heating block for digestion, preheat the heating block to 150 °C.

Sample digestion

- Add 5 mL prepared faecal sludge sample into each digestion tube. Label each tube with the corresponding sample identification.
- Add 5 mL distilled water into another digestion tube (blank).
- Add 3 mL potassium dichromate digestion solution into each tube.
- Add 7 mL sulphuric acid reagent (with silver sulphate) into each tube. Pour the acid down the wall of the flask while the flask is tilted. If the sample is too concentrated, it will turn green and further dilution of the sample must then be performed.
- Digest the samples at 150 °C for 2 hrs. Follow the manufacturer's instructions for the microwave digester or for the heating block.

Titration procedure

- Let the samples cool to room temperature in the digester after digestion.
- Pour the content of each digestion tube into an individual 100 mL Erlenmeyer.
- Record the starting volume of the FAS titrant standard in the burette.
- Add 2 drops of ferroin indicator to each Erlenmeyer.
- Titrate the excess dichromate in the digested mixture with standard ferrous ammonium sulphate.
- Titrate from a bright green-orange to a red-brown end-point.
- Record the final volume of the FAS titrant standard in the burette. The difference is the volume delivered.
- Repeat the above procedure with the blank sample.

8.6.2.2.9 Calculations

Molarity of FAS =

$$\frac{\text{Volume } K_2Cr_2O_7 \text{ solution titrated (mL)}}{\text{Volume FAS used in titration (mL)}} \times 0.10$$

COD (mg/L) =

$$\left(\frac{\text{Volume FAS used in blank titration (mL)}}{\text{Sample volume (mL)}}\right) \times$$

(Molarity of FAS × 8,000 × DF) −

$$\left(\frac{\text{Volume FAS used in titration (mL)}}{\text{Sample volume (mL)}}\right) \times$$

(Molarity of FAS × 8,000 × DF)

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COD on dry basis (g/g) =

$$\frac{\text{COD (mg/L)} \times 0.001}{\text{Total solids (g/g)}}$$

Where:

FAS = Ferrous ammonium sulphate (see the method description)

8,000 = Milliequivalent weight of oxygen × 1,000 mL/L

DF = Dilution factor (V/M), if used

M = Mass of sludge used in sample preparation

V = Total volume (L) of diluted sample

8.6.2.2.10 Data set example

In Ward *et al*. (2021), COD was measured using the closed reflux titrimetric method for 465 faecal sludge samples from 421 pit latrines and septic tanks in Lusaka, Zambia. For septic tanks, the median COD was 53.3 g/L and the average COD was 72.1 g/L, with a standard deviation of 56.9 g/L. For pit latrines, the median COD was 121.1 g/L and the average COD was 122.6 g/L, with a standard deviation of 65.5 g/L. The heterogeneity of faecal sludge is reflected in the high standard deviations. As a quality control measure, laboratory triplicates were done for every 10th sample. The relative standard error on the replicates was 8%. The entire raw data set is included with publication[13].

Two faecal sludge samples were collected in Durban, South Africa: one from a ventilated improved pit latrine and another one from a urine diversion toilet. These were analysed in four replicates using Method 8.6.2.2. The average COD (g/g dry sample) were 0.77 and 0.64 for VIP and UDDT toilets, respectively. The results for COD are presented in Table 8.8 (source: unpublished data UKZN PRG).

Table 8.8 COD values for VIP and UDDT toilets analysed by the COD closed reflux titrimetric method.

	VIP	UDDT
Samples	COD (g/g dry sample)	COD (g/g dry sample)
1	0.7769	0.6305
2	0.7967	0.6244
3	0.8099	0.6335
4	0.7879	0.6365
Average	0.7728	0.6374
SD	0.0272	0.0122

8.6.3 Fat and fibre

Crude fat and crude fibre are components of the organic matter in a sample. Characterisation of crude fat and crude fibre content is useful in assessing the resource recovery potential of faecal sludge. Crude protein is not covered in this section, but may also be of interest for characterising samples for resource recovery applications. Methods for characterisation for crude protein can be found in FAO (2011) and Regulation (EC) No 152/2009 – Determination of the Content of Crude Protein. These methods, however, could require additional method development for faecal sludge samples.

8.6.3.1 Crude fat – Soxhlet extraction method[14]

8.6.3.1.1 Introduction

In the proximate system of analysis, crude fat is the fraction of the sample that is soluble in organic solvents, such as ether. The crude fat fraction contains mostly fatty acids, but can also include waxes. Crude fat can be used to characterise resource recovery end products from faecal sludge treatment, for example black soldier fly larvae, to determine their value as animal fodder and/or use as bio-oil or a feedstock for biodiesel. Crude fat can also be measured directly on dewatered or dried faecal sludge to determine its suitability as a feedstock or co-feedstock for larvae rearing or vermiculture. Since several lipid extraction methods may be considered, the source material and lipid type present in the sample play an important role

[13] https://doi.org/10.25678/00037X

[14] This method is adapted from Crude Fat - Ether Extract Method (FAO, 2011) including recommendations from Regulation (EC) No 152/2009 Determination of Crude Oils and Fats. This method should be cited as: Crude Fat - Ether Extract Method (FAO, 2011) as adapted in Velkushanova *et al.* (2021).

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in determining the appropriate extraction method. The most widely used is the Soxhlet extraction method. This method uses a selected solvent of choice, such as petroleum ether, to extract fat from a dried, ground sample. Then petroleum ether is distilled, leaving behind fat as residue. The fat is then dried and quantified by weighing, and is reported as a mass fraction (% crude fat).

8.6.3.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Petroleum ether is flammable and has a very low flash point. Ensure that electrical equipment is earthed (grounded) and that sparks are not generated in the vicinity. Ensure that the ether is fully evaporated before heating flasks in the oven, to avoid fire or explosion.
- Do not inhale petroleum ether vapour. Always use a fume hood when decanting or handling open containers. Ensure that no solvent escapes out of the condenser (the water in the condenser must be cool). Check the temperature of the water bath regularly. Ensure the joints are sealed and the glassware is not cracked. Conduct the extraction in a well-ventilated area.
- Acids must be handled with care.
- Eye and hand protection must be used when preparing acids. Ensure when handling concentrated acids that an acid-proof lab coat and acid-proof gloves are used. For more detailed information on selecting the correct type of glove, consult the glove comparison chart (Berkeley Environment[D], Health & Safety).
- Never add water to acid. Add acid to water (slowly and carefully).
- Be extra cautious when heating acids and always use a fume hood during digestion.
- Inspect the glassware before heating acids and bases. Never use cracked glassware.

8.6.3.1.3 Required chemicals

- Petroleum ether (40-60 °C, evaporation residue ≤ 20 mg/L).
- Hydrochloric acid (3 M) (if hydrolysis pre-treatment is necessary).

8.6.3.1.4 Required apparatus and instruments

- Pestle and mortar
- Extraction thimbles and glass wool or fat-free cotton wool
- 250 mL round-bottomed flask
- Silicon carbide chips or glass beads
- Soxhlet extractor
- Condenser fitted to the Soxhlet
- Retort stand and clamps
- Heating block (heating mantle, hot water bath – able to maintain 40-60 °C)
- Cooling unit and water pump (circulate cold water through the condenser – approx. 15 °C)
- Rotary evaporator
- Vacuum grease
- Desiccator with dry desiccant
- Analytical balance
- Fume hood
- Drying oven or vacuum oven
- Beaker or conical flask (if hydrolysis pre-treatment is necessary)
- Hot plate (if hydrolysis pre-treatment is necessary)
- Buchner funnel filtration apparatus and vacuum pump with fat-free filter paper (if hydrolysis pre-treatment is necessary)
- Litmus paper (if hydrolysis pre-treatment is necessary)

8.6.3.1.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operation conditions, and interferences that are specific to this method include:

- Commercially available oils can be used to prepare quality control standards. Quality control samples should be stored in a refrigerator (2-8 °C) and changed every 6 months.
- This method is for samples with a crude fat content lower than 20%. Samples with a higher fat content should be pre-treated with a preliminary extraction (see FAO, 2011).

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- Depending on the composition of the sample, oils and fats may not be fully extractable without prior hydrolysis pre-treatment. When developing this method for a new faecal sludge type or feedstock, it will be necessary to perform crude fat analysis with and without prior hydrolysis. If the amount of crude fat measured with prior hydrolysis is higher than the result achieved without hydrolysis, the amount obtained with hydrolysis should be considered the true crude fat value and prior hydrolysis should be incorporated as part of the method (EC, 2009).
- Ensure the desiccant in the desiccator is not saturated, otherwise samples may absorb water while cooling down in the desiccator. Desiccant should be routinely dried in the oven at 105 °C (or at a different temperature, depending on the manufacturer's instructions), prior to the colour indicating that the desiccant is nearly saturated.
- Always keep the lid of the desiccator on and use a lubricant on the rim to ensure airtight sealing. Do not overload the desiccator.
- Ensure the samples are fully cooled in a desiccator to ambient temperature prior to weighing.
- Follow the quality control recommendations for calibration of the analytical balance and the drying oven described in Method 8.6.1.1.

8.6.3.1.6 Sample preservation

To preserve, samples can be freeze-dried, oven-dried at 100 ± 3 °C, or dried in a vacuum oven at 80 ± 2 °C to a constant weight, following Method 8.6.1.1. Freeze-dried or oven-dried samples can be stored in a cool, dry place for an extended period of time.

8.6.3.1.7 Sample preparation

- Determine the amount of faecal sludge required for analysis - *e.g.* 5.0 g of dried, ground sludge is required per crude fat analysis. It is recommended to prepare at least 11 g of dried, ground sludge to allow for duplicate measurements and determination of the moisture content of a dried sample. Ensure that a sufficient amount of faecal sludge is dried to allow for at least 11 g of dried, ground sample, taking into account that some sample may be lost during the grinding and sieving. The amount of wet sludge required will be dependent on the TS of the sample.
- Before drying, uniformly mix all the faecal sludge using a stainless steel rod (or other appropriate tool) in order to have thoroughly-mixed representative samples. If desired, samples may also be blended (see Section 8.4.2).
- Freeze-dry or oven-dry (100 ± 3 °C) or vacuum-dry (80 ± 2 °C) samples to a constant weight, following Method 8.6.1.1.
- Grind the dried samples (*e.g.* with a pestle and mortar or coffee grinder) to pass through a 1 mm sieve.
- Measure the moisture content of the dried, ground sample using either Method 8.6.1.1 or Method 8.6.1.5. Note the moisture content of the final dried sample.

8.6.3.1.8 Analysis protocol

Calibration

Standard curve and error estimation:

- Use commercially available oil as a standard reference (a known value of the fat present).
- Add 2 g defatted sand/silica to a thimble, add 0.00625 g oil to the sand in the thimble, add more defatted sand/ silica to make the total weight of 5 g ~ 0.125% crude fat.
- Continue as follows:
  0.0125 g ~ 0.25%
  0.025 g ~ 0.5%
  0.05 g ~ 1%
  0.075 g ~ 1.5%
  0.1 g ~ 2%
  0.125 ~ 2.5%
  0.15 ~ 3%
  0.175 ~ 3.5%
  0.2 ~ 4%
- Perform Soxhlet extraction on each dilution of the standard oil.
- Plot a curve of the % crude fat determined.
- Determine if there is any error and the degree of error in the method.

Hydrolysis pre-treatment (optional, based on sample characteristics)

- Weigh out 5 g of the dried and ground sample into a beaker or conical flask. Record the exact weight of the sample to 4 decimal places (W_1).
- Add 100 mL of hydrochloric acid and silicon carbide chips or glass beads to the beaker or

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conical flask. Cover the beaker with a watch glass or connect a reflux condenser to the conical flask.
- Boil the mixture gently on a hot plate for 1 hr. Swirl occasionally to keep the material from sticking to the sides of the beaker or flask.
- Cool and filter through a pre-moistened fat-free double filter paper using a Buchner funnel with a vacuum.
- Wash the residue with cold distilled water until the filtrate is neutral (check with Litmus paper).
- Carefully transfer the filter paper and residue into an extraction thimble and dry in a drying oven for 1 hr at 100 ± 3 °C or a vacuum oven at 80 ± 2 °C.
- Remove the thimble with filter paper from the drying oven, cover with glass wool or fat-free cotton wool and proceed with the extraction (see the next section).

Extraction
- Weigh out 5 g dried and ground sample into a thimble. Record the exact weight of the sample to 4 decimal places (W_1).
- Cover the thimble with a wad of glass wool or fat-free cotton wool, then place the thimble in the Soxhlet chamber.
- Add silicon carbide chips or glass beads (5) to a 250 mL round-bottomed flask.
- Weigh the 250 mL round-bottomed flask and record to 4 decimal places (W_2).
- Pour 150 mL petroleum ether into the 250 mL round-bottomed flask.
- Connect the 250 mL round-bottomed flask to the Soxhlet apparatus (see Figure 8.4).
- Ensure the 250 mL round-bottomed flask, Soxhlet and condenser are connected and the joints are greased with vacuum grease (always grease after adding the contents in the Soxhlet and the round-bottomed flask). Ensure that the 250 mL round-bottomed flask sits snugly in the heating pocket.
- Connect the water cooling system to the condenser and ensure there is a flow of cold water (approximately 15 °C) through the condenser and back into the cooling bath (the condenser: the bottom nozzle - water flows in, and the top nozzle - water flows out), see Figure 8.4.
- Extract for at least 6 hr, to get at least 60 siphons.
- Turn off the heating mantle and allow the 250 mL round-bottomed flask to cool down to room temperature.

Drying
- Remove the 250 mL round-bottomed flask and connect to the rotary evaporator.
- Evaporate the solvent until the 250 mL round-bottomed flask is almost free from solvent.
- Leave the 250 mL round-bottomed flask overnight in a fume hood, to ensure all the solvent is evaporated.
- Dry the 250 mL round-bottomed flask with residue for 1.5 hr in the drying oven set at 100 ± 3 °C.
- Cool in the desiccator until room temperature.
- Weigh the 250 mL round-bottomed flask with residue and record to 4 decimal places (W_3).

8.6.3.1.9 Calculation

$$\% \text{ crude fat} = \frac{W_3 - W_2}{W_1} \times 100\%$$

Where:

W_1 = Initial weight of sample (g)
W_2 = Weight of 250 mL round-bottomed flask (g)
W_3 = Weight of 250 mL round-bottomed flask (g) with fat residue

8.6.3.1.9 Data set example

Gold *et al*. (2020) quantified lipids in human faeces to assess their potential as a feedstock for black soldier fly larvae rearing. Lipids were extracted from freeze-dried samples using ether extraction with petroleum ether at 40-60 °C following hydrolysis with 3 M hydrochloric acid following Regulation (EC) No 152/2009 Determination of Crude Oils and Fats. Crude fat content in human faeces was measured at 20.9 % of the dry mass. Nyakeri *et al*. (2019) used ether extraction to quantify crude fat in black soldier fly larvae reared using faecal sludge as a feedstock. Larvae were dried and ground, and the residue was dried at 110 °C after extraction, instead of the 80 °C specified in this method. Analyses were performed in triplicate. The average crude fat content in larvae fed with faecal sludge was reported as 184 ± g/kg, which can be converted to 18.4% of dry mass.

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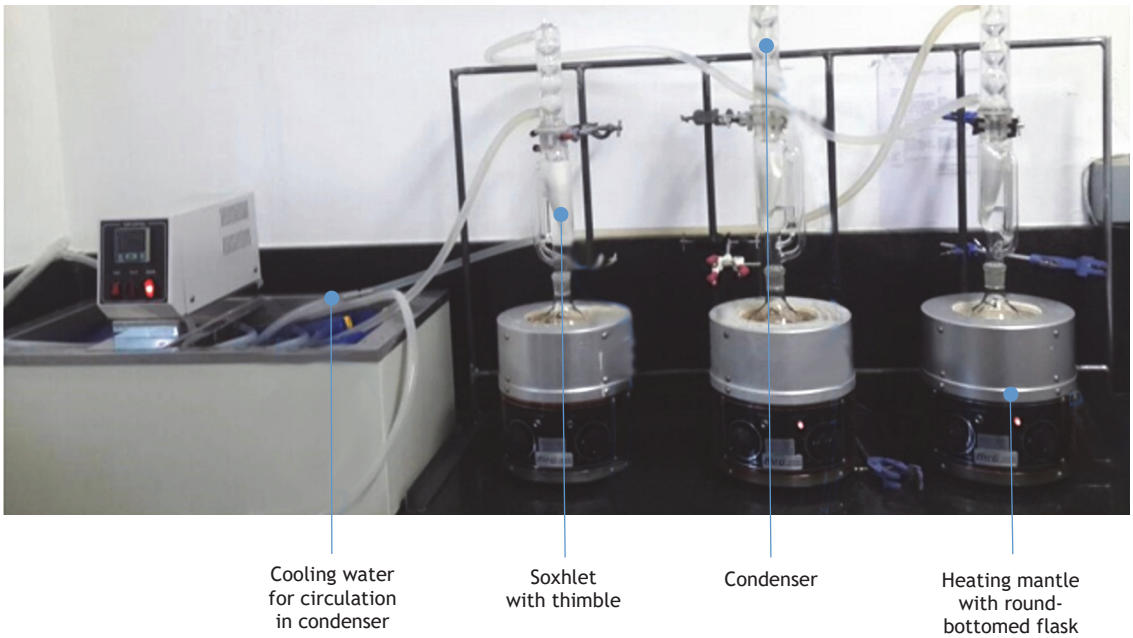

Figure 8.4 Soxlet setup (photo: UKZN PRG).

8.6.3.2 Crude fibre – filtration method[15]

8.6.3.2.1 Introduction

Determinations of crude fibre are used for quality control and specifications of animal feeds by regulatory agencies (Mertens, 2003). This method involves the extraction of fibre from a defatted sample using dilute sulphuric acid followed by potassium hydroxide solution. The mass difference after extraction, filtration, and incineration in a muffle furnace is equal to the mass of crude fibre (reported as % crude fibre). While this method is the standard for regulatory agencies, and is robust and easy to measure for many feedstocks and in many laboratories, crude fibre is not reflective of actual available dietary fibre, and does not fully capture all cellulose, pentosans, and lignins in the sample. Other methods of characterising dietary fibre may be more appropriate, depending on the purpose of characterisation, including acid detergent fibre (ADF), neutral detergent fibre (NDF), and total dietary fibre (TDF). These methods are described in detail in FAO (2011) and Novotny *et al*. (2017).

This method is applicable for determination of samples with a crude fibre content higher than 1%. If the sample contains >10% fat, the fat must first be extracted with petroleum ether prior to beginning the analysis. Fat extraction can be performed as part of the characterisation of crude fat, described in Method 8.6.3.1. The defatted sample from the crude fat analysis can be subsequently analysed for crude fibre following this method.

8.6.3.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Concentrated sulphuric acid and potassium hydroxide must be handled with care.

[15] This method is adapted from the Crude Fibre – Filtration Method described in the FAO Quality Assurance for Animal Feed Analysis Laboratories Manual (FAO, 2011) including suggestions from Novotny *et al*. (2017). This method should be cited as: Crude Fibre – Filtration Method (FAO, 2011) as adapted in Velkushanova *et al.* (2021).

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- Eye and hand protection must be used when preparing acids and bases. Ensure when handling concentrated acids and bases that an acid/base-proof lab coat and acid/base-proof gloves are used. For more detailed information on selecting the correct type of glove, consult the glove comparison chart (Berkeley Environment, Health & Safety[16]).
- Never add water to acid. Add acid to water (slowly and carefully).
- Be extra cautious when heating acids and bases and always use a fume hood during digestion.
- Inspect the glassware before heating acids and bases. Never use cracked glassware.
- Do not dispose of the following reagents in the same container: sulphuric acid and potassium hydroxide; sulphuric acid and petroleum; and potassium hydroxide and petroleum.

8.6.3.2.3 Required chemicals

- Dilute potassium hydroxide (0.23 M)
  - Potassium hydroxide (KOH) pellets
  - Distilled water
- Dilute sulphuric acid (0.15 M)
  - Concentrated sulphuric acid (H2SO4) (98%)
  - Distilled water
- Petroleum ether (if de-fatting is required)
- Acetone (technical quality)

8.6.3.2.4 Required apparatus and instruments

- Sintered glass Gooch crucibles (40-100 µm pore size recommended for faecal sludge samples)
- Buchner/side arm flask for the Gooch crucibles
- Beakers (500 mL)
- Volumetric flasks (1 L)
- Litmus paper (neutral)
- Desiccator with dry desiccant
- Analytical balance with four decimal places
- Vacuum pump
- Filtration manifold with variable pressure
- Fume hood
- Hot plate
- Drying oven
- Muffle furnace

8.6.3.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- A control standard should be analysed with each batch of samples. The control standard should have similar crude fibre and fat content to the samples analysed in the laboratory (*e.g.* a commercial animal feed can be used as a control standard for a laboratory analysing faecal sludge for suitability as a feed for black soldier fly larvae). A range of ± 2 standard deviations of the average value of the measurements of the control standard is acceptable (FAO, 2011).
- Duplicate measurements of each sample are recommended. Acceptable differences between duplicates are no more than ± 0.3% for samples with less than 10% crude fibre, and no more than ± 3% for samples with ≥ 10% crude fibre (FAO, 2011)
- Ensure the desiccant in the desiccator is not saturated, otherwise the samples may absorb water while cooling down in the desiccator. The desiccant should be routinely dried in the oven at 105 °C (or at a different temperature, depending on the manufacturer's instructions), prior to the colour indicating that the desiccant is nearly saturated.
- Always keep the lid of the desiccator on and use a lubricant on the rim to ensure airtight sealing. Do not overload the desiccator.
- Ensure the samples are fully cooled in the desiccator to ambient temperature prior to weighing.
- Care should be taken during quantitative transfer from the beakers following digestion to ensure that no material is lost. This is one of the greatest sources of error in the determination of crude fibre content (Novotny *et al*., 2017).
- The pore size of the Gooch crucibles can impact the results, as coarse membranes will allow small fibre particles to escape, while fine membranes may clog during filtration. The pore sizes suggested in Section 8.6.3.2.4 are based on the method development with faecal sludge, but

[16] https://ehs.berkeley.edu/workplace-safety/glove-selection-guide#comparison

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additional trials with specific sludge to be analysed may be necessary to determine the best pore size for a specific sample. The pore size of the Gooch crucibles should be reported with the results. Filtration of a sample should not take more than 10 min. Information about modifications to this method to deal with difficult-to-filter samples can be found in Novotny *et al*. (2017).

- Crucibles should be checked periodically to screen for cracks or clogging. It should take approximately 180 seconds for 50 mL of water to pass through a crucible without a vacuum. Times less than 120 sec indicate cracks or leaks, while times longer than 240 sec indicate clogs. Cracked crucibles should be discarded. Clogged crucibles should be cleaned by soaking in 6 N HCl for 30 min, then re-evaluated for filtration time (Novotny *et al*., 2017).
- Follow the quality control recommendations for calibration of the analytical balance and drying oven described in Method 8.6.1.1, and for calibration of the muffle furnace described in Method 8.6.1.2.

8.6.3.2.6 Sample preservation

To preserve them, samples can be freeze-dried or oven-dried (at 80 °C if crude fat will also be analysed in the sample, otherwise dry at 103 °C) to constant weight, following Method 8.6.1.1. Freeze-dried or oven-dried samples can be stored for an extended period in a cool, dry place.

8.6.3.2.7 Sample preparation

- Determine the amount of faecal sludge required for analysis. 1 g of dried, ground sludge is required per crude fibre analysis. It is recommended to prepare at least 3 g of dried, ground sludge to allow for duplicate measurements and determination of moisture content. Ensure that a sufficient amount of faecal sludge is dried to allow for at least 3 g of dried, ground sample, taking into account that some sample may be lost during grinding and sieving. The amount of wet sludge required will be dependent on the TS of the sample.
- Before drying, uniformly mix all the faecal sludge using a stainless-steel rod (or other appropriate tool) in order to have thoroughly-mixed, representative samples. If desired, samples may also be blended (see Section 8.4.2).
- Freeze-dry or oven-dry (103 °C) samples to a constant weight, following Method 8.6.1.1.
- Grind the dried samples (*e.g.* with pestle and mortar or coffee grinder) to pass through a 1 mm sieve.
- Measure the moisture content of the dried, ground sample using either Method 8.6.1.1 or Method 8.6.15. Moisture content should not exceed 10%. If moisture content is higher than 10%, the sample should be dried until the moisture content is below 10%. Note the moisture content of the final dried sample.

8.6.3.2.8 Analysis protocol

Preparation of reagents, chemicals, and standard solutions

- Dilute potassium hydroxide (0.23 M)
  - Add 12.9042 g KOH pellets to a 1 L volumetric flask.
  - Fill to the 1 L mark with distilled water and swirl to fully dissolve the KOH pellets.
- Dilute sulphuric acid (0.15 M)
  - Add some distilled water to a 1 L volumetric flask.
  - Add 8.1582 mL concentrated sulphuric acid (98%) to the 1 L volumetric flask.
  - Top up the 1 L volumetric flask to the mark with distilled water.

Preparation of equipment

- Place a clean Gooch crucible in the furnace at 550 ±20 °C for 15 min prior to use to remove any potential residual organic material from previous measurements. Cool the Gooch crucible in the desiccator until it reaches room temperature.
- Weigh out 1 g dried and ground sample into the Gooch crucible. Record the exact weight of the sample to 4 decimal places (W_1).

Defatting pre-treatment

Perform defatting pre-treatment if the sample has more than 10% fat, and fat has not already been extracted for the crude fat analysis.

- Place the Gooch crucible on the filtration manifold.
- Add 30 mL petroleum ether.

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- Filter using the vacuum pump.
- Repeat 2 more times.
- Dry the residue in the air.

Digestion and filtration

- Acid digestion and filtration:
  - Transfer the residue quantitatively to a 500 mL beaker using hot distilled water.
  - Add 150 mL 0.15 M sulphuric acid.
  - Add a glass rod to avoid bumping, see Figure 8.5.
  - Boil on a heating mantle for 30 ± 1 min under a fume hood (maintain the volume with hot distilled water).
  - Leave to cool.
  - Filter through the Gooch crucible using the vacuum pump, see Figure 8.6.
  - Wash the residue 5 times, each time with 10 mL hot distilled water (check with litmus paper for neutrality).
  - Just cover the residue with acetone, and leave for a few minutes.
  - Apply slight suction to remove the acetone.
- Base/alkaline digestion and filtration:
  - Transfer the residue quantitatively to a 500 mL beaker using hot distilled water.
  - Add 150 mL 0.23 M potassium hydroxide.
  - Add a glass rod to avoid bumping, see Figure 8.5.
  - Boil on a heating mantle for 30 ± 1 min in a fume hood (maintain the volume with hot distilled water).
  - Leave to cool.
  - Filter through the Gooch crucible using the vacuum pump, see Figure 8.6.
  - Wash the residue with hot distilled water until the rinsings are neutral (check with litmus paper for neutrality).
  - Wash with 30 mL acetone and vacuum filter, and repeat 3 times in total.

Oven drying

- Place the Gooch crucible in the drying oven at 103 ± 2 °C for 4 hr.
- Remove from the oven and place in the desiccator to cool to room temperature.
- Weigh the Gooch crucible + dry the residue immediately after removing from the desiccator. Record the weight to 4 decimal places (W_2).

Muffle furnace

- Place the Gooch crucible in the muffle furnace at 550 ± 20 °C for 2 hr.
- Remove from the furnace and place in the desiccator to cool to room temperature.
- Weigh the Gooch crucible + incinerated residue immediately after removing from the desiccator. Record the weight to 4 decimal places (W_3).

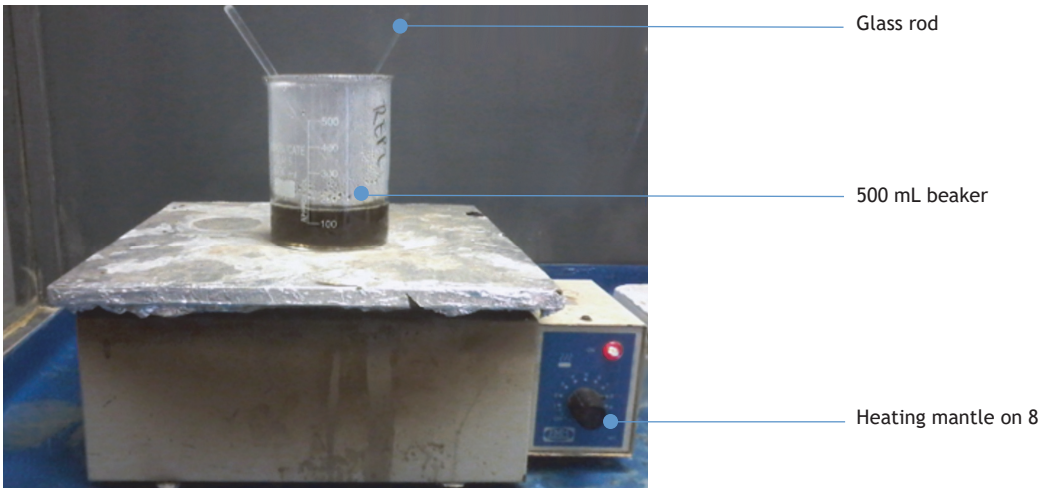

Figure 8.5 Acid-base digestion setup (photo: UKZN PRG).

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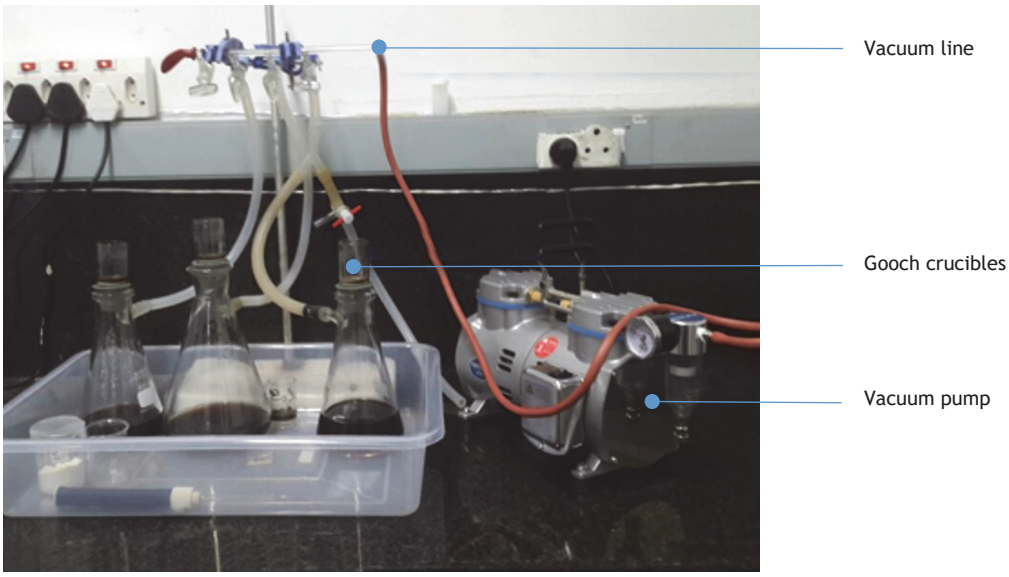

Figure 8.6 Vacuum filtration setup (photo: UKZN PRG).

8.6.3.2.9 Calculation

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{W_1} \times 100\%$$

Where:

W_1 = Weight of the sample (g)

W_2 = Weight Gooch crucible and residue after drying (g)

W_3 = Weight Gooch crucible and residue after incineration (g)

8.6.3.2.10 Data set example

An example of a different acid digestion fibre method for the determination of fodder quality for plants grown on planted drying beds is presented in Gueye *et al*. (2016). Acid digestion fibre (ADF) was measured by boiling a sample of forage in a detergent under acid conditions (pH = 2) and filtering the boiled sample through filter paper. In addition to fibre, TKN was determined by the Kjeldahl digestion method, crude protein (CP) was calculated as TKN × 6.25, crude cellulose (CC) was determined by first digesting in sulfuric acid (0.26 N), and then potassium hydroxide (0.23 N). TP was extracted by dry ashing in a muffle furnace diluted with an acid mix (HCL/HNO_2) and analysed by the molybdate procedure.

8.6.4 Nitrogen

Measurements of the different forms of nitrogen indicate the stabilisation and nutrient availability in treatment and resource recovery processes. Nitrogen is present in water and wastewater in different concentrations and chemical forms, including inorganic forms (ammonia, ammonium, nitrite and nitrate) and organic nitrogen. Organic nitrogen consists of a complex mixture of compounds including amino acids, amino sugars, and proteins. Total nitrogen consists of the sum of organic and inorganic fractions, and Kjeldahl nitrogen consists of organic nitrogen and ammonia/ammonium, which can be determined together analytically. In faecal sludge, nitrogen concentrations can be 10-100 times greater than observed in influent to sewer-based, municipal wastewater treatment plants, so special care needs to be taken with sample preparation and method selection. For more information on the nitrogen cycle, refer to Strande *et al*. (2014).

Ammonia exists either as free ammonia (NH_3) or in the ionic form ammonium (NH_4^+). In aqueous solutions, a pH-dependent equilibrium exists between the two forms, and thus ammonia occurs partly in the form of ammonium and partly as free ammonia. In

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faecal sludge treatment processes, it is important to monitor ammonia measurements because high ammonia concentrations can inhibit biological processes, such as anaerobic digestion and stabilisation ponds. In planted drying beds, high ammonia concentrations can inhibit plant growth, which is important for treatment processes. The leachate from planted and unplanted drying beds typically also still has high concentrations of ammonia. Due to the ability to inhibit biological activity, ammonia concentrations can also be used to slow the growth of pathogens, and during alkaline stabilisation of faecal sludge, the addition of lime increases the pH, which results in greater NH_3 concentrations (than NH_4^+) (Englund and Strande, 2019). In this book, methods for determination of ammonia include test strips, and spectrophotometric and titrimetric methods. Other methods not yet included in this book are the ammonia selective electrode method and flow injection analysis.

Nitrite, which is an intermediate of the oxidation of ammonia to nitrate (nitrification), is usually present in low to non-detectable concentrations in wastewater and faecal sludge. However, in the event of an imbalance in the nitrification process, nitrite may accumulate. Nitrite is toxic and effluents with high nitrite concentration can adversely affect aquatic life when dilution in the receiving water is insufficient. In aerobic treatment processes, accumulation of nitrite may signify deficiencies in the treatment process. In this book, methods for determination of nitrite include nitrite colorimetric and spectrophotometric methods.

Nitrate and ammonium are considered to be plant-available nutrients. However, ammonia/ammonium is rapidly oxidised to nitrate in the environment, and nitrate can leach rapidly through soils. Nitrate becomes toxic and affects public health when it enters the food chain through contamination of surface or groundwater. Eutrophication and algal blooms of surface water are direct environmental impacts of pollution from excess nutrients. Nitrate management in faecal sludge treatment is thus essential to protect public and environmental health. In this book, methods for determination of nitrate concentrations include colorimetric and spectrophotometric methods.

8.6.4.1 Total nitrogen – spectrophotometric method[17]

Total nitrogen can be determined by several different methods, including in-line UV/persulfate digestion and oxidation with flow injection. The method described here is total nitrogen quantified with spectrophotometry, based on the oxidation of all the nitrogenous compounds with the persulfate digestion to nitrate, followed by a cadmium reduction process to measure the nitrate.

Commercial test kits based on standard methods for measuring total nitrogen are available, with pre-packaged individual aliquots of necessary chemical in pillows (dry chemicals) and vials (liquid chemicals). Commonly used total nitrogen test kits from manufacturers such as Hach and Merck employ a variety of methods. The example provided here is the Hach total nitrogen spectrophotometric test[D] (Hach, 2020) for samples with concentrations of 2-150 mgN/L, which is based on the manufacturer's protocol for water and wastewater using the standard method 4500-N C (Rice *et al.,* 2017). For faecal sludge, samples must be diluted to prevent the false high readings associated with turbid solutions.

8.6.4.1.1 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

[17] This method should be cited as: Method 4500-N C (Rice et al., 2017), and if test kits are used, also as per the manufacturer's directions including any modifications.

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8.6.4.1.2 Required chemicals

- Deionised or distilled water (free from nitrogen)
- Ammonia nitrogen standard solution (100 mg/L NH_3-N)
- Total nitrogen persulfate reagent powder pillow (supplied by the manufacturer)
- Total nitrogen reagent A powder pillow (supplied by the manufacturer)
- Total nitrogen reagent B powder pillow (supplied by the manufacturer).

8.6.4.1.3 Required apparatus and instruments

- Spectrophotometer (*e.g.* DR6000)
- Digester or heating block (capable of heating to 150 °C)
- 2 total nitrogen hydroxide digestion reagent vials (supplied by the manufacturer)
- TN reagent C vial (supplied by the manufacturer)
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipettes and pipette tips
- Volumetric flask (1 L)
- Glass beakers (50 or 100 mL)
- Test tube rack
- Funnel
- Finger cot.

8.6.4.1.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with the Hach ammonia nitrogen standard solution with every test batch (or on a daily or weekly basis, depending on the testing load).
  Note: quality control for other methods can also include nitrate standard solutions.
- Measure a blank sample for every test batch and subtract the blank values from the sample results.
- During the reaction, undissolved powder will remain at the bottom of the sample cell after the reagent dissolves. This deposit will not affect results.
- Common interferences in faecal sludge may include chloride and bromide ions. For specific concentrations refer to the manufacturer's instructions.
- Suspended solids and turbid solutions interfere with measurement, thus faecal sludge samples should be diluted with an appropriate dilution factor based on the type of sludge for accurate measurements. Always use serial dilutions.

8.6.4.1.5 Sample preservation

- Samples should be analysed as soon as possible after sampling. For analysis within 48 hr of collection, sample must be refrigerated at 4 °C (Rice *et al*., 2017).
- Samples can be stored for a maximum of 28 days by preserving with concentrated sulphuric acid and refrigerating at 4 °C.
- For samples preserved with sulphuric acid, pH must be adjusted to 7 with sodium hydroxide solution of a known normality (5.0 N NaOH is recommended) before analysis.
- Samples must be thawed to room temperature before the analysis is performed.

8.6.4.1.6 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- A turbid solution can falsely increase the spectrophotometric reading. Turbid samples should be diluted to prevent false high measurements.
- Samples containing more than 150 mgN/L must be diluted with nitrogen-free distilled water.
- The results can be reported on a mass per volume basis (gN/L).

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.

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- Total solids analysis should be performed on the samples so that the results of the total nitrogen measurement can be expressed on a mass per mass basis (gN/gTS).

8.6.4.1.7 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary. For this method, calibration can be performed as follows:

- Prepare a series of at least four different dilutions of the standard solution, making sure to include the lowest and highest concentrations of the kit testing range with serial dilutions, or dilutions with a uniform interval including the lowest and highest concentrations.
- Determine the total nitrogen concentration of the standard solutions.
- Multiply the answer by the dilution factor and report the results in mgN/L.
- Prepare a calibration curve by plotting the instrument response against the standard concentration.

Procedure

For measuring the range of 2-150 mgN/L:

- Preheat the digester or heating block to 150 °C.
- With the aid of a funnel, add the contents of one total nitrogen persulfate reagent powder pillow to each of the two total nitrogen hydroxide digestion reagent vials, ensuring that the reagent does not stick to the lips of the vials.
- Pipette 0.5mL of the sample into one of the vials.
- Pipette 0.5mL of deionised or nitrogen-free water into the second vial as a blank.
- Cover the vials with caps and shake vigorously for at least 30 sec to mix. The undissolved powder does not affect the accuracy of the test.
- Place the vials in the heating block and digest for 30 min.
- Remove the vials from the heating blocks after 30 min, place them onto a test tube rack and allow them to cool to room temperature.
- Add one total nitrogen (TN) reagent A powder pillow to each vial and shake for 30 sec.
- Allow the mixture to react for 3 min using a set timer.
- Add one TN reagent B powder pillow to each vial and shake vigorously for 15 sec. The solution will turn yellow but with some undissolved powder which does not affect the accuracy of the test.
- Allow a 2 min reaction time using a set timer and pipette 2 mL of the sample into a TN reagent C vial.
- Mix the solution by slowly inverting the vials approximately 10 times and allow a reaction time of 5 min using a set timer.
- Wipe the vials with a laboratory tissue so it is clean (*e.g.* to remove water spots, fingerprints, etc), and measure in a spectrophotometer.

8.6.4.1.8 Calculations

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

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8.6.4.1.9 Data set example

While developing methods to reliably estimate faecal sludge qualities and quantities, Strande *et al*. (2018) measured the total nitrogen of faecal sludge (in addition to other physicochemical parameters) from pit latrines and septic tanks located in households, non-households, and public toilets. 180 samples were analysed, with an uneven distribution and high variability. Hence, the median rather than the mean of the results were used for the statistical analysis. In spite of the high variability in the results, as illustrated in Figure 8.7 of the TN results for pit latrines and septic tanks, there were differences based on households, non-households and public toilets, and the pit latrines in general had a higher TN concentration. The complete raw data set is available at the link provided below[18].

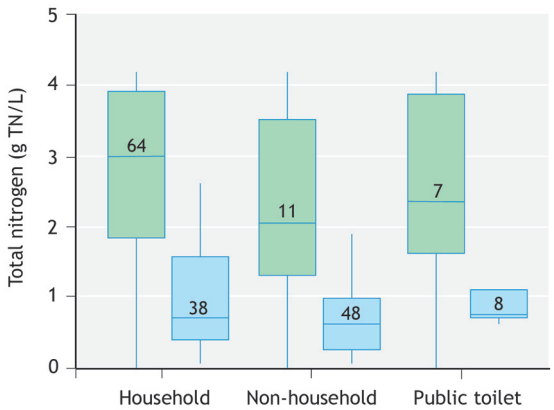

Figure 8.7 Total nitrogen for septic tanks (blue boxes) and pit latrines (green boxes) from households, non-households, and public toilets (Strande *et al.*, 2018).

8.6.4.2 Ammonium – colorimetric (test strip) method[19]

8.6.4.2.1 Introduction

Measurement of ammonium is important for assessing the impact on receiving water bodies, evaluating treatment performance, and to check for inhibitory concentrations in treatment processes. In the test strip method, ammonium ions react with Nessler's reagent to form a yellow-brown compound. The concentration of ammonium is measured semi-quantitatively by visual comparison of the reaction zone of the test strip with the fields of a colour scale. This method is based on the specific manufacturer's protocol for water and wastewater. The example provided here is one of many ammonium colorimetric test methods for samples with concentrations of 10-400 mg/L NH_4^+. Ammonium measurement results are expressed either as mg/l NH_4^+ or NH_4-N, and it is important to note how the results are expressed by the selected test method. For faecal sludge, samples must be diluted to prevent masking of the resultant colour change.

8.6.4.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.4.2.3 Required chemicals

The required chemicals will be specific to each manufacturer's test kit; specific information on test kits can be found on the manufacturers' websites.

- Distilled water
- Reagent NH4-1 (provided with the test kit)
- Ammonium standard solution Centipur®

8.6.4.2.4 Apparatus and instruments

- Test strips
- Glass beakers (50 or 100 mL)
- Balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Volumetric flask (250 mL)
- Absorbent paper towel
- Filter paper (adequate for removing solids from the sample, for example 0.45µm filter for liquid samples)

[18] https://doi.org/10.25678/0000tt

[19] This method should be cited as the specific manufacturer's method along with any modifications. The example used here is the Merck MQuant Ammonium Test (Merck, 2020j)[D].

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8.6.4.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with an ammonium standard solution with every test batch (or on a daily or weekly basis depending on the testing load).
- Dilute the ammonium standard solution with distilled water to 100 mgNH_4^+/L.
- The test strips are stable, and can be stored up to the date stated on the package, when stored closed at 2 to 8°C.
- The colour of the reaction zone might continue to change after the specified reaction time has elapsed. This should not be considered in the measurement, which should always be recorded at the stated time.
- Common interferences in faecal sludge include nitrate, nitrite, phosphate, potassium and magnesium. For specific concentrations of interference, refer to the manufacturer's instructions.
- For faecal sludge, samples should be diluted with an appropriate dilution factor, based on the type of sludge, to prevent interference with the colour of the test strips. Always use serial dilutions.
- The ammonium test strip method is used for qualitative to semi-quantitative measurement. For a quantitative measurement, refer to Method 8.7.5.6.

8.6.4.2.6 Sample preservation

Samples should be analysed as soon as possible after sampling. For analysis within 24 hours of collection, the sample must be refrigerated at 4 °C. Refrigeration reduces biological activity, which can increase NH_4^+ concentration.

8.6.4.2.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Solid samples must be dissolved in water before further dilution is performed.
- Filter samples to prevent interference in colour.
- Samples containing more than 400 mgNH_4/L must be diluted with deionised or distilled water.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Filter samples through a 0.45µm filter paper and measure nitrite concentration.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Total solids analysis should be performed on the samples so that the results of the nitrite measurement can be expressed on a mass per mass basis (gNH_4^+-N/gTS).

8.6.4.2.8 Analysis protocol

For measuring range of 10-400 mgNH_4^+/L:

- Rinse the test vessel several times with the sample. Fill the test vessel with 5 mL of the sample. Add 10 drops of the reagent NH4-1 and swirl.
- Immerse the reaction zone of the test strip in the measurement sample for 3 seconds.
- Allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel and after 10 seconds determine which colour field on the label the colour of the reaction zone coincides with most closely. Read off the corresponding result in mg/L NH_4^+.

8.6.4.2.9 Calculation

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A \left(\frac{mg}{L}\right) \text{ x DF}$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

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Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.4.3 Ammonium – phenate spectrophotometric method[20]

8.6.4.3.1 Introduction

There are various methods to measure ammonia; selection of the appropriate method is based on the concentration and the presence of interferences. In this method, ammonia first reacts with hypochlorite to form monochloramine. Chloramine then reacts with phenate to form 5-aminophenate which is oxidised to indophenol, a reaction catalysed by sodium nitroprusside. Indophenol has an intense blue colour that is measured spectrophotometrically. This method measures both ammonium ions and dissolved ammonia in a concentration range of 2–150 mgNH_4-N/L.

It is also possible to conduct this method with commercially available test kits. Commonly used test kits from manufacturers such as Hach, Merck, and Hanna employ different methods in the kits used for ammonia measurement; the Nessler method, the salicylate method and the phenate method are common methods. For example, the Hach test kit uses the salicylate method while Merck kits are based on the phenate method. The test kits are also based on standard methods, with pre-packaged individual aliquots of necessary chemical in pillows (dry chemicals) and vials (liquid).

The example provided here is the Merck ammonium spectrophotometric test kit (Merck, 2020f)[D] for samples with concentrations of 2-150 mgNH_4-N/L, and uses the manufacturer's protocol for water and wastewater based on the standard method 4500 (Rice *et al*., 2017). For faecal sludge, samples must be diluted and filtered to prevent false-high readings associated with turbid solutions.

8.6.4.3.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.4.3.3 Required chemicals

- Distilled water.
- Hypochlorite, and phenate and sodium nitroprusside.
  - Reagent NH_4-1 (supplied by the manufacturer).
  - Reagent NH_4-2 (supplied by the manufacturer).
- Ammonium standard solution CRM (supplied by the manufacturer).

8.6.4.3.4 Required apparatus and instruments

- Spectrophotometer equipped with 1 cm or larger cuvette
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipettes and pipette tips
- 10 mm cuvettes (2 packs)
- Volumetric flask (1 L)
- Glass beakers (50 or 100 mL)
- Glass storage bottle
- Blue micro spoon (supplied by the manufacturer)
- Glass test tubes (30 mL or 50 mL)
- Filter paper (adequate for removing solids from sample, for example 0.45µm filter for liquid samples)

[20] This method should be cited as: Method 4500 (Rice *et al*., 2017), and if test kits are used, also the manufacturer's directions including any modifications.

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8.6.4.3.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with an ammonium standard solution with every test batch (or on a daily or weekly basis, depending on the testing load).
- Due to the specific temperature range dependence of the colour reaction, ensure that the temperature of the reagents is maintained between 20-30 °C.
- Ammonium-free samples turn yellow on addition of reagent NH_4-2. Thus, blank samples (distilled water) should be tested with every test batch.
- Colour of the measurement solution remains stable for up to 60 minutes after the end of the reaction time, thus the spectrophotometric measurement should be conducted within that timeframe.
- Common interferences in faecal sludge include magnesium and nitrite. For specific concentrations refer to the manufacturer's instructions.
- For faecal sludge, samples should be diluted with an appropriate dilution factor and filtered based on the type of sludge for accurate measurements. Always use serial dilutions.

8.6.4.3.6 Sample preservation

- Samples should be analysed as soon as possible after sampling to prevent losses due to volatilisation or biological activity. For analysis within 24 hours of collection, sample must be refrigerated at 4 °C.
- Samples can be frozen at -20 °C or preserved by acidifying to pH ≤ 2 and kept at 4 °C for long-term storage (Rice *et al.*, 2017).
- If acidification is used in storing samples, neutralise the samples with KOH or NaOH immediately before ammonium determination.

8.6.4.3.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Turbidity will falsely increase the spectrophotometric reading, thus samples might have to be filtered to prevent false-high measurements. Report any preparation steps with the results.
- Samples containing more than 150 mgNH_4-N/L must be diluted with distilled water.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper and measure ammonium concentration according to the analysis protocol.
- Total solids analysis should be performed on the samples so that the results of the ammonium measurement will be reported as gNH_4-N/gTS.

8.6.4.3.8 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary. For this method, calibration can be performed as follows:

- Prepare a series of at least four different concentrations of a standard solution making sure to include the lowest and highest concentration of the kit testing range. It is typical to do serial dilutions or dilutions with a uniform interval including the lowest and highest concentrations.
- Take a reading for the prepared ammonium concentrations of the dilutions of the standard.
- Multiply the answer by the appropriate dilution factor and report the results in mgNH_4-N/L.
- Prepare a calibration curve by plotting the instrument response against the standard concentration.

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Procedure

For measuring range of 2.0–75 mgNH_4-N/L (2.6–96.6 mgNH_4^+/L):

- Pipette 5.0 mL of reagent NH_4-1 into a glass test tube.
- Add 0.2 mL of the diluted, filtered sample into the test tube and mix by agitating.
- Add 1 level blue micro spoon of reagent NH_4-2 and shake vigorously until completely dissolved.
- Allow the solution to stand for 15 minutes in a test tube rack and fill the 10 mm cuvette with the sample.
- Wipe the cuvette with a soft tissue to remove water spots and fingerprints and measure in the spectrophotometer.

For measuring ranges of > 75-150 mgNH_4-N/L (> 96.6-193 mgNH_4^+/L):

- Pipette 5.0 mL of reagent NH_4-1 into a test tube.
- Add 0.1 mL of the diluted, filtered sample into the test tube and mix by agitating.
- Add 1 level blue micro spoon of reagent NH_4-2 and shake vigorously until completely dissolved.
- Allow the solution to stand for 15 min in a test tube rack and fill the 10 mm cuvette with the sample.
- Wipe the cuvette with a soft tissue to remove water spots and fingerprints and measure in the spectrophotometer.

8.6.4.3.9 Calculations

Result of the analysis (mg/L NH_4-N) = measurement value, A (mg/L) × dilution factor

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A \left(\frac{mg}{L}\right) \text{ x } DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

DF = Dilution factor (F/I)

F = Final diluted volume (L)

I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.4.3.10 Data set example

In evaluating the effect of drying on the physical and chemical characteristics of faecal sludge for its reuse, Septien *et al*. (2020) measured the ammonium concentration of faecal sludge from pit latrines before and after drying at different temperatures using a Merck Spectroquant®,D. Raw faecal sludge samples had ammonium concentration of 24 ± 4 g/g TS. By using the convective drying rig, the ammonia concentration reduced to 4 ± 2 g/g TS when it was dried at 40 °C.

Hach test kits using the salicylate method and a Hach-Lange DR2800 spectrophotometer, following the manufacturers' directions, were used to evaluate 60 samples in Hanoi, Vietnam, and 180 in Kampala, Uganda, for NH_4^+-N concentrations. In Hanoi concentrations ranged from 54 to 1,700 NH_4^+-N/L, and in Kampala, Uganda from 24 to 3,000 mgNH_4^+-N/L (Englund *et al*., 2020; Strande *et al*., 2018). The complete raw data set is available using the link below[21].

[21] https://doi.org/10.25678/0000tt

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8.6.4.4 Ammonia – distillation and titration method[22]

8.6.4.4.1 Introduction

Acid-base titrations are useful in determining concentrations of acids and bases in samples. An acid or a base is neutralised with a known volume of corresponding acid or base. The exact concentration of the titrant solution must be known in order to establish the concentration of the titrated solution. The endpoint of this reaction is when the solution reaches neutral pH, where the determinant in the sample is equivalent to the analyte, and is only visible when an indicator is used. This is used to calculate the concentration of other determinants from the known ratio of the reaction.

Ammonia exists either as free ammonia (NH_3) or ammonium ions (NH_4^+). In aqueous solutions, a pH-dependent equilibrium exists between the two forms and thus ammonia occurs partly in the form of ammonium ions and partly as free ammonia. Measurement of ammonia is important for assessing the impact on receiving water bodies, evaluating treatment performance and nutrient management, and checking for inhibitory concentrations in treatment processes. The sample is adjusted to pH 9.5 using a borate buffer to allow the measurement of organic nitrogen compounds. The sample containing ammonium nitrogen is distilled in boric acid and then titrated using 0.02N H_2SO_4 as follows:

$$H_2SO_4 + 2\ NH_4OH \rightarrow 2\ H_2O + (NH_4)_2SO_4$$

This method is based on the Standard Methods for the Examination of Water and Wastewater (Rice *et al*., 2017). Based on local experience, the method might need to be adapted for different types of faecal sludge. For example, the UKZN PRG in Durban adapted the sample volume to 70 mL, based on the characteristics of faecal sludge in Durban and the semi-automated distillation setup used in the laboratory.

8.6.4.4.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Handle concentrated acid with care.
- Use eye and hand protection when preparing acid or handling colour reagents. Ensure when handling concentrated acid that an acid-resistant apron or lab coat and acid-resistant gloves and are used. Information on selecting the correct type of glove can be found following the link below[23].
- Prepare and keep the reagents in a fume hood during use.

8.6.4.4.3 Required chemicals

Ammonia-free distilled water
- Distilled water
- Concentrated sulphuric acid, or bromine or chlorine, depending on method used; see Section 8.6.4.4.8.

Ammonium chloride standard
- Ammonia-free distilled water
- NH_4Cl

NaOH solutions (1 N and 0.1 N)
- Ammonia-free distilled water
- NaOH

Borate buffer solution
- 0.1 N NaOH solution
- 0.025 M disodium tetraborate ($Na_2B_4O_7$) solution - (9.5 g $Na_2B_4O_7 \cdot 10H_2O$ hydrous/L or 5.0 g $Na_2B_4O_7$ anhydrous/L)

Mixed indicator solution
- Methyl red indicator
- 95% organic polar solvent (isopropyl alcohol or ethanol)
- Methylene blue

Indicator boric acid solution

[22] This method is based on Method 4500-NH3 B and C of the Standard Methods for the Examination of Water and Wastewater and should be cited as: Rice *et al*. (2017) as described in Velkushanova *et al*. (2021).

[23] https://ehs.berkeley.edu/workplace-safety/glove-selection-guide#comparison, (Berkeley Environment, Health & Safety).

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- Ammonia-free distilled water
- Mixed indicator solution
- H_3BO_3

Standard sulphuric acid titrant (0.02 N)

- Ammonia-free distilled water
- Concentrated sulphuric acid
- Anhydrous sodium carbonate
- Methylene blue-methyl red mixed indicator solution.

8.6.4.4.4 Required apparatus and instruments

- Distillation unit
- pH meter
- Filter paper with 0.45 µm pore size
- Drying oven that can reach temperature 270 ºC
- Analytical balance
- Blender
- Pipettes and tips
- 1 L volumetric flask
- 50 mL beakers
- Plastic bottle
- Titration setup with burette in metal clamp (or automatic titration unit).

8.6.4.4.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Check the accuracy of the measurement procedure by using a standard solution of known ammonia concentration. This calibration should be done with every set of samples. Dilute and reanalyse any samples exceeding the highest standard on the calibration curve.
- Prepare and analyse blanks with every sample set.
- Urea and cyanates hydrolyse when distilled at pH 9.5, which amounts to 7% for urea and 5% for cyanates at this pH. On standing, glycine, urea, glutamic acid, cyanates and acetamide hydrolyse only very slowly in solution.

8.6.4.4.6 Sample preservation

- Samples should be analysed as soon as possible after sampling. For analysis within 24 hr of collection, samples must be refrigerated at 4 °C. For longer storage durations of up to 28 days, the pH should be reduced to below pH 2 with concentrated sulphuric acid and stored at 4 ºC, or the sample should be frozen at -20 ºC without acidifying. If acid preservation has been used, neutralise the samples to pH 7 with NaOH or KOH immediately before carrying out the analysis.
- Storage of reagents:
  - Ammonium-free water is very difficult to store without interference by gaseous ammonia, and storage should therefore be avoided.
  - Prepare a fresh solution of ammonium chloride monthly.
  - Prepare mixed indicator solution monthly.
  - Prepare indicator boric acid solution monthly.
  - Standard sulphuric acid titrant should not be stored longer than one week.

8.6.4.4.7 Sample preparation

This method is valid within a concentration range of 5-100 mg/L NH_3-N:

This method is valid for all sludge types (liquid, slurry, semi-solid, and solid), as long as they are pre-diluted using serial dilutions within the specified range. Ideally, dilutions should aim to be within the range of 10-25 mg/L NH_3-N. Use ammonium-free distilled water for making dilutions.

For liquid faecal sludge:

- Filter turbid samples using 0.45 µm filter.

For slurry to solid faecal sludge:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Total solids analysis should be performed on the samples so that the results of the ammonia measurement can be expressed on a mass per mass basis (gNH_3-N/gTS).

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8.6.4.4.8 Analysis protocol

Preparation of reagents, chemicals and standard solutions

- Ammonia-free water

Eliminate traces of ammonia in distilled water by adding 0.1 mL concentrated sulphuric acid to 1 L distilled water and then redistill. Alternatively, treat distilled water with enough bromine or chlorine to produce a free halogen residual of 2-5 mg/L and redistill after standing for 1 hr. Discard the first 100mL of distillate, and check the ammonia concentration in the collected water before use.

- Ammonium chloride standard
  - Prepare ammonium chloride standard by weighing and dissolving 3.819 g of NH_4Cl into a 1 L volumetric flask with distilled water and dilute the flask content to the mark with ammonia-free distilled water. This makes an ammonium chloride standard solution where 1.0 mL = 1 mg N = 1.22 mg NH_3.
  - Then prepare a working standard solution by transferring 10 mL stock solution into 1 L in a volumetric flask and further dilute with ammonia-free distilled water to give a concentration of 12 mg/L NH_3.
- N NaOH
  - Weigh 4 g of NaOH and dissolve in 1 L of ammonia-free distilled water.
- 1 N NaOH
  - Weigh 40 g NaOH and dissolve in 1 L of ammonia-free distilled water.
- Borate buffer solution
  - Add 88 mL of 0.1 N NaOH solution to 500 mL of 0.025 M disodium tetraborate ($Na_2B_4O_7$) solution (9.5 g $Na_2B_4O_7 \cdot 10H_2O$ hydrous/L or 5.0 g $Na_2B_4O_7$ anhydrous/L) into a 1 L volumetric flask and dilute to the mark with ammonia-free distilled water.
- Mixed indicator solution
  - Weigh and dissolve 200 mg methyl red indicator in 100 mL of 95% organic polar solvent (isopropyl alcohol or ethanol) in a beaker. In a separate beaker, dissolve 100 mg methylene blue in 50 mL of the same solvent. Then combine the solutions.
- Indicator boric acid solution
  - Weigh 20 g H_3BO_3 and dissolve in ammonia-free distilled water. Add this to a 1 L volumetric flask, add 10 mL mixed indicator solution, and dilute to the mark.
- Standard sulphuric acid titrant (0.02 N)
  - First make a 0.1 N sulphuric acid solution by diluting 2.71 mL concentrated sulphuric acid to 1L with distilled water.
  - Dilute 200 mL 0.1 N sulphuric acid solution to 1 L with distilled water to make a 0.02 N solution.
  - Allow it to cool down to ambient temperature.
- Sulphuric acid titrant standardisation
  - Dry anhydrous sodium carbonate (Na_2CO_3) at 270 °C, cool down in a desiccator, and weigh 1.325 g. Dissolve in ammonia-free distilled water and make up to 500 mL in a volumetric flask (0.05 N solution). Do not keep longer than 1 week.
  - Titrate the sulphuric acid solution against 25 mL of sodium carbonate solution using 5 drops of methylene blue-methyl red mixed indicator.
  - Calculate the exact normality of the sulphuric acid:

    Normality of H_2SO_4 solution =

$$\frac{25 \times 0.05}{\text{Volume of } H_2SO_4 \text{ used}}$$

8.6.4.4.9 Calibration

- Prepare a 1,000 mgNH_3-N/L stock solution by dissolving 3.819 g ammonium chloride in 1 L ammonia-free distilled water. The stock solution is diluted to make up four-point standard solutions covering the required range of NH_3-N concentrations.
- Prepare a calibration curve by plotting the instrument response against the standard concentration and calculating the linear regression line. Compute the sample concentration by adjusting the sample response with the offset of the standard curve. Report only those values that fall between the lowest and the highest calibration standards. Dilute and reanalyse the samples exceeding the highest standard. Report the results in mg/L.

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Preparation of the setup

Add 500 mL ammonia-free water and 20 mL borate buffer to a distillation flask and adjust the pH to 9.5 with 6.0 N NaOH solution. Add a few glass beads and use this mixture to steam-clean the distillation apparatus until the distillate shows no traces of ammonia.

Procedure

- Add the sample to a distillation flask. Use the sample volume as specified here:

Expected ammonia nitrogen in the sample (mg/L)	Sample volume to be used (mL)
5 – 10	250
11 – 20	100
21 – 50	50
51 – 100	25

- Add ammonia-free distilled water to make up 500 mL.
- Add 25 mL borate buffer to the distillation flask.
- Distil at a rate of 6-10 mL/min, ensuring the tip of the delivery tube is below the surface of the acid-receiving solution, and collect the distillate into 50 mL of indicator boric acid solution.
- Titrate ammonia in the distillate with standardised 0.02 N sulphuric acid:
  - Note the starting volume of the sulphuric acid in the burette.
  - Titrate until the indicator turns pale purple.
  - Note the final volume of sulphuric acid in the burette.
- Process a blank in the same way and apply the necessary correction to the results.

8.6.4.4.10 Calculations

Liquid and slurry samples:

NH_3-N (mg/L) =

$$\frac{(A - B) \times 280}{\text{Sample volume (mL)}} \times DF$$

NH_3-N on dry basis (g/g) =

$$\frac{NH_3\text{-N (mg/L)}}{\text{Total solids concentration (mg/L)}}$$

Where:

A = Volume of H_2SO_4, titrated for sample, mL

B = Volume of H_2SO_4, titrated for blank, mL

Sulphuric acid: standard solution (0.02 N, 1 mL = 0.28 mgNH_3-N)

1 L = 280 mgNH_3- N

DF = Dilution factor (F/I)

F = Final diluted volume (L)

I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

NH_3-N on dry basis (g/g) =

$$\frac{(A - B) \times 280}{\text{Sample volume (mL)}} \times \frac{V}{M} \times \frac{1}{1{,}000} \times \frac{1}{\text{Total solids content (g/g)}}$$

Where:

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.4.4.11 Data set example

Spit *et al*. (2014) sampled three pit latrines in Blantyre, Malawi, and determined the ammonia using the titration method. Each sample was analysed in triplicate and the results were reported volumetrically, as shown in Table 8.9.

Table 8.9 The results from the ammonia and TS analysis from three pit latrines in Blantyre, Malawi.

		NH_3-N (g/L)	TS (%)
Pit 1	1	22.0	10.5
	2	30.0	12.0
	3	26.0	26.0
	Average	26.0	16.2
	SD	4.0	8.5
Pit 2	1	36.0	19.0
	2	31.0	18.0
	3	30.7	19.3
	Average		
	SD	5.5	1.5
Pit 3	1	33	25.0
	2	36	22.0
	3	26	22.0
	Average	31.7	23.0
	SD	5.1	1.7

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Two faecal sludge samples were collected from two ventilated improved pit latrines in Durban, South Africa. They were analysed in four replicates each, using Method 8.6.4.5. The average ammonia content (g/g dry sample) was 0.71 and 0.74 for samples one and two respectively. The results for ammonia are presented in Table 8.10 (source: unpublished data UKZN PRG).

Table 8.10 VIP faecal sludge analysis using the ammonia distillation and titration method.

Replicate	NH_3-N (g/g dry sample) TS = 2 g/L	NH_3-N (g/g dry sample) TS = 5 g/L
1	0.0070	0.0074
2	0.0072	0.0075
3	0.0071	0.0075
4	0.0071	0.0074
Average	0.0071	0.0074
SD	0.0002	0.0001

8.6.4.5 Nitrite – colorimetric (test strip) method[24]

8.6.4.5.1 Introduction

Nitrite is a toxic intermediate of nitrification. Wastewater effluents with high nitrite concentration can adversely affect aquatic life when dilution in the receiving water is insufficient. In aerobic faecal sludge treatment processes, accumulation of nitrite signifies deficiencies in the treatment process. This method describes the Merck 110007 MQuant Nitrite Test[D] as an example of a commercially available nitrite colorimetric test strip method for samples with concentrations of 2-80 mgNO_2^-/L. It is based on the manufacturer's protocol for water and wastewater. In the presence of an acidic buffer, nitrite ions react with aromatic amine to form a diazonium salt, which in turn reacts with N-(1-naphthyl)-ethylenediamine to form a red-violet azo dye. The nitrite concentration is measured semi-quantitatively by visual comparison of the reaction zone of the test strip with the fields of a colour scale. Nitrite measurement results are expressed as either mgNO_2^-/L or mgNO_2-N/L, and it is important to note how the results are expressed by the selected test method. Depending on the expected nitrite concentration in the sample, kits with the appropriate measurement range should be selected. For faecal sludge, samples must be diluted to prevent masking of the resultant colour change.

8.6.4.5.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.4.5.3 Required chemicals

The required chemicals will be specific to each manufacturer's test kit; specific information on test kits can be found on the manufacturer's websites.

- Distilled water.
- Nitrite standard solution Certipur® (1,000 mg/L NO_2^-).

8.6.4.5.4 Required apparatus and instruments

- Test strips.
- Glass beakers (50 or 100 mL).
- Balance with weighing boats (slurry to solid samples).
- Blender (slurry to solid samples).
- Volumetric flask (250 mL).
- Filter paper (adequate for removing solids from the sample, for example 0.45µm filter for liquid samples).

8.6.4.5.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

[24] Test strip methods should be cited as the specific manufacturer's method along with any modifications. The example used here is the Merck MQuant Nitrite Test (Merck, 2020h)[D].

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- Dilute the standard nitrite solution with distilled water to 20 $mgNO_2^-/L$, and analyse according to the analysis protocol.
- Perform quality control with nitrite standard solution with every test batch (or on a daily or weekly basis depending on the testing load).
- The test strips are stable up to the date stated on the pack when stored closed at +2 to +8 °C.
- The colour of the reaction zone may continue to change after the specified reaction time has elapsed. This must not be considered in the measurement, which should always be recorded at the stated time.
- Common interferences in faecal sludge include potassium, magnesium, and nitrate. For specific concentrations refer to the manufacturer's instructions.
- For faecal sludge, samples should be diluted with an appropriate dilution factor, based on the type of sludge, to prevent interference with the colour of the test strips. Always use serial dilutions.
- The nitrite colorimetric test method is used for qualitative to semi-quantitative measurement. For a quantitative measurement, refer to Method 8.6.4.6.

8.6.4.5.6 Sample preservation

- Samples should be analysed immediately after sampling to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3. For short-term preservation of 1 to 2 days, samples should be stored at 4 °C or frozen at −20 °C (Rice *et al.,* 2017).
- Samples should be thawed to ambient temperature before analysis.

8.6.4.5.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Solid samples must be dissolved in a known volume of water before further dilution is performed.
- Filter samples through a 0.45 µm filter paper to prevent interference in colour.
- Samples containing more than 80 $mgNO_2^-/L$ must be diluted with distilled water.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Filter samples through a 0.45µm filter paper and measure nitrite concentration.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Total solids analysis should be performed on the samples so that the results of the nitrite measurement can be expressed on a mass per mass basis (gNO_2-N/gTS).

8.6.4.5.8 Analysis protocol

For measuring range of 2-80 $mgNO_2^-/L$

- Immerse the reaction zone of the test strip in the diluted, filtered sample (15 - 30 °C) for 1 second. Shake off excess liquid from the strip and after 15 sec determine which colour field on the label the colour of the reaction zone coincides with most exactly. Read off the corresponding result in $mgNO_2/L$ or $mgNO_2$-N/L.

8.6.4.5.9 Calculation

The dilution factor used must be stated.

Result of the analysis (mg/L NO_2^-) = measurement value, A (mg/L) × dilution factor (DF)

Liquid and slurry samples:

$$\text{Final concentration } (\text{mg/L}) = A\left(\frac{\text{mg}}{\text{L}}\right) \times \text{DF}$$

$$\text{Dry basis } \left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{Final concentration } \left(\frac{\text{mg}}{\text{L}}\right)}{\text{Total solids concentration } \left(\frac{\text{mg}}{\text{L}}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

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Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.4.6 Nitrite – spectrophotometric method[25]

8.6.4.6.1 Introduction

Nitrite can be measured using different methods, such as chromatography or spectrophotometry. This method describes nitrite measurement by spectrophotometry. The principle is that in an acidic solution nitrite ions react with sulfanilic acid to form a diazonium salt. The diazonium salt reacts with N-(1-naphthyl) ethylenediamine dihydrochloride to form a red-violet azo dye at a pH of 2.0-2.5, which is then determined spectrophotometrically. Nitrite measurement results are expressed in different ways (*i.e.* mg/L NO_2^- or mg/L NO_2-N), and it is important to know how the results are expressed by the selected test method. Spectrophotometric measurements ranging from 0.01-1.0 mg/L is applicable for this method. However, for lower concentrations < 0.01 mg/L, spectrophotometric measurements can be made by adapting this method using a 5 cm light path and a green colour filter. The sample is filtered prior to analysis, as solids interfere with the spectrophotometric method, based on the assumption that NO_2^- will be solution, and not with the solids fraction. It is possible to conduct this method with commercially available test kits. The test kits are based on standard methods, with pre-packaged individual aliquots of the necessary chemicals in pillows (dry chemicals) and vials (liquid chemicals). Commonly used test kits from manufacturers such as Hach, Merck, and Hanna vary slightly in the methods they use for nitrite measurement. The ferrosulphate method and diazotization method are most commonly used in test kits.

The example provided here is the Merck Spectroquant Nitrite Test spectrophotometric test kit (Merck, 2020e)[D] for samples with concentrations of 0.02-1.0 mgNO_2-N/L, and it is based on the manufacturer's protocol for water and wastewater using the standard method 4500 (Rice *et al.,* 2017). For faecal sludge, samples must be diluted and filtered to prevent false high readings associated with turbid solutions.

8.6.4.6.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.4.6.3 Required chemicals

- Distilled water (free from nitrogen)
- Reagent NO_2-1 (supplied by the manufacturer)
- Nitrite standard solution CRM, 0.200 mg/L NO_2-N (supplied by the manufacturer).

8.6.4.6.4 Required apparatus and instruments

- Spectrophotometer equipped with 1 cm or larger cuvettes
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipette and pipette tips
- 10 mm cuvettes
- Volumetric flask (1 L)
- Glass beakers (50 or 100 ml)
- Glass storage bottle
- pH test strips
- Blue micro-spoon (supplied by the manufacturer)
- Glass test tubes (30 mL or 50 mL)
- Laboratory cleaning tissues
- Filter paper (adequate for removing solids from the sample, for example a 0.45µm filter for liquid samples).

[25] This method should be cited as: Method 4500-NO2–B (Rice *et al.,* 2017) and, if test kits are used, also as per the manufacturer's directions including any modifications.

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8.6.4.6.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with nitrite standard solution with every test batch (or on a daily or weekly basis depending on the testing load).
- For spectrophotometric measurements, the cuvettes must be clean. Before analysis, wipe with a laboratory cleaning tissue.
- Measurement of turbid solutions yields false high readings. For faecal sludge, samples should be diluted with an appropriate dilution factor through serial dilutions and filtered based on the type of sludge for accurate measurements.
- The pH of the measurement solution must be within the range 2.0-2.5.
- The colour of the measurement solution remains stable for up to 60 min after the end of the reaction time; thus, the spectrophotometric measurement should be conducted within that timeframe.
- Common interferences in faecal sludge include magnesium and nitrates. For specific concentrations refer to the manufacturer's instructions.

8.6.4.6.6 Sample preservation

- Samples should be analysed immediately after sampling to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3. For short-term preservation of 1 to 2 days, samples should be stored at 4 °C or frozen at −20 °C (Rice *et al.,* 2017). Samples should be thawed to room temperature before analysis.

8.6.4.6.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Samples containing concentrations of nitrite beyond the range of the test kit must be diluted with distilled water.
- The nitrite content of samples can be estimated prior to dilution with the MQuant nitrite test kit.
- Filter the samples through a 0.45 µm filter paper.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Then blend for 30 sec on the highest speed.
- Transfer the content of the blender into a volumetric flask, rinse the blender twice with a known volume of distilled water and dilute to the mark with distilled water.
- Transfer the volumetric flask content into a plastic bottle and store in a dark cold room or refrigerator.
- Filter the samples through a 0.45 µm filter paper and measure the nitrite concentration.
- Total solids analysis should be performed on the samples so that the results of the nitrite measurement can be reported as gNO_2-N/gTS.

8.6.4.6.8 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration, since calibration procedure differs between instruments. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary.

- Prepare a series of at least four different concentrations of a standard solution, making sure to include the lowest and highest concentration of the kit testing range. It is typical to do serial dilutions or dilutions with a uniform interval including the lowest and highest concentrations.
- Determine the nitrite concentration of the standard solutions.
- Multiply the answer by the dilution factor and report the results in $mgNO_2$-N/L.
- Prepare a calibration curve by plotting the instrument response against the standard concentrations.

Procedure

This method is for a measuring range of 0.02-1.0 mg/L NO_2-N

- Pipette 5ml of the sample into a test tube.
- Add 1 level blue micro-spoon of reagent NO_2-1 to the test tube.

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- Shake vigorously until the reagent is completely dissolved. The pH of the solution must be between 2-2.5; check this with a pH test strip.
- Leave the solution to stand for 10 min, then fill the 10 mm cuvette with the sample.
- Wipe the cuvette with a soft tissue to remove water spots and fingerprints and then measure in a spectrophotometer.

8.6.4.6.9 Calculation

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A \left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

DF = Dilution factor (F/I)

F = Final diluted volume (L)

I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.4.6.10 Data set example

Septien *et al*. (2020) evaluated the effect of drying on the physical and chemical characteristics of faecal sludge for resource recovery. The nitrite concentration of faecal sludge from pit latrines before and after drying at different temperatures were measured using a Merck Spectroquant®,D. The sample was prepared by blending it with water, centrifugation, recovering the liquid fraction and using the commercial test kit. Nitrite in a solid sample dissolves and remains in a soluble state which can be measured. In this example, nitrite was measured in the faecal sludge sample which was further subjected to drying in infra-red and convective drying rigs. For duplicate samples, nitrite concentration of 0 g/kg dry solid was measured for untreated faecal sludge and dried faecal sludge samples after drying in a convective drying rig at 30 °C, 60 °C and 80 °C. Nitrite is usually oxidised to nitrate and thus concentrations are expected to be very low to zero in faecal sludge samples. However, nitrite concentrations in untreated faecal sludge for infra-red drying were 13 ± 0 g/kg dry solid. After drying, the concentration of nitrite decreased to 2.3 ± 0.1 g/kg dry solid. The decrease in nitrite and other nitrogen forms determined in this work did not translate into a decrease in total nitrogen, and thus drying of faecal sludge was concluded to induce changes in the chemical forms of nitrogen.

8.6.4.7 Nitrate – colorimetric (test strip) method[26]

8.6.4.7.1 Introduction

Nitrate is a major source of available nitrogen for plants and microorganisms. However, nitrate becomes toxic and affects public health when it contaminates surface or groundwater used as drinking water. Eutrophication and algal blooms of surface water are also environmental impacts of nitrate pollution. Nitrate management in faecal sludge treatment is thus essential to protect public and environmental health. This method describes the Merck MQuant® Nitrate Test[D] as one example of a commercially available nitrate colorimetric test strip method, for samples with concentrations of 10-500 mgNO_3^-/L. It is based on the manufacturer's protocol for water and wastewater. In this method, nitrate ions are reduced to nitrite ions in the presence of a reducing agent. In the presence of an acidic buffer, nitrite ions react with aromatic amine to form a diazonium salt, which in turn reacts with N-(1-naphthyl)-ethylenediamine to form a red-violet azo dye. Nitrate measurement results are expressed as

[26] Test strip methods should be cited as the specific manufacturer's method along with any modifications. The example used here is the Merck MQuant Nitrate Test (Merck, 2020g)[D].

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either $mgNO_3^-/L$ or $mgNO_3$-N/L, and it is important to note how the results are expressed by the selected test method. The nitrate concentration is measured semi-quantitatively by visual comparison of the reaction zone of the test strip with the fields of a colour scale. Depending on the expected nitrate concentration in the sample, kits with the appropriate measurement range should be selected. For faecal sludge, samples must be diluted to prevent masking of the resultant colour change.

8.6.4.7.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.4.7.3 Required chemicals

The required chemicals will be specific to each manufacturer's test kit; specific information on test kits can be found on the manufacturer's websites.

- Distilled water,
- Nitrate standard solution Certipur® (1,000 $mgNO_3^-/L$),
- Amidosulfuric acid.

8.6.4.7.4 Required apparatus and instruments

- Test strips.
- Glass beakers (50 or 100 mL).
- Balance with weighing boats (slurry to solid samples).
- Blender (slurry to solid samples).
- Volumetric flask (250 mL).
- Filter paper (adequate for removing solids from sample, for example 0.45 µm filter for liquid samples).

8.6.4.7.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Dilute the nitrate standard solution with distilled water to 250 $mgNO_3^-/L$, and analyse according to the analysis protocol.
- Perform quality control with nitrate standard solution with every test batch (or on a daily or weekly basis depending on the testing load).
- The test strips are stable up to the date stated on the pack when stored closed at +2 to +8 °C.
- The colour of the reaction zone may continue to change after the specified reaction time has elapsed. This must not be considered in the measurement, which should always be recorded at the stated time.
- Observe critically the second reaction zone or alert zone of the test strip, which changes colour in the presence of nitrite ions.
- Common interferences in faecal sludge include potassium, magnesium, and nitrite. For specific concentrations refer to the manufacturer's instructions.
- For faecal sludge, samples should be diluted with an appropriate dilution factor, based on the type of sludge, to prevent interference with colour of the test strips. Always use serial dilutions.
- The nitrate colorimetric test method is used for qualitative to semi-quantitative measurement. For a quantitative measurement, see Method 8.6.4.8.

8.6.4.7.6 Sample preservation

- Samples should be analysed immediately after sampling. For short-term preservation of 1 to 2 days, samples should be stored at 4 °C or frozen at -20 °C. Acid preservation for long-term storage is not encouraged because nitrate and nitrite in acid-preserved samples cannot be analysed as individual species (Rice *et al*., 2017).
- Samples should be thawed to ambient temperature before analysis.

8.6.4.7.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Solid samples must be dissolved in a known volume of water before further dilution is performed.
- Filter the samples to prevent interference in colour.
- Samples containing more than 500 $mgNO_3^-/L$ must be diluted with distilled water.

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For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper and measure the nitrate concentration according to the analysis protocol.
- Total solids analysis should be performed on samples so that the results of the nitrate measurement can be expressed as gNO_3-N/gTS.

8.6.4.7.8 Analysis protocol

For measuring range of 10-500 $mgNO_3/L$:

- Immerse the reaction zone of the test strip in the diluted, filtered sample (15-30 °C) for 1 second. Shake off the excess liquid from the strip and after 1 min determine which colour field on the label is closest to the colour of the reaction zone. Read off the corresponding result in $mgNO_3^-/L$ or $mgNO_3$-N/L.
- If the nitrite alert zone changes colour, eliminate nitrite interference by adding 5 drops of 10% aqueous amidosulfuric acid solution to 5 mL of each sample. Shake well and repeat the nitrate measurement.

8.6.4.7.9 Calculation

The dilution factor used must be stated.

Result of analysis (mg/L NO_3^-) = measurement value, A (mg/L) × dilution factor

Liquid samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

8.6.4.8 Nitrate – cadmium reduction spectrophotometric method[27]

8.6.4.8.1 Introduction

The principle of this method is that nitrate interacts with cadmium particles in aqueous solutions which converts all the nitrate in the sample into nitrite. The nitrite formed is determined by diazotising with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured azo dye that is measured spectrophotometrically. Nitrate measurements are easily influenced by interfering components such as dissolved organic matter, surfactants, nitrite and various inorganic compounds in water. The concentration ranges for the different methods used for nitrate measurement are also limited. For instance, the concentration ranges of the electrode method, the cadmium reduction spectrophotometric method and the automated cadmium reduction method are 0.14 to 1,400 mg NO_3-N/L, 0.01 to 1.0 mg NO_3-N/L and 0.001 to 10 mg NO_3-N/L, respectively. This means

[27] This method should be cited as: Method 4500-NO3-E (Rice *et al*., 2017), and if test kits are used, also as per the manufacturer's directions including any modifications.

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that higher concentrations should be diluted to the range of the selected method. Methods for nitrate determination are based on the oxidizing properties of nitrate. However, other oxidants present in water and wastewater may interfere making nitrate measurement difficult.

It is possible to conduct this method with commercially available test kits. The test kits are based on standard methods, with pre-packaged individual aliquots of necessary chemical in pillows (dry chemicals) and vials (liquid chemicals). Commonly used test kits from manufacturers such as Hach, Merck, and Hanna employ different methods in the kits used for nitrate measurement. The cadmium reduction method is used in Hach and Hanna test kits.

The example provided here is the Hach nitrate spectrophotometric test kit (Hach, 2020)[D] for samples with concentrations of 0.3-30 mgNO_3-N/L, which is based on the manufacturer's protocol for water and wastewater using the standard method 4500-NO_3-E (Rice *et al*., 2017). For faecal sludge, samples must be diluted and filtered to prevent false high readings associated with turbid solutions.

8.6.4.8.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.4.8.3 Required chemicals

- Distilled water (free from nitrogen)
- NitraVer 5 nitrate reagent powder pillow (supplied by the manufacturer)
- Nitrate nitrogen standard solution 10.0 mg/L (supplied by the manufacturer).

8.6.4.8.4 Required apparatus and instruments

- Spectrophotometer (*e.g.* DR6000)
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipettes and pipette tips
- Sample cell
- Volumetric flask (1 L)
- Glass beakers (50 or 100 mL)
- Filter paper (adequate for removing solids from the sample, for example a 0.45µm filter for liquid samples).

8.6.4.8.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with a nitrate standard solution with every test batch (or on a daily or weekly basis, depending on the testing load).
- Measure a blank sample for every test batch and subtract the blank values from the sample results.
- This method is technique-sensitive and thus shaking time and technique influence the colour development.
- Deposits of unoxidized metal will remain at the bottom of the sample cell after the reagent dissolves. The deposit will not affect the results.
- Common interferences in faecal sludge include chloride, ferric ion, and nitrite. For specific concentrations refer to the manufacturer's instructions.
- Suspended solids and turbid solutions interfere with measurement, thus faecal sludge samples should be diluted with an appropriate dilution factor and filtered based on the type of sludge for accurate measurements. Always use serial dilutions.

8.6.4.8.6 Sample preservation

- Samples should be analysed as soon as possible after sampling. For analysis within 48 hr of collection, sample must be filtered and refrigerated at 4 °C (Rice *et al.*, 2017).
- Samples must be thawed to room temperature before the analysis is performed.

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8.6.4.8.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Turbid solutions falsely increase the spectrophotometric reading. Therefore, turbid samples should be filtered with a 0.45 µm filter paper to prevent false high measurements.
- Samples containing more than 30 $mgNO_3$-N/L must be diluted with distilled water.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper and measure the nitrate concentration according to the analysis protocol.
- Total solids analysis should be performed on samples so that the results of the nitrate measurement can be expressed as gNO_3-N/gTS.

8.6.4.8.8 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary. For this method, calibration can be performed as follows:

- Prepare a series of at least four different concentrations of a standard solution making sure to include the lowest and highest concentration of the kit testing range. It is typical to do serial dilutions or dilutions with a uniform interval including the lowest and highest concentrations.
- Determine the nitrate concentration of the standard solutions.
- Multiply the answer by the dilution factor and report the results in $mgNO_3$-N/L.
- Prepare a calibration curve by plotting the instrument response against the standard concentration.

Procedure

For measuring range of 0.3-30.0 $mgNO_3$-N/L:

- Pipette 10.0 mL of the sample into the sample cell.
- Add the contents of one NitraVer 5 nitrate reagent powder pillow and cover the sample cell with a stopper.
- Shake the sample cell vigorously for 1 min using a timer. It is important to note that not all the powder will dissolve. The undissolved powder will not affect the test results.
- Allow the solution to sit for 5 min making sure to use a set timer. An amber colour develops in the presence of nitrate.
- While waiting for the 5 min reaction time, prepare a blank solution.
- After the 5 min reaction time is over, fill a second cell with the sample solution.
- Wipe the sample cell with a laboratory tissue to clean it (*e.g.* to remove water spots and fingerprints) and measure in a spectrophotometer.
- Measure the nitrate concentration of the blanks and the sample.
- The sample reading should be taken within 1 min after the reaction time.

8.6.4.8.9 Calculation

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

DF = Dilution factor (F/I)

F = Final diluted volume (L)

I = Initial sample volume (L)

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Slurry, semi-solid and solid samples:

$$\text{Dry basis}\left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content}\left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.4.8.10 Data set example

Strande *et al*. (2018) determined the nitrate concentrations of faecal sludge from pit latrines and septic tanks located in households, non-households, and public toilets using the Hach nitrate test kits. As shown in Figure 8.8, 180 samples were analysed and the results showed an uneven distribution and a high variability. Hence, the median rather than mean of the results was used for the statistical analysis. Similar to other forms of nitrogen determined, nitrate concentrations in pit latrines from households, non-households and public toilets were all found to be higher compared to septic tanks. The complete raw data set is available at the link below[28].

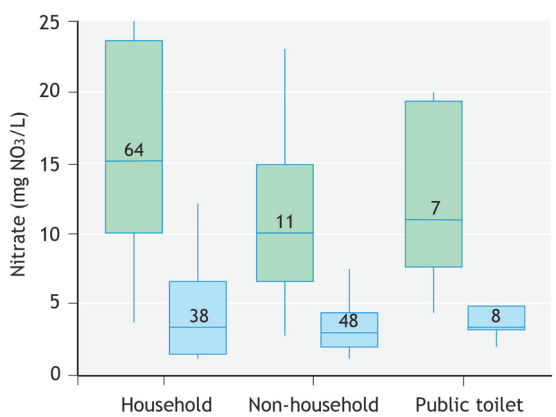

Figure 8.8 Nitrate concentrations in septic tanks (blue boxes) and pit latrines (green boxes) from households, non-households, and public toilets (Strande *et al.*, 2018).

8.6.4.9 Total Kjeldahl nitrogen – distillation and titration method[29]

8.6.4.9.1 Introduction

Kjeldahl nitrogen is defined as the total organic nitrogen together with the inorganic compounds ammonia and ammonium (NH_3/NH_4^+). In the presence of sulphuric acid, potassium sulphate, and cupric sulphate catalyst, amino nitrogen in the organic matter in the sample and free ammonia are converted into ammonium during digestion. Upon addition of a base, ammonium is converted into ammonia, and the ammonia is then distilled from an alkaline medium and absorbed in boric or sulphuric acid. The ammonia may be determined spectrophotometrically, by ammonia selective electrode or by titration with a standard mineral acid. The titration method is described here. The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentrations.

Degradation:

Organic N in sample + $H_2SO_4 \rightarrow (NH_4)_2SO_4(aq) + CO_2(g) + SO_2(g) + H_2O(g)$

Liberation of ammonia:

$(NH_4)_2SO_4(aq) + 2NaOH \rightarrow Na_2SO_4(aq) + 2H_2O(l) + 2NH_3(g)$

Capture of ammonia:

$B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^+ + B(OH)_4^-$

Back-titration:

$B(OH)_3 + H_2O + Na_2CO_3 \rightarrow NaHCO_3(aq) + NaB(OH)_4(aq) + CO_2(g) + H_2O$

All dilutions and reagents should be made with ammonia-free distilled water.

Various apparatus is available for Kjeldahl analysis, ranging from low-tech heating blocks for digestion to fully automatic Kjeldahl setups that do digestion and/or distillation and titration. This method might need to be adapted based on the type of apparatus used, and for automated systems the

[28] https://doi.org/10.25678/0000tt

[29] This method is adapted from Method 4500-Norg B and C of the Standard Methods for the Examination of Water and Wastewater and should be cited as: Rice *et al*. (2017), as described in Velkushanova *et al.* (2021).

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manufacturer's instructions should be followed. In addition, the method might need to be adapted for different types of faecal sludge, based on local experience and the specific characteristics of faecal sludge. The total Kjeldahl nitrogen distillation and titration (TKN) method is widely accepted and has been tested for multiple substances; however, some steps in the process are affected by the composition of the sample and need to be adjusted accordingly (*e.g.* amounts of acid used, and digestion temperature and time). A useful guide with further information on considerations for Kjeldahl analysis can be found in 'A Guide to Kjeldahl Nitrogen Determination Methods and Apparatus' by Labconco Corporation (1998), and the Labconco website[30]. Methods should always be adapted according to rigorous quality control principles. For example, UKZN PRG in Durban uses a semi-automated digestion setup, and for samples with a high organic/fat content they mix samples overnight on the digestion apparatus prior to digestion.

8.6.4.9.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Use eye and hand protection when preparing acid or handling colour reagents. Ensure when handling concentrated acid that an acid-proof lab coat and acid-proof gloves and goggles are used. For more detailed information on selecting the correct type of glove, consult the glove comparison chart provided by Berkeley Environment, Health & Safety[31].
- Prepare and keep reagents in a fume hood during use.
- Fumes are generated during the digestion step. Take care to not inhale these fumes! Ideally, this procedure is conducted in a fume hood.

8.6.4.9.3 Required chemicals

- Preparation of ammonia-free water
  - Distilled water
  - Concentrated sulphuric acid, or bromine or chlorine, depending on the method used; see Section 8.6.4.9.8.
- Digestion reagent
  - K_2SO_4
  - $CuSO_4$
  - Alternatively, commercial pre-mixed Kjeldahl tablets or powder, free of Hg and Se, are available and can be used instead of mixing digestion reagent manually.
- Boric acid – 4%
  - Boric acid
  - Ammonia-free distilled water
- Concentrated sulphuric acid – 98%
- Sulphuric acid – 0.1 N
  - Concentrated sulphuric acid
  - Ammonia-free distilled water
- Sodium hydroxide (NaOH) – 35%
  - NaOH
  - Ammonia-free distilled water
- Mixed indicator
  - Methyl red indicator
  - Bromocresol green indicator
  - Ethanol.

8.6.4.9.4 Apparatus and instruments

- Analytical balance
- (Kjeldahl) digestion apparatus (can be gas or electric, should be able to reach temperatures between 375-385 ºC), or semi-automated Kjeldahl device
- Kjeldahl flasks or digestion tubes that fit the digestion apparatus used
- Boiling stones
- 100 mL volumetric flask
- 1 L volumetric flasks
- 250 mL Erlenmeyer flasks
- Plastic bottle
- Distillation setup
- Titration setup with burette in metal clamp (or automatic titration unit).

[30] https://www.labconco.com/articles/a-brief-introduction-to-kjeldahl-nitrogen-determ

[31] https://ehs.berkeley.edu/workplace-safety/glove-selection-guide#comparison

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8.6.4.9.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Check the accuracy of the measurement procedure by using a standard solution of known nitrogen concentration. This calibration should be done with every set of samples. Dilute and reanalyse any samples exceeding the highest standard on the calibration curve.
- Prepare and analyse blanks with every set of samples.
- Do not use $HgCl_2$ for sample preservation because it interferes with the ammonia removal liberation process.
- Avoid nitrate: during Kjeldahl digestion, nitrate in excess of 10 mg/L can oxidise a portion of the ammonia released from the digested organic nitrogen, producing N_2O and resulting in a negative interference. According to USEPA (2001), no known method exists to prevent this interference, but its effect can be predicted on the basis of preliminary nitrate determination of the sample.
- Control the system temperature, the inorganic salts and solids: the acid and salt content of the Kjeldahl digestion reagent is intended to produce a digestion temperature of approximately 380 °C.
- If the sample contains a very large quantity of salts or inorganic solids, the temperature may rise to 400 °C during digestion at which point pyrolytic loss of nitrogen occurs. To prevent this, add controlled amounts (*e.g.* 1 mL H_2SO_4/g salt) of sulphuric acid to maintain an acid-salt balance.
- Samples containing chloride should be dechlorinated prior to analysis.

8.6.4.9.6 Sample preservation

- The most reliable results are obtained from fresh samples. If immediate analysis is not possible, preserve the samples for Kjeldahl digestion by acidifying between pH 1.5 and 2.0 with concentrated sulphuric acid and storing in a refrigerator at 4 °C. Let the samples return to room temperature before starting the analysis. If acid preservation was used, neutralise the samples to pH 7 with NaOH or KOH immediately before starting the analysis.
- Storage of reagents:
  - Mixed indicator solution should be freshly prepared every month.
  - Sulphuric acid titrant should not be stored longer than one week.

8.6.4.9.7 Sample preparation

For liquid faecal sludge:

- Filter turbid samples using a 0.45 µm pore size filter.

For slurry to solid faecal sludge:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper and measure the TKN concentration according to the analysis protocol.
- Total solids analysis should be performed on samples so that the results of the TKN measurement can be expressed as gTKN/gTS.

8.6.4.9.8 Analysis protocol

Preparation of reagents, chemicals and standard solutions

Ammonia-free water

- Eliminate traces of ammonia in the distilled water by adding 0.1 mL concentrated sulphuric acid to 1 L distilled water and redistill. Alternatively, treat the distilled water with enough bromine or chlorine to produce a free halogen residual of 2-5 mg/L and redistill after standing for 1 hr. Discard the first 100 mL of distillate, and check the ammonia concentration in the collected water before use.

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Boric acid - 4%

- Dissolve 40g of boric acid in the distilled water in a 1L volumetric flask, and dilute up to the mark.

Sulphuric acid - 0.1 N

- Dilute 2.71 mL 98% concentrated sulphuric acid in the distilled water in a 1 L volumetric flask, and dilute up to the mark.

Mixed indicator

- Weigh 0.02 g methyl red and 0.10 g bromocresol green indicator in a 100 mL volumetric flask and fill to the mark with ethanol.

Calibration

- Prepare a 80 mg N/L stock solution by dissolving 0.4285 g glycine in 1 L ammonia-free distilled water. The stock solution is diluted to make up seven-point standard solutions covering the required range of nitrogen concentrations, including a blank.
- Test the standard solutions following the TKN procedure.
- Prepare a calibration curve by plotting the instrument response against the standard concentration and calculating the linear regression line. Compute the sample concentration by adjusting the sample response with the offset of the standard curve. Report only those values that fall between the lowest and the highest calibration standards. Dilute and reanalyse those samples exceeding the highest standard. Report the results in mg/L.

Procedure

- Include standards and a blank with every analysis batch.

Digestion

- Transfer 50 mL of the homogenised diluted sample into a 300 mL Kjeldahl flask or digestion tube (depending on the digestion apparatus used), and add 5 boiling stones.
- Use either 1 Kjeldahl tablet or mix 3.5 g K_2SO_4 and 0.5 g $CuSO_4$ together, and add to the flask. Then slowly add 10 mL of concentrated sulphuric acid and swirl to dissolve.
- Digest the samples at 380 °C for 1 hr under a fume hood. Fumes will appear above the liquid.
- Wait for the samples to cool to ambient temperature.

Distillation

- Add 50 mL distilled water and 50 mL NaOH to the flasks after digestion. The mixture should be above pH 11 before distillation.
- Prepare the absorption solution by placing 25 mL of 4% boric acid in a 250 mL Erlenmeyer flask, then insert under the condenser outlet with the tip below the surface of the boric acid.
- Distil the sample into the boric acid.

Titration

- Fill the burette with 0.1 N sulphuric acid and note the starting volume.
- Add 3 drops of mixed methyl red-bromocresol green indicator to the distillate and titrate the distillate with sulphuric acid until the colour changes from blue to pale pink.
- Read off the final volume on the burette and note down.

8.6.4.9.9 Calculation

Liquid and slurry samples:

$$\text{TKN (mg/L)} = \frac{(A - B) \times 0.1 \times 14 \times 1{,}000}{\text{Sample volume (mL)}} \times \text{DF}$$

Where:

A = Volume H_2SO_4 titrated in sample (mL)
B = Volume H_2SO_4 titrated for blank (mL)
0.1 = Normality of sulphuric acid used in titration
14 = Atomic weight of nitrogen
1,000 = Conversion from g to mg
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

TKN on dry basis (g/g) =

$$\frac{\text{TKN (mg/L)}}{\text{Total solids concentration (mg/L)}}$$

Slurry, semi-solid and solid samples:

TKN on dry basis (g/g) =

$$\frac{(A - B) \times 0.1 \times 14 \times 1{,}000}{\text{Sample volume (mL)}} \times \frac{V}{M} \times \frac{1}{1{,}000} \times \frac{1}{\text{Total solids content (g/g)}}$$

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Where:
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

8.6.4.9.10 Data set example

Faecal sludge samples were collected from a ventilated improved pit (VIP) latrine and from a urine diversion toilet (UDDT) in Durban, South Africa, and were analysed using the total Kjeldahl nitrogen distillation and titration method. Each sample was analysed in four replicates and reported gravimetrically. The average TKN values (g/g dry sample) were 0.02 and 0.03 for the VIP and UDDT, respectively. The results for TKN are presented in Table 8.11 (source: unpublished data UKZN PRG).

Table 8.11 TKN values (g/g dry sample) for a VIP and a UDDT toilet analysed by the titrimetric method.

	TKN (g/g dry sample)	
Replicate	VIP	UDDT
1	0.0212	0.0298
2	0.0204	0.0275
3	0.0188	0.0289
4	0.0200	0.0292
Average	0.0201	0.0289
SD	0.0010	0.0010

8.6.5 Phosphorus

Phosphorus is important for monitoring the nutrient availability in both faecal sludge treatment and resource recovery processes. It is an essential nutrient for the growth of plants and organisms, and also a potential environmental pollutant (Tchobanoglous *et al.*, 2003). Phosphorus is present in faecal sludge as orthophosphates, polyphosphates, and organically bound phosphates (Strande *et al.,* 2014).

There are three ways of determining phosphorus in wastewater or faecal sludge: orthophosphate, acid hydrolysable phosphate, and total phosphorus, which are used to analyse orthophosphate, polyphosphates (condensed phosphate), and organic phosphate. Orthophosphate is also called reactive or available phosphorus, as it is soluble, readily interacts with other positive elements or compounds, and is bioavailable. It is the only phosphorus form that can be determined directly without preliminary hydrolysis or oxidative digestion of the sample. To measure the concentration of polyphosphates, acid hydrolysis of samples at boiling water temperature is used to convert the dissolved and particulate phosphates into dissolved orthophosphate which is then quantified. Organic phosphates can be determined by an oxidative destruction of the organic matter in the sample, which converts organic phosphates into orthophosphates, which are then quantified. Total phosphorus concentrations will be greater than the orthophosphate concentration. In this book, a spectrophotometric method for determining total phosphorus and orthophosphate concentrations is described.

8.6.5.1 Total phosphorus and orthophosphate - spectrophotometric method[32]

8.6.5.1.1 Introduction

The determination of total phosphorus and orthophosphate is based on the principle that in sulphuric acid solution, orthophosphate ions react with molybdate ions to form phosphomolybdic acid. This molybdate compound is reduced by ascorbic acid to form an intense phosphomolybdenum blue (PMB) that is then quantified with a spectrophotometer. To measure total phosphorus, other phosphorus forms are initially converted into orthophosphate before measurement.

Commercial test kits based on standard methods for measuring total phosphorus and orthophosphates are available, with pre-packaged individual aliquots of any necessary chemicals in pillows (dry chemicals) and vials (liquid chemicals). Commonly used total phosphorus and orthophosphate test kits from manufacturers such as Hach and Merck employ a variety of methods. For example, the Merck test kit uses the ascorbic acid method. The example provided here is the Merck phosphate spectrophotometric test[D]

[32] This method should be cited as: Method 4500-P E (Rice *et al*., 2017), and if test kits are used, also as per the manufacturer's directions including any modifications.

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which is used for the determination of total phosphorus and orthophosphate for samples with concentrations of 0.05-5 mg PO_4-P/L, and it is based on the manufacturer's protocol for water and wastewater using the standard method 4500-P E (Rice *et al*., 2017). Phosphate measurement results are expressed as either mg PO_4^{3-}/L or mg PO_4-P/L (or gTP/gTS or gPO_4-P/gTS with TS analysis) and it is important to note how the results are expressed by the selected test method. For faecal sludge, samples must be diluted (for total phosphorus) and filtered (for orthophosphate) to prevent false high readings associated with turbid solutions.

8.6.5.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.5.1.3 Required chemicals

- Deionised or distilled water
- Total phosphorus standard solution (4 mg/L PO_4^-P)
- Reagent P-1K (supplied by the manufacturer)
- Reagent P-2K (supplied by the manufacturer)
- Reagent P-3K (supplied by the manufacturer).

8.6.5.1.4 Required apparatus and instruments

- Spectrophotometer (*e.g.* Merck, Hatch, and Hanna)
- Digester or heating block (capable of heating to 150 °C)
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipettes and pipette tips
- Reaction cells with reagent (supplied by the manufacturer)
- Volumetric flask (1 L)
- Glass beakers (50 or 100 mL)
- Test tube rack
- Glass storage bottle
- Laboratory cleaning tissues
- Filter paper (adequate for removing solids from the sample, for example, a 0.45µm filter for liquid samples).

8.6.5.1.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with the total phosphorus standard solution with every test batch (or on a daily or weekly basis, depending on the testing load).
- Measure a blank sample for every test batch and subtract the blank values from the sample results.
- For orthophosphate analysis, samples must be pre-treated by filtration with a 0.45 µm filter to remove most of the turbidity which interferes with spectrophotometric measurement.
- Filters may contribute a significant amount of phosphate to samples with low phosphate concentration. Thus, filters should be washed with distilled water before use.
- For faecal sludge samples, the filtration step removes struvite (magnesium ammonium phosphate) that has potentially precipitated during storage due to the reactions of urine and phosphorus in the faecal sludge.
- For total phosphorus, the sample should be diluted without filtration.
- Acid-washed glassware (cleaned with HCL) should be used for determining low concentrations of orthophosphates. Avoid using detergents containing phosphate.
- Phosphate can adsorb on glass surfaces, so glassware needs to be carefully cleaned to prevent contamination.
- Common interferences in faecal sludge for this method include ammonium, nitrites, sodium, and chemical oxygen demand. For specific concentrations of concern, refer to the manufacturer's instructions.
- Suspended solids and turbid solutions interfere with the spectrophotometric measurement; therefore, faecal sludge samples should be diluted with an appropriate dilution factor based on the

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type of sludge for accurate measurements. Always use serial dilutions.

- The colour remains stable for at least 60 min following the end of the reaction time, which takes 5 min.

8.6.5.1.6 Sample preservation

- Samples must be filtered immediately after collection to prevent hydrolysis of polyphosphates. For short-term preservation of 1 to 2 days, samples should be stored at 4 °C or frozen at -20 °C. For longer storage of up to 28 days, 40 mg$HgCL_2$/L may be added. However, $HgCL_2$ is a hazardous substance and the appropriate H&S precautions must be observed.
- Samples with low phosphorus concentration must be stored in glass bottles and not plastic bottles unless they are kept frozen. Phosphates may be adsorbed onto the walls of both glassware and plastic bottles. However, washing glassware with acids prevents adsorption and thus it can be used for storage of samples. For plastic bottles, adsorption can still occur during storage under refrigerated conditions unless samples are kept frozen (Rice *et al.,* 2017).

8.6.5.1.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Turbid solutions falsely increase the spectrophotometric reading. Therefore, turbid samples should be diluted and/or filtered with a 0.45µm filter paper to prevent false high measurements.
- For orthophosphate analysis, the samples should be filtered and diluted where necessary using serial dilutions.
- For total phosphorus, the samples should not be filtered and appropriate dilutions should be used with serial dilutions.
- Samples containing more than 5 mgPO_4-P/L must be diluted with distilled water.

For slurry, semi-solid or solid samples:

- Weigh out between 1.8 g and 2.0 g of the thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Dilute with an appropriate dilution factor to achieve a concentration between 0.05 and 5.0 mg/L total phosphorus or orthophosphate and filter the samples through a 0.45µm filter paper for orthophosphate measurement.
- Total solids analysis should be performed on the samples so that the results of the total phosphorus or orthophosphate measurement can be expressed as gTP/gTS or gPO_4-P/gTS.

8.6.5.1.8 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary. For this method, calibration can be performed as follows:

- Prepare a series of at least four different concentrations of a standard solution, making sure to include the lowest and highest concentration of the kit testing range. This can be done with serial dilutions, or dilutions with a uniform interval throughout the range.
- Determine the total phosphorus concentration of the standard solutions.
- Multiply the spectrophotometer readings by the appropriate dilution factor and report results in mg TP/L.
- Prepare a calibration curve by plotting the instrument response against the concentration of the standards.

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Procedure

- Orthophosphate measurement

For measuring range of 0.05-5.0 mg/L PO_4-P:

- Pipette 5 mL of the sample into a test tube.
- Add 5 drops of reagent P-2K and mix.
- Add 1 dose of reagent P-3K and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), then pour the sample into the cell (fill it), and measure in the spectrophotometer.

- Total P measurement

For measuring range of 0.05-5.0 mg/L PO4-P:

- Pipette 5 mL of the sample into a reaction cell.
- Add 1 dose of reagent P-1K, close the cell tightly, and mix.
- Heat the cell at 120 °C in a preheated heating block for 30 min.
- Allow the closed cell to cool to room temperature in a test tube rack.
- Do not cool with cold water as this action will result in cracking of the glass, loss of the sample, and risk of acid spills.
- Shake the tightly closed cell vigorously after cooling.
- Add 5 drops of reagent P-2K, close the cell tightly, and mix.
- Add 1 dose of reagent P-3K, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), and then wipe the cell with a laboratory tissue to remove water spots and fingerprints and measure in a spectrophotometer.

8.6.5.1.9 Calculation

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

DF = Dilution factor (F/I)

F = Final diluted volume (L)

I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)$$
$$\text{Total solids content } \left(\frac{g}{g}\right)$$

Where:

A = Spectrophotometric measurement value (mg/L)

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.5.1.10 Data set example

Phosphate was measured by Septien *et al*. (2020) while evaluating the effect of drying on the physical and chemical characteristics of faecal sludge for resource recovery. Phosphate concentrations of faecal sludge from pit latrines were measured before and after drying under convective and infra-red drying rigs using a Merck Spectroquant. Different concentrations of phosphate were determined for untreated faecal sludge samples. Before drying in the convective drying rig, average phosphate in untreated faecal sludge was 2.4 ± 0.7 g PO_4^{3-}/g dry solid. The untreated faecal sludge used for infra-red drying was 13 ± 0 gPO_4^{3-}/g dry solid. Drying under the convective drying rig did not affect the phosphate concentrations because 2.3 ± 0.9, 2.5 ± 0.6 and 2.1 ± 0.7 g PO_4^{3-}/g dry solid were measured after drying at 40 °C, 60 °C and 80 °C, respectively. For infra-red drying, phosphate concentrations were slightly reduced to 11 ± 0 g PO_4^{3-}/g dry solid after drying at 80% medium infra-red for 9 min. It was concluded from the study that drying does not affect the phosphorus concentration because most phosphorus forms are bound to the solids in faecal sludge. This further suggests that dried faecal sludge pellets present an attractive nutrient composition for agricultural applications.

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Phosphate was measured with the Hach test kit for 60 samples in Englund *et al*. (2020) and phosphate and total phosphorus for 180 samples in Strande *et al*. (2018) and reported as mg/L. The range of observed PO_4 in septic tanks in Hanoi, Vietnam was 3.5 to 33 mg PO_4-P/L. The range of PO_4 was 4-367 mgPO_4-P/L and TP was 6-2,040 mg TP/L for a range of household and non-household sources in pit latrines and septic tanks in Kampala, Uganda as shown in Figure 8.9. Both complete raw data sets can be downloaded using the link below[33].

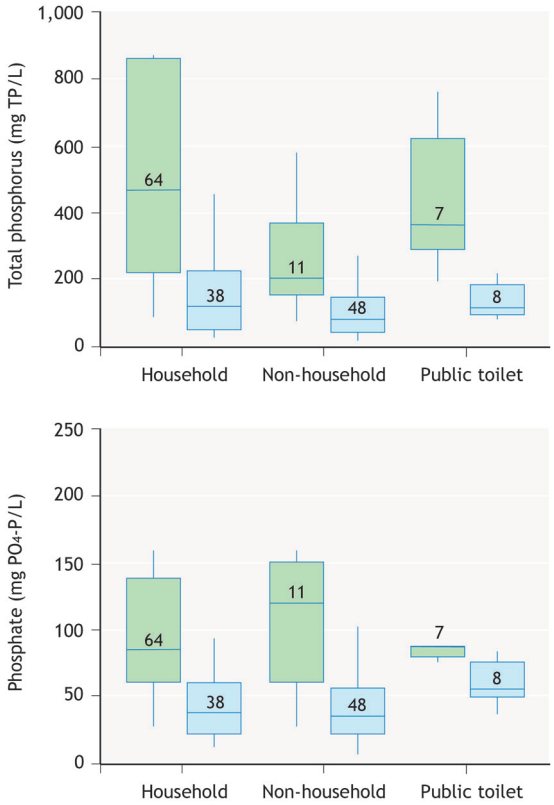

Figure 8.9 Range of total phosphorus (above) and phosphate (bellow) concentrations in faecal sludge samples from Kampala, Uganda. 180 samples consisting of pit latrines (green boxes) and septic tanks (blue boxes) (*Strande et al., 2018*).

8.6.5.2 Orthophosphate - colorimetric (test strip) method[34]

8.6.5.2.1 Introduction

Similar to nitrates, phosphorus is a major nutrient that contributes to eutrophication and it is present in aqueous solutions mainly as orthophosphate, polyphosphate or organically-bound phosphate. Concentrations of phosphorus in faecal sludge are usually 2-5 times higher than in wastewater and thus orthophosphate determination is crucial in faecal sludge treatment. The colorimetric method described here is one of many orthophosphate colorimetric test methods for samples with concentrations of 10-500 mg/L PO_4^{3-}. It is based on the Merck MQuant Phosphate Test[D] protocol for water and wastewater. In this method, orthophosphate ions (PO_4^{3-}) react with molybdate ions in the presence of a sulphuric solution to form molybdophosphoric acid, which is reduced to phosphomolybdenum blue (PMB). The phosphate concentration is measured semi-quantitatively by visual comparison of the reaction zone of the test strip with the fields of a colour scale. Phosphate measurement results are expressed as either mgPO_4^{3-}/L or mgPO_4-P/L or mgP_2O_5/L and it is important to note how the results are expressed by the selected test method. Depending on the expected phosphate concentration in the sample, kits with the appropriate measurement range should be selected. For faecal sludge, samples must be diluted and filtered to prevent masking of the resultant colour change.

8.6.5.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

[33] https://doi.org/10.25678/0000tt

[34] Test strip methods should be cited as the specific manufacturer's method along with any modifications. The example used here is the Merck MQuant Phosphate Test (Merck, 2020i)[D].

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8.6.5.2.3 Reagents, chemicals and standard solutions

The required chemicals will be specific to each manufacturer's test kit; specific information on test kits can be found on the manufacturers' websites.

- Distilled water.
- Reagent PO_4^{3-}-1.
- Standard phosphate solution Certipur® (1,000 mg/L PO_4^{3-}).

8.6.5.2.4 Apparatus and instruments

- Test strips.
- Absorbent towel.
- Glass beakers (50 or 100 mL).
- Balance with weighing boats (slurry to solid samples).
- Blender (slurry to solid samples).
- Volumetric flask (250 mL).
- Filter paper (adequate for removing solids from sample, for example 0.45 µm filter for liquid samples).

8.6.5.2.1 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Dilute the phosphate standard solution with distilled water to 100 mg/L PO_4^{3-}, and analyse according to the analytical protocol.
- Perform quality control with phosphate standard solution with every test batch (or on a daily or weekly basis depending on the testing load).
- The test strips are stable up to the date stated on the pack when stored closed at +15 to +25 °C.
- The colour of the reaction zone may continue to change after the specified reaction time has elapsed. This must not be considered in the measurement, which should always be recorded at the stated time.
- Common interferences in faecal sludge include nitrite and nitrate. For specific concentrations refer to the manufacturer's instructions.
- For faecal sludge, samples should be diluted with an appropriate dilution factor, based on the type of sludge, to prevent interference with the colour of the test strips. Always use serial dilutions.
- The orthophosphate colorimetric test method is used for qualitative to semi-quantitative measurement. For a quantitative measurement, refer to Method 8.7.3.10 (the total phosphorus and orthophosphate spectrophotometric method).

8.6.5.2.6 Sample preservation

- Samples must be filtered immediately after collection to prevent hydrolysis of polyphosphates. For short-term preservation of 1 to 2 days, samples should be stored at 4 °C or frozen at -20 °C. For longer storage up to 7 days, 40 mg$HgCL_2$ /L may be added. However, $HgCL_2$ is a hazardous substance and the appropriate H&S precautions must be observed.
- Samples with low phosphorus concentration must be stored in glass bottles and not plastic bottles unless they are kept frozen. Phosphates may be adsorbed onto the walls of the plastic bottles if used for storage under refrigerated conditions (Rice *et al*., 2017).
- Samples should be thawed to ambient temperature before analysis.

8.6.5.2.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Solid samples must be dissolved in a known volume of water before further dilution is performed.
- Filter the samples through a 0.45 µm filter paper to prevent interference in the colour.
- Samples containing more than 500 mgPO_4^{3-}/L must be diluted with distilled water.

For slurry, semi-solid or solid samples:

- Weigh out between 1.8 g and 2.0 g of the thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.

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- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper and measure the orthophosphate concentration according to the analytical protocol.
- Filter the samples through a 0.45 µm filter paper and measure the PO_4^{3-} concentration.
- Total solids analysis should be performed on the samples so that the results of the total phosphorus or orthophosphate measurement can be expressed as g PO_4^{3-}/gTS or gPO_4-P/gTS.

8.6.5.2.8 Analysis protocol

This method is valid for a measuring range of 10-500 mgPO_4^{3-}/L.

- Immerse the reaction zone of the test strip in the diluted, filtered sample (15-30 °C) for 1 sec. Allow the excess liquid to run off via the long edge of the strip onto an absorbent paper towel.
- Add 1 drop of reagent PO_4^{3-}-1 and place on the reaction zone and allow to react for 15 sec.
- Allow the excess liquid to run off via the long edge of the strip onto an absorbent paper towel and after 1 min determine with which colour field on the label matches the colour of the reaction zone most closely. Read off the corresponding result in mgPO_4^{3-}/L or mgPO_4-P/L.

8.6.5.2.9 Calculation

Liquid samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

8.6.6 pH and electrical conductivity

pH, electrical conductivity (EC), and alkalinity are measurements of solution properties in faecal sludge samples. pH is a measure of how acidic or basic an aqueous solution is. The pH scale is logarithmic, and is inversely related to the concentration of hydrogen ions in the solution. A pH of 7 is neutral, an acidic solution has a lower pH (< 7) and a high concentration of hydrogen ions, while a basic solution has a high pH (>7) and a low concentration of hydrogen ions. Biological treatment processes and conditioning for improved dewatering commonly require a pH between 6 and 9, and therefore pH is a standard design and operational parameter for faecal sludge treatment. pH values outside of this range may indicate contamination by other solid or liquid wastes, and can contribute to issues with biological processes or problems with corrosion. EC is a measure of dissolved salts, and can be used as a proxy for salinity. The concentration of dissolved salts influences the flocculation and dewatering properties of faecal sludge, and high ECs may inhibit biological treatment processes. EC is also an important parameter in resource recovery from faecal sludge as irrigation or soil conditioner, as high concentrations of dissolved salt may be harmful to crops and contribute to the accumulation of salinity in soils. Measurements of pH and EC are temperature-dependent, and thus temperature should always be recorded together with pH and EC. Alkalinity is also an important solution property of faecal sludge, and is important for biological reactions as it is a measure of the capacity of the solution to resist acidification. A method for measuring alkalinity in faecal sludge is not included in this section, but a thorough explanation of how to measure alkalinity can be found in Method 2320 of the Standard Methods for the Examination of Water and Wastewater (Rice *et al.,* 2017).

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8.6.6.1 pH – electrode method[35]

8.6.6.1.1 Introduction

This method is an electrometric measurement procedure to determine the pH of faecal sludge samples with different TS contents. This method is preferred over the colorimetric test strip method for faecal sludge due to its increased accuracy and precision, and the fact that pH readings obtained with this method are not influenced by the colour of the sample and can be made at higher TS contents than the pH test strip method. In this method, different sample preparation and measurement steps may be necessary depending on the TS of the sample. If the electrode does not produce a stable reading when inserted into a sample, the sample needs to be diluted before measuring the pH. For most types of faecal sludge (liquid, slurry, semi-solid, and some solid samples), dilution will not be necessary. However, for some solid samples or samples that have been dewatered or dried, dilution will be required.

8.6.6.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.6.1.3 Required chemicals

- Distilled water
- pH standard buffer solutions.

8.6.6.1.4 Required apparatus and instruments

- pH meter including a potentiometer
- Glass electrode and reference electrode with a temperature sensor and compensation (should be accurate to 0.1 pH unit with pH range of 0 -14)
- Beakers
- Magnetic stirrer with stir bar
- Analytical balance capable of weighing 0.1 g (if pre-dilution of the sample is required).

8.6.6.1.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- The pH meter and electrode must be calibrated with minimum two points that encompass the expected pH of the samples and are at least 3 pH units apart (USEPA 2004a). For faecal sludge samples, it is recommended to calibrate with three buffer solutions at pH 4, 7, and 10.
- Only fresh buffer solutions should be used. Changing all the solutions daily is good practice.
- If the measured pH of a sample is outside of the calibration range (*e.g.* below 4 or above 10), a calibration should be performed with an additional buffer to extend the calibration range, and the sample should be measured again.
- USEPA recommends repeat measurements on successive aliquots of sample (duplicate or triplicate measurements). Replicates should differ by no more than 0.1 pH units (USEPA 2004a).
- Typically, pH values should be reported to the nearest 0.1 pH unit (Rice *et al*., 2017).
- Samples should always be measured at room temperature, and the actual temperature should always be measured and recorded together with pH.
- If the sample temperature is more than 2 °C different from the buffer solutions, the measured pH values must be corrected. The mode of correction depends on the type of instrument, and is either done automatically or adjusted manually (see the manufacturer's instructions) (USEPA 2004a).
- The electrode should not touch the stir bar, bottom, or sides of the beaker during the pH measurement.

[35] This method is adapted from USEPA Method 9040C (USEPA 2004a), USEPA Method 9045D (USEPA 2004b), Method 4500 from the Standard Methods for the Examination of Water and Wastewater (Rice *et al.,* 2017), and the UK Method for Measuring the pH of Food Products (Vijayakumar and Adedeji, 2017). This method should be cited as described in Velkushanova *et al.* (2021).

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- Allow the electrodes sufficient time to stabilise while calibrating or measuring. A stability indicator on most meters prompts the user when readings should be taken.
- Electrodes must be rinsed thoroughly (and be fully cleaned) between measurements of different samples. Errors will occur if the electrode is coated with oily material or particulate matter. To clean electrodes, first rinse with distilled water. If coated with an oily material, the electrodes may not rinse free and should instead:
  - be cleaned with an ultrasonic bath, or,
  - be washed with detergent, rinsed several times with water, placed in 1:10 HCl so that the lower third of the electrode is submerged, and then thoroughly rinsed with water, or,
  - be cleaned as per the manufacturer's instructions.
  - It should be noted that adding distilled water to the sample may change the pH. If the pH of a sample can be measured without adding water, it should not be diluted.

8.6.6.1.6 Sample preservation

Samples should be analysed as soon as possible.

8.6.6.1.7 Sample preparation

For liquid and slurry samples:

- Homogenise the sample with a blender.
- Pour the sample into a beaker, ensuring the liquid level is high enough for complete immersion of the sensing elements of the electrode while allowing for enough space at the bottom of the beaker for the magnetic stir bar to avoid colliding with the electrode, as shown in Figure 8.10.
- Allow the sample to reach room temperature.

For semi-solid and solid samples:

- Homogenise the sample with a blender.
- If blending the sample produces a paste-like consistency, add the sample to a beaker, ensuring the level is high enough for complete immersion of the sensing elements of the electrode.
- Allow the sample to reach room temperature.
- If the sample is too dry to form a paste after blending, water addition may be necessary - follow the sample preparation steps for very dry samples.

For dry samples (e.g. very 'thick' or 'dry' solid samples, dried end products such as pellets):

- Method development to establish the appropriate dilution will be required, and dilution should be reported with the results. Presented here is an example of dilutions used by the UKZN PRG laboratory.
- If the sample is too dry to produce a paste when blended, make a 1:1 dilution with distilled water by weighing equal masses of the sample and distilled water into a beaker (common masses are 20 g sample + 20 g distilled water).
- Cover the beaker and continuously stir the suspension for 5 min.
- If the sample absorbs all of the added distilled water, begin the sample preparation again using a 2:1 dilution (*e.g.* 20 g sample + 40 g distilled water). Report the dilution with the results.
- After stirring, let the diluted suspension stand for about 15 min to allow most of the suspended solids to settle out, or filter or centrifuge to isolate the aqueous phase for pH measurement.
- If there is an oily layer floating at the top of the supernatant, decant the oily phase before measuring the pH of the liquid phase. The electrode will need to be cleaned if it becomes coated with oily material.

8.6.6.1.8 Analysis protocol

Calibration

- Pour fresh buffer solutions into separate beakers with magnetic stir bars: for typical faecal sludge samples, pH buffer solutions at pH 4, 7, and 10 are used.
- Buffer solutions should be stirred gently with the magnetic stirrer during calibration.
- Calibrate the pH meter according to the manufacturer's instructions using standard buffer solutions.

Measurement

For liquid and slurry samples:

- Rinse the electrode with distilled water. Dry the electrode with a laboratory tissue.
- Turn on the magnetic stir bar and place the electrode into the beaker containing the sample. Ensure that the sensing elements of the electrode are completely immersed, and do not allow the

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magnetic stir bar to collide with the electrode (see Figure 8.10).

- If not using a magnetic stir bar, create a stirring motion with the electrode to ensure movement of the sample across the sensing element and to homogenise the sample.
- Once the reading has stabilised, record the pH and temperature of the sample.
- Thoroughly rinse the electrode with distilled water prior to measuring the pH of the next sample.

For semi-solid and solid samples:

- Rinse the electrode with distilled water. Dry the electrode with a laboratory tissue.
- Dip the electrode into the paste-like sample, ensuring that the sensing elements of the electrode are completely immersed.
- Allow the reading to stabilise, then record the pH and temperature.
- Take two additional readings at different locations in the sample, and record. Report the average of the three readings.
- Thoroughly rinse the electrode with distilled water prior to measuring the pH of the next sample.

For dry samples requiring water addition:

- Rinse the electrode with distilled water. Dry the electrode with a laboratory tissue.
- Place the electrode into a beaker that contains the settled diluted sample. Ensure that the sensing elements of the electrode are completely immersed in the supernatant.
- Once the reading has stabilised, record the pH and temperature of the sample.
- Thoroughly rinse the electrode with distilled water prior to measuring the pH of the next sample.

8.6.6.1.9 Calculation

No calculation required - direct reading.

8.6.6.1.10 Data set example

Ward *et al*. (2021) measured the pH of 465 faecal sludge samples collected from pit latrines and septic tanks in Lusaka, Zambia. pH was measured either on the day of collection or on the following day, with samples stored at 4 °C. Samples were homogenised with a blender before measuring the pH. The median pH value for all the samples was 7.7. TS values of the samples in this study ranged from < 0.5% ds to approximately 20% DS. None of the samples required dilution prior to pH measurement. The entire raw data set is included with publication[36].

Figure 8.10 Analysis of pH for liquid faecal sludge sample. Note position of pH electrode: submerged in the well-mixed sample, but with enough clearance above the magnetic stir bar (photo: Eawag).

[36] https://doi.org/10.25678/00037X

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8.6.6.2 Electrical conductivity – electrode method[37]

8.6.6.2.1 Introduction

Electrical conductivity (EC) is a measure of the ability of an aqueous sample to conduct electric current. Samples with higher concentrations of ions have higher EC, and EC can be used as a proxy measurement for dissolved salt concentrations. EC in faecal sludge is reported in units of millisiemens per centimetre or microsiemens per centimetre. The symbol for siemens is written either as 'S' or 'mho' so EC measurements for faecal sludge may be reported as any of the following: mS/cm, mmhoS/cm, µS/cm, µmho/cm.

Different sample preparation and measurement steps may be necessary depending on the TS concentration of the sample. If the sample does not have high enough water content for a reading to be obtained, dilution will be required. For most types of faecal sludge (liquid, slurry, semi-solid, and some solid samples), dilution will not be necessary. However, for some solid samples or samples that have been dewatered or dried, dilution will be required.

8.6.6.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.6.2.3 Required chemicals

- Reagent-grade water with low conductivity compared to the samples being measured (see Section 1080 in Rice *et al*. (2017) for instructions on how to prepare reagent-grade water)
- Standard potassium chloride (KCl) solution, 0.01 M (or molarity specified by the manufacturer).

8.6.6.2.4 Required apparatus and instruments

- Conductivity meter (capable of measuring conductivity with an error no more than 1% or 1 µS/cm, whichever is greater
- Conductivity electrode with a temperature sensor
- Beakers
- Analytical balance capable of weighing 0.1 g (if pre-dilution of the sample is required)

8.6.6.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Multi-point calibrations are generally not required for conductivity meters. The conductivity cell is calibrated by determining the cell constant using a one-point calibration, commonly with 0.01 M KCl, which gives an EC reading of 1412 µS/cm at 25 °C.
- Conductivity is highly temperature-dependent. It is important to understand whether the conductivity meter compensates for temperature differences, or whether readings must be manually adjusted.
- Samples should be allowed to reach room temperature before measuring EC, and the actual temperature should always be recorded along with the EC measurements. See Method 2510B in Rice *et al*. (2017) for instructions on manual adjustment of EC readings based on temperature.

8.6.6.2.6 Sample preservation

Samples should be analysed as soon as possible.

8.6.6.2.7 Sample preparation

For liquid and slurry samples:

- Homogenise the sample with a blender.
- Pour the sample into a beaker, ensuring the liquid level is high enough for complete immersion of the sensing elements of the electrode.
- Allow the sample to reach room temperature.

[37] This method is based on Method 2510B of the Standard Methods for the Examination of Water and Wastewater (Rice *et al*., 2017), with specific adaptations to deal with the extended range of TS present in faecal sludge samples. This method should be cited as: Method 2510B (Rice *et al.,* 2017), as adapted in Velkushanova *et al.* (2021).

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For semi-solid and solid samples:

- Homogenise the sample with a blender.
- If blending the sample produces a paste-like consistency, add the sample to a beaker, ensuring the level is high enough for complete immersion of the sensing elements of the electrode.
- Allow the sample to reach room temperature.
- If the sample is too dry to form a paste after blending, water addition may be necessary - follow the sample preparation steps for very dry samples.

For dry samples (e.g. very 'thick' or 'dry' solid samples, dried end products such as pellets):

- Method development to establish the appropriate dilution will be required, and dilution should be reported with the results. Presented here is the example of dilutions used by the UKZN PRG laboratory.
- If the sample is too dry to produce a paste when blended, make a 1:1 dilution with reagent water by weighing equal masses of the sample and distilled water into a beaker (common masses are 20 g sample + 20 g distilled water).
- Cover the beaker and continuously stir the suspension for 5 min to allow the salts to solubilise.
- If the sample is hygroscopic and absorbs all of the added reagent water, begin the sample preparation again using a 2:1 dilution (*e.g.* 20 g sample + 40 g distilled water). Report the dilution with the results.
- After stirring, let the diluted suspension stand for approximately 15 min to allow most of the suspended solids to settle out, or filter or centrifuge to isolate the aqueous phase for EC measurement.
- If there is an oily layer floating at the top of the supernatant, decant the oily phase before measuring the EC of the liquid phase. The electrode will need to be cleaned if it becomes coated with an oily material.
- Note: the suggested dilutions are based on methods developed for faecal sludge at UKZN PRG, and may not be appropriate for every sample. Method development to establish the appropriate dilution will be required, and dilution should be reported with the results.

8.6.6.2.8 Analysis protocol

Calibration

- Rinse the electrode with reagent water, then rise three times with 0.01 M KCl standard solution.
- Pour fresh room-temperature 0.01 M KCl standard solution into a beaker, ensuring the liquid level is high enough for complete immersion of the sensing elements of the electrode.
- Calibrate the conductivity meter according to the manufacturer's instructions.

Measurement

For liquid and slurry samples:

- Rinse the electrode with distilled water. Dry the electrode with a laboratory tissue.
- Place the electrode into the beaker containing the sample. Ensure that the sensing elements of the electrode are completely immersed.
- Once the reading has stabilised, record the EC and temperature of the sample.
- Thoroughly rinse the electrode with distilled water prior to measuring the EC of the next sample.

For semi-solid and solid samples:

- Rinse the electrode with distilled water. Dry the electrode with a laboratory tissue.
- Dip the electrode into the paste-like sample, ensuring that the sensing elements of the electrode are completely immersed.
- Allow the reading to stabilise, then record the EC and temperature.
- Take two additional readings at different locations in the sample, and record. Report the average of the three readings.
- Thoroughly rinse the electrode with distilled water prior to measuring the EC of the next sample.

For dry samples requiring water addition:

- Rinse the electrode with distilled water. Dry the electrode with a laboratory tissue.
- Place the electrode into the beaker that contains the settled diluted sample. Ensure that the sensing elements of the electrode are completely immersed in the supernatant.
- Once the reading has stabilised, record the EC and temperature of the sample.
- Thoroughly rinse the electrode with distilled water prior to measuring the EC of the next sample.

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8.6.6.2.9 Calculations

No calculation required - direct reading.

8.6.6.2.10 Data set example

Ward *et al*. (2021) measured the EC of 465 faecal sludge samples collected from pit latrines and septic tanks in Lusaka, Zambia. EC was measured either on the day of collection or on the following day, with samples stored at 4 °C. Samples were homogenised with a blender before measuring the EC. The median EC value for pit latrine samples was 14.5 mS/cm, and the median value for septic tank samples was 1.8 mS/cm. The TS values of samples in this study ranged from < 0.5% DS to approximately 20% DS. None of the samples required dilution prior to the EC measurement. The entire raw data set is included with publication.

8.6.7 Elemental analysis

Elemental analysis is a process where a sample is analysed for chemical elements. Examples of metal analysis include macro- and micro-nutrients that are necessary for treatment performance and plant and animal growth (*e.g.* boron, chlorine, copper, iron, manganese, molybdenum, and zinc), and heavy metals for compliance in land application or incineration (*e.g.* arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, and zinc). Examples of ultimate analysis include total carbon to estimate energy content or carbon sequestration in biochar, and total carbon and nitrogen for stabilisation of compost. Methods for chlorine and chloride are also covered in this section.

8.6.7.1 Metals – overview

Although some laboratories have methods that are routinely used for analysis of metals in faecal sludge, in general, there is a need for methods to be further developed based on types of faecal sludge, different objectives for the analysis, and available laboratory capacity and analytical machines. Provided here is a general overview of the analysis of metal concentrations, some examples of methods, and references to refer to when developing methods for these specific needs. General considerations for laboratory method development are covered in Section 8.1.

Appropriate forms of sample preparation and analysis will depend on the objectives of the analysis. For example, metals in the effluent of a treatment plant prior to discharge in a receiving water body, in contrast to metal concentrations in compost prior to land application. Preliminary treatment prior to analysis of metals will typically be required. As discussed in *Section 3000 Metals* of the Standard Methods for the Examination of Water and Wastewater, metals can be operationally defined as dissolved, suspended, total, or acid-extractable (Rice *et al*., 2017). Sample preparation will depend on which of these is being analysed (*e.g.* a 0.45 µm membrane filter for suspended metals; a filtered solution for dissolved metals). Acid digestions are required for all samples other than very clear samples such as drinking water with a turbidity < 1 NTU. The USEPA 3050B method for acid digestion is most commonly used for slurry to solid faecal sludge samples, and in general sediments, sludge and soil samples including readily oxidisable organic matter. A nitric acid digestion can be adequate for relatively more 'clean' samples (*e.g.* metals loosely adsorbed on particulate matter), whereas further digestion with additional acids may be required with more difficult to oxidise samples, or if quantifying total metals (*i.e.* dissolved and particulate, organic and inorganically bound).

To evaluate the accuracy and precision of the digestion, it is important to use standards as positive controls that have similar organic characteristics and metal concentrations to evaluate the percentage recovery of total metals (*e.g.* National Institute of Standards and Technology (NIST) standards for soils and sludge, and standard biochars (UK Bioresearch Center (UKBRC)). It is also important to use consistent conditions for each sample (weights and volumes), and report the type of acid digestion along with the results. Great care needs to be taken during the sampling and laboratory preparation and analysis in order not to introduce metals into the sample.

Metals can then be quantified; Table 3010:I in Rice *et al*. (2017) summarises which methods can be used for the quantification of different metals. The table includes colorimetric and instrument methods, including atomic absorption (AA), flame photometry,

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and inductively coupled plasma mass spectrometry (ICP-MS)). The choice of analytical instrument will depend on what instruments are available, the required detection limits and range of concentrations, and the sample matrix and potential interferences.

Metals - instrument methods

Instrument methods are not covered in more detail, as they will be specific to the available laboratory equipment and the manufacturer's operating directions. The instrument used to quantify results following sample preparation and digestion will depend on availability, type of metals, required level of detection, and sample matrix. As mentioned above, the corresponding method for extraction must also be matched to the type of instrument. Table 3010:I in Rice *et al*. (2017) summarises which instrument methods can be used for the quantification of different metals. In general, the AES and MS detectors can detect lower concentration ranges than AA detectors. For example, the optimal concentration range for lead for FLAA with direct aspiration atomic absorption is 1-20 mg/L (Method 3111), whereas for the ICP-MS it is 42-4,700 µg/L (Method 3125 (Rice *et al*., 2017)). It is important to note that the given concentrations are for what is analysed with the instrument (*e.g.* following digestion), and their translation to corresponding concentrations in faecal sludge needs to be back-calculated based on the method and moisture content.

General considerations for the operation of instruments includes running calibration curves covering the entire range of the analysed concentrations, quantifying MDLs using a selected set of standards appropriate to the metals being analysed, spiked samples (*e.g.* known concentrations of standards in HNO_3), and instrument blanks. The mean concentration and standard deviation for each sample need to be calculated (a calculation of the sample variation, with replicates), and the percentage recovery of standards, spiked digestion, and spiked instrument samples need to be reported. All the blanks must be below the method detection limit.

Metals - colorimetric and spectrophotometric methods

Some elements can be quantified with more simple instrumentation, using colorimetric methods and quantification with a spectrophotometer (see the methods in Section 3500 and Table 3010:I) (Rice *et al*., 2017). Commercially available test kits are also available for many of these methods. However, the applicability for faecal sludge is limited, as the methods are not applicable with high organic content samples, which reduces the digestion capacity. Possible applications are if metal concentrations are high enough that following dilution to reduce organics prior to digestion they are still above the MDL, relatively clear liquid samples such as effluents of treatment processes, or samples that are filtered for analysis of dissolved metal concentrations. Also of concern are interference from colour and turbidity, and chemicals used in sample preparation that could complex with metals (*e.g.* EDTA, citric acid). Preliminary tests and sample digestions are always required in advance to validate the method for the specific type of sample, along with using the appropriate controls and blanks, and suspended solids have to be uniformly distributed in the sample prior to digestion. Concentration ranges for the Hach Crack Set digestion method include: iron (0.24-7.2 mg/L), lead (0.12-2.40 mg/L), nickel (0.12-7.2 mg/L), cadmium (0.02-0.3 mg/L) and zinc (0.24-7.2 mg/L). If using commercially available test kits, it is always important to follow the manufacturer's directions and to document how the method has been validated and adapted for each use.

8.6.7.2 Metals – acid digestion for environmentally available metals[38]

8.6.7.2.1 Introduction

The USEPA Method 3050B for metal analysis of sediments, sludge, and soil samples is commonly used for analysis of metals in faecal sludge. It is not considered to be a total digestion; the results are considered to be representative of metal concentrations that could become environmentally available (USEPA, 1996). The method can be coupled with instrumental methods for analysis, either flame atomic absorption spectrometry (FLAA) or

[38] This method should be cited as USEPA Method 3050B (1996).

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inductively-coupled plasma atomic emission spectrometry (ICP-AES), *OR* Graphite Furnace AA (GFAA) or inductively-coupled plasma mass spectrometry (ICP-MS), depending on the modifications in the protocol. Hence, it is important to follow each step in the method specific for the different types of instrumental analysis. For GFAA or ICP-MS the final digest is diluted to 100 mL, whereas for ICP-AES or FLAA hydrochloric acid is added for a final reflux. Typical faecal sludge sample sizes for analysis are 1-2 g wet weight or 1 g dry weight. The complete method can be downloaded using the link below[39]. This method is intended for acid-extractable, environmentally-available metals in solid, semi-solid and slurry faecal sludge samples. For more liquid samples (*e.g.* liquid and slurry, depending on characteristics), it is necessary to determine if suspended and/or dissolved metals are most relevant to quantify, and then carry out extractions on the filter and/or filtered sample (see Section 3030 in Rice *et al*., 2017).

8.6.7.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- When working with concentrated acid special protective measures need to be taken, for example working in a fume hood, and wearing an acid-safe laboratory coat, goggles, and gloves. Spills need to be neutralised prior to cleaning, per established laboratory protocols.

8.6.7.2.3 Required chemicals

The reagent blank must be less than the MDL.

- Reagent water.
- Nitric acid (concentrated HNO_3).
- Hydrochloric acid (concentrated HCl).
- Hydrogen peroxide (30% H_2O_2).

8.6.7.2.4 Required apparatus and instruments

- Digestion vessels - 250 mL.
- Vapour recovery device (*e.g.* watch glasses, solvent handling system, refluxing device).
- Drying oven.
- Thermometer - accurate measurement to at least 125 °C.
- Filter paper - Whatman No. 41 or equivalent.
- Centrifuge and centrifuge tubes.
- Analytical balance - accurate to 0.01 g.
- Heating source - able to maintain 90-95 °C (*e.g.* hot plate, block digester, microwave).
- Funnel.
- Graduated cylinder.
- Volumetric flasks - 100 mL.
- Standard laboratory glassware and utensils.

8.6.7.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Important measures include acid washing of all the glassware to prevent background contamination of metals, and special consideration to not include metal contamination during the sample preparation and analysis.
- To ensure precision and accuracy, ongoing laboratory and analytical control measures are necessary, including ensuring that reagent blanks do not have background metal concentrations greater than the MDL.
- A method blank must be taken through the entire digestion and analytical procedure to ensure no background contamination above the MDL is introduced during the process.
- Duplicate spiked samples in the sample matrix should be analysed periodically, and always when analysing a new sample matrix/type of faecal sludge.
- In addition to standard quality control measures such as calibration, standards, duplicates and blanks, it is also important to use standards as positive controls in the digestion that have similar organic characteristics and metal concentrations

[39] https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf.

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(*e.g.* NIST standards for soils and sludge). Examples of recoveries from NIST standards for hot-plate and microwave heat sources, and total digestion values are provided in tables 3, 4, and 5 in the USEPA method (1996).

8.6.7.2.6 Sample preservation

Samples should be refrigerated upon arrival at the laboratory (kept on ice during collection and transport), and analysed as soon as possible. Prior to analysis, the samples need to be air-dried. Dried samples can be stored for the longer term, if they can be stored in conditions that ensure they remain dry; if samples are damp or moist, biological degradation will continue.

8.6.7.2.7 Sample preparation

It can also be difficult to obtain representative results with wet and damp faecal sludge from the extraction process, so sub-samples are oven-dried, crushed, and ground prior to analysis to reduce sub-sample variability.

Solid, semi-solid and slurry samples (TS > 5%):

- Mix the dried sample well and take a representative sub-sample.
- Oven-dry the sub-sample at 105 °C to dryness (*i.e.* weight does not change), and then grind the sample (*e.g.* rolling pin, pestle and mortar, or coffee grinder), and then sieve to 2 mm (*e.g.* USS #10).
- If debris, rubbish, rocks, etc. are removed by sieving, this should be noted in the results.
- Weigh out the thoroughly-mixed samples of 1-2 g in the digestion vessel.

8.6.7.2.8 Analysis protocol

For a step-by-step protocol and a flow chart of modifications depending on the instrument method, refer to USEPA (1996)[40]. In general, the following steps are carried out.

- A series of reflux reactions with HNO_3 are repeated until brown fumes are no longer produced (*i.e.* samples heated without boiling for specified times, with the vapour condensed and returned to the flask).
- As an alternative to using hot plates or block digesters, the method can be modified for microwave digestion of samples for analysis with GFAA or ICP-MS.
- Samples are then warmed with aliquots of H_2O_2 until there is minimal effervescence.
- The H_2O_2 is then reduced by heating without boiling for 2 hours (or microwave modification).
- After cooling, dilute to 100 mL with water and remove particulates with filtration or centrifugation for analysis with GFAA or ICP-MS.
- For analysis with FLAA or ICP-AES, an additional reflux with HCl is required (or microwave modification) and filter with Whatman No. 41 paper (or equivalent).

Modifications to the method need to be carefully developed and documented, and will depend on the available resources. For example, the UKZN PRG laboratory has a microwave digestion system (Milestone Ethos One, Italy)[D], which allows for a specific mass of a prepared sample (between 0.1 and 1 g, depending on TS content and digestion time) to be digested with 10 mL of Aqua regia (9 mL concentrated nitric acid + 3 mL concentrated hydrochloric acid) in a closed polytetrafluoroethylene (PTFE) vessel for 105 min with the following pre-settings:

05:00 min at 1,000 w and at 90 °C
60:00 min at 1,000 w and at 130 °C
10:00 min at 1,000 w and at 40 °C
30:00 min at 1,000 w and at 30 °C.

8.6.7.2.9 Calculations

The dilution factor must be taken into consideration for the total recovery of the analyte. An example calculation for the determination of the mass fraction of cadmium (mg/kg TS, also called 'dry mass') in a slurry, semi-solid, or solid faecal sludge sample is provided here:

[40] https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf

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Slurry, semi-solid and solid (mg/kg TS):

$$\frac{5\text{ mg Cd}}{\text{L}} \times \frac{100\text{ mL extracted}}{1\text{ g dried sludge}} \times \frac{1000\text{ g}}{\text{kg}} \times \frac{\text{L}}{1000\text{ mL}} = 5\ \frac{\text{mg Cd}}{\text{kg DS}}$$

8.6.7.2.10 Data set example

Examples in the literature of reported concentrations of metals in faecal sludge include 60 samples from Hanoi, Vietnam with a range of 1.7-64 g/L TS. Samples were analysed with inductively-coupled plasma (ICP) based on Standard method 3120 Metals by plasma emission spectroscopy (Rice *et al*., 2017), and concentrations of 0.1-41 mg/L Ni, 0.1-3-3 mg/L Pb, 2-2,000 mg/L Fe, and 1-118 mg/L Zn were reported (Englund *et al*., 2020, the complete raw data set is available using the link below[41]).

Examples of reported mass fractions of metals in biochar from faecal sludge in composting toilets include 188.8 (± 3.1) mg Zn/kg DS, < 5 mg/kg DS Cd, and < 12.5 mg/kg DS for Cu, Cr, Pb and Ni. Samples were analysed with ICP-OES following a microwave digestion (ultraCLAVE 4, MLS GmbH, Germany) at 250 °C and 120 bar for ten minutes, with 0.2 g sample, 5 mL HNO_3, 1 mL $H2O_2$ and 0.3 mL hydrofluoric acid (HF) (Bleuler *et al*., 2020).

Examples of total metals in faeces and faecal sludge from pit latrines in Colorado, USA and Kampala, Uganda have been reported gravimetrically as a percentage of metal oxides in the ash (% ash). Samples were dried at 105 °C, pulverised and homogenised, then incinerated at 550 °C and the resulting ash was digested in aqua regia and hydrofluoric acid using a microwave digester prior to analysis with ICP-OES, following ASTM D6357-11 (ASTM 2011). P, Mn, Fe, Mg, Si, Al, Ca, Ti, Na, and K were analysed (Hafford *et al*., 2018).

8.6.7.3 Ultimate analysis – total carbon, hydrogen, nitrogen, oxygen, and sulphur[42]

8.6.7.3.1 Introduction

Ultimate analysis is the quantification of the major organic elemental composition of a sample or material. It includes elemental carbon, hydrogen, nitrogen and oxygen, and sometimes sulphur, halogens or ash. Samples are combusted in an ultimate analyser at a range of high temperatures in a pure oxygen environment, and then quantified by the subsequent release of gasses (*e.g.* CO_2, SO_2, NO_2). Ultimate analysis is used for compliance with standards for fuel, to routinely analyse plants, soil samples, food and feed, or to evaluate the stability of organic matter based on the carbon-to-nitrogen ratio (C:N) (*e.g.* compost). The step-by-step procedure will vary depending on the laboratory and available equipment. One example is the total carbon, nitrogen and sulphur analysis used by the UKZN PRG laboratory in Durban, South Africa, that is described here. The LECO-TruMac-CNS Series 928 analyser[D] is a carbon, nitrogen and sulphur analyser utilising a pure oxygen environment in a ceramic horizontal furnace regulated at high temperatures (1,100 to 1,450 °C). The combustion gas collection and handling system uses a helium carrier gas and a thermal conductivity cell for the detection of nitrogen.

8.6.7.3.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Check gas pressures before opening the gas cylinders. Exercise standard safety protocols for working with pressurised gas cylinders (*e.g.* keep them safely tethered at all times, and never transport without the gas cap in place).

[41] https://doi.org/10.25678/0000tt

[42] This method should be cited as the specific method that is carried out in each laboratory, including the manufacturer's make and model of analyser, and the exact method of the sample preparation. Existing standard methods for coal and coke include ISO 17247:2020 and ASTM D3176–15

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- Ensure the furnace door seals during combustion to avoid risk of fire or burns.

8.6.7.3.3 Required chemicals

Gas cylinders

- Pure oxygen (35 psi, 241 kPa).
- Helium (35 psi, 241 kPa).
- Air (40 psi, 276 kPa).

8.6.7.3.4 Required apparatus and instruments

- CNS analyser equipped with furnace, computer and auto-sampler.
- Analytical balance accurate to four decimal places.
- Flow meter and regulators.
- Nickel liners (size specific to the instrument).
- Ceramic boats or crucibles (size specific to the instrument).

8.6.7.3.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Calibrate the instruments daily using a certified acetanilide/EDTA standard containing nitrogen (6.48 ± 0.09), carbon (72.48 ± 0.25) and sulphur (7.47 ± 0.05), measured in g for solid and mL for liquid samples.
- Test replicates of 3 different masses ranging from 0.1 g to 0.5 g.
- No sample dilution is required, but it should be ensured that all solid waste is removed, and the sample must be thoroughly mixed.
- Nickel liners should be used for liquid and slurry samples with high moisture content (TS < 5%) as the ceramic boats are porous.
- Use NIST standards 'Montana soil' and 'sludge standard' and/or UKBRC standard biochars depending on the analysis, along with acetanilide and benzoic acid as internal standards, and report percentage recoveries.

8.6.7.3.6 Sample preservation

Samples should be refrigerated upon arrival at the laboratory (and kept on ice during collection and transport), and analysed as soon as possible. Some types of ultimate analysers require samples to be air-dried prior to analysis, while other analysers are able to characterise wet samples. If an analyser requires air-dried samples, it is recommended to dry samples before storage. Dried samples can be stored for the longer term, if they can be stored in conditions that ensure they remain dry; if samples are damp or moist, biological degradation will continue.

8.6.7.3.7 Sample preparation

As discussed in Chapter 3, it is difficult to obtain representative samples of faecal sludge; hence, a carefully designed plan needs to be followed to ensure representativeness. It can also be difficult to obtain representative results with wet and damp faecal sludge, so sub-samples are frequently oven-dried, crushed, and ground prior to analysis to reduce sub-sample variability. The analyser used by the UKZN PRG is able to take wet samples, but requires a nickel liner for the crucible as it is permeable.

8.6.7.3.8 Analysis protocol

Instrument setup

- Switch on the instrument and auto-sampler.
- Switch on the computer and software.
- Set the furnace temperature to 1,350 °C.
- Turn on the gasses.
- Perform an instrument check.
- Check the furnace temperature (1,350 °C)
- A system check is done through the software according to the instructions of the supplier.
- Check for leaks (oxygen and helium (1,263 Hg) independently).

Analysis parameters

- Furnace temperature = 1,350 °C.
- Cooler temperature = 5 °C.
- Dehydration time = 0 sec.
- Purge cycles = 2 sec.
- Equilibration time = 30 sec.

Blank analysis

- Condition system by analysing 3-5 blanks of empty crucibles.

Solid, semi-solid and slurry samples (TS > 5%)

- Weigh 0.1 g of sample into the crucible; enter the mass and sample name into the software.
- Place the crucible into the auto-sampler.
- Start the analysis, according to the manual supplied by the manufacturer.

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Liquid (TS < 5%)

- Place a nickel boat liner on the crucible (crucible is porous).
- Weigh 0.1–0.15 g of the liquid sample into the cover of the crucible; enter the mass and sample name into the software.
- Place the crucible into the auto-sampler.
- Start the analysis following the manual supplied by the manufacturer.

8.6.7.3.9 Calculations

There are no required calculations; the results are automatically converted by the software programme, including conversions of units (ppm, %, mg/L).

Report values as % based on dry weight basis, and the C:N as ratio as weight:weight.

8.6.7.3.1 Data set example

Example 1

Carbon, nitrogen and sulphur were analysed in duplicate on dried, pulverised samples with a HEKAtech Eurovector and a Leco TruSpec CHNS Marco Analyser[D], to evaluate the use of dried faecal sludge as a dry combustion fuel in kilns. Elevated concentrations of nitrogen, sulphur and chlorine indicate potential for dioxin, furan, NOx, N_2O, SO_2, HCl, HF and CxHy formation during combustion (Gold *et al*., 2017). Results from this study are summarised in Table 8.12.

Table 8.12 Ultimate analysis of dried faecal sludge (Gold *et al*., 2017).

	Kampala, Uganda		Dakar, Senegal	
	Average	SD	Average	SD
Carbon %	27.8	3.1	28.8	3.4
Hydrogen %	4.2	0.5	4.2	0.4
Nitrogen %	3.2	0.4	3.0	0.6
Sulphur %	0.7	0.1	1.7	0.0

Example 2

Three samples were collected – two of them were faecal sludge samples from ventilated improved pit latrines and urine diversion toilets in Durban, South Africa, the third sample was fresh faeces also from donors in Durban. These samples were analysed in six replicates each, using Method 8.6.7.3. The values were similar to those obtained by Gold *et al*., (2017). The average CNS content for the VIP was: 21.05% for carbon, 2.06% for nitrogen and 0.65% for sulphur (Table 8.13). For the UDDT it was: 5.25% for carbon, 0.46% for nitrogen and 0.15% for sulphur (Table 8.14), and for the fresh faeces: 18.47% for carbon, 5.25% for nitrogen and 3.97% for sulphur (Table 8.15) (source: unpublished data UKZN PRG).

Table 8.13 Example of CNS values for VIP faecal sludge (UKZN PRG).

Samples	Mass	Carbon (%)	Nitrogen (%)	Sulphur (%)
1	0.2095	19.180	1.9100	0.6120
2	0.2009	19.513	1.9629	0.5990
3	0.2025	20.878	1.9743	0.6600
4	0.2056	24.753	2.3859	0.7770
5	0.2018	21.093	2.0852	0.6510
6	0.2020	20.907	2.0305	0.6270
Average	0.2037	21.0540	2.0581	0.6543
SD	0.0032	1.9811	0.1714	0.0643

Table 8.14 Example of CNS values for UDDT faecal sludge (UKZN PRG).

Samples	Mass	Carbon (%)	Nitrogen (%)	Sulphur (%)
1	0.2051	4.0186	0.3549	0.1320
2	0.2071	4.8903	0.4033	0.1360
3	0.2077	5.0185	0.5597	0.1370
4	0.2012	5.0963	0.4172	0.1440
5	0.2076	5.3617	0.3953	0.1540
6	0.2093	7.095	0.6611	0.2150
Average	0.2063	5.2467	0.4653	0.1530
SD	0.0028	1.0138	0.1187	0.0313

Table 8.15 Example of CNS values for fresh faeces (UKZN PRG).

Samples	Mass	Carbon (%)	Nitrogen (%)	Sulphur (%)
1	0.1075	16.3180	2.3198	7.6700
2	0.1135	18.0580	2.5307	5.5500
3	0.1085	18.0510	2.4342	8.9100
4	0.1036	19.3000	2.2742	0.1320
5	0.1137	19.1050	2.8293	1.5200
6	0.1085	20.0080	2.5805	0.0656
Average	0.1092	18.4733	2.4947	3.9746
SD	0.00351	1,1856	0.1841	3.5722

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8.6.7.4 Chlorine – colorimetric (test strip) method[43]

8.6.7.4.1 Introduction

Chlorine is used as a disinfectant to reduce microbial load in drinking water and wastewater. Excessive chlorine in wastewater effluents cam form carcinogenic chloro-organics that affect aquatic organisms. In faecal sludge treatment, chlorine measurement is essential as it influences electrical conductivity in the effluent. In this method, chlorine oxidizes an organic compound to a violet dye. The chlorine concentration is measured semi-quantitatively by visual comparison of the reaction zone of the test strip with the fields of a colour scale. The example provided here is the is the Merck MQuant Chlorine - Test[D], one of many chlorine colorimetric test methods for samples with concentrations of 25-500 $mgCl_2/L$. For faecal sludge, samples must be filtered and/or diluted to prevent masking of the resultant colour change by the faecal sludge sample.

8.6.7.4.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.7.4.3 Required chemicals

- Distilled water
- Dichloroisocyanuric acid sodium salt dehydrate (for the quality control procedure)

8.6.7.4.4 Apparatus and instruments

- Test strips
- Glass beakers (50 or 100 mL)
- Balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Volumetric flask (250 mL)
- Filter paper (adequate for removing solids from the sample, for example 0.45µm filter for the liquid samples)

8.6.7.4.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- To make a standard solution, dissolve 1.85 g of dichloroisocyanuric acid sodium salt dihydrate in distilled water, make up to 1,000 mL with distilled water, and mix. This corresponds to approximately 1,000 mg/L free chlorine.
- Perform quality control on the standard on a daily or weekly basis (depending on the testing load).
- The test strips are stable up to the date stated on the pack when stored closed at +2 to +8 °C.
- The colour of the reaction zone can continue to change after the specified reaction time has elapsed. This gives an incorrect measurement.
- Common interferences in faecal sludge include sodium and nitrites (for specific concentrations refer to the manufacturer's instructions).
- For faecal sludge, filter and/or dilute the samples based on the type of sludge to prevent interference in colour with the test strips. Always use serial dilutions.
- The chlorine test strip method is used for qualitative to semi-quantitative measurement. For a quantitative measurement, see Method 8.6.7.5.
- The colorimetric test kit must be selected based on the expected range of chlorine concentration in the sample.

8.6.7.4.6 Sample preservation

- Samples for chlorine analysis must not be stored. Samples must be analysed immediately after sampling without exposure to excessive light and agitation. In aqueous solutions, chlorine is unstable and its concentration decreases with storage, agitation, and exposure to light (Rice *et al*., 2017).

[43] This method should be cited as the specific manufacturer's method along with any modifications. The example used here is the Merck MQuant Chlorine Test Kit (Merck, 2020l)[D]

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8.6.7.4.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Solid samples must be dissolved in a known volume of water before further dilution is performed.
- Filter the samples to prevent interference in the colour.
- Samples containing more than 500 $mgCl_2/L$ must be diluted with distilled water.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to a blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Filter samples through a 0.45µm filter paper and measure nitrite concentration.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Total solids analysis should be performed on the samples so that the results of the nitrite measurement can be expressed on a mass per mass basis (gCl_2/gTS).

8.6.7.4.8 Analysis protocol

The measuring range of this method is 25-500 $mgCl_2/L$:

- Immerse the reaction zone of the test strip in the diluted sample (15-25 °C) for 2 seconds. Shake off excess liquid from the strip after exactly 10 seconds to determine with which colour field on the label the colour of the reaction zone coincides most closely. Read off the corresponding result in $mgCl_2/L$.
- Always analyse samples together with the standard solution to ascertain the reliability of the results.

8.6.7.4.9 Calculation

Liquid samples:

Result of analysis ($mg/L\ Cl_2$)

$$\text{Final concentration}\left(\frac{mg\ Cl_2}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis}\left(\frac{g}{g}\right) = \frac{\text{Final concentration}\left(\frac{mg}{L}\right)}{\text{Total solids concentration}\left(\frac{mg}{L}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis}\left(\frac{g}{g}\right) = \frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)$$
$$\text{Total solids content}\left(\frac{g}{g}\right)$$

Where:

A = Colorimetric measurement value (mg/L)
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

8.6.7.5 Chlorine – spectrophotometric method[44]

8.6.7.5.1 Introduction

This method for quantifying chlorine in faecal sludge is based on the principle that in a weakly acidic buffer solution, free chlorine reacts with dipropyl-p-phenylenediamine (DPD) in the presence of potassium iodide to form a red-violet dye. The degree of the colour that is formed in solution is then measured spectrophotometrically. Commercially available test kits for measuring chlorine based on standard methods are available with pre-packaged individual aliquots of the necessary chemicals in pillows (dry chemicals) and vials (liquid chemicals). Commonly used test kits from manufacturers such as Hach, Merck, and Hanna vary slightly in the methods they use for chlorine measurement. The example provided here is the Merck Spectroquant® Chlorine Cell Test (Merck, 2020b)[D] for samples with

[44] This method should be cited as: Method 4500 CL2-G (Rice *et al*., 2017), and if test kits are used, also as per the manufacturer's directions including any modifications.

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concentrations of 0.03-6.0 mgCl_2/L, and it is based on the manufacturer's protocol for water and wastewater using the standard method 4500 (Rice *et al*., 2017). For faecal sludge, samples must be diluted and filtered to prevent false high readings associated with turbid solutions.

8.6.7.5.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.7.5.3 Required chemicals

- Distilled water (free from chlorine)
- Bottle of reagent Cl_2-1 (supplied by the manufacturer)
- Chlorine standard solution (3.00 mg/L Cl_2) can be purchased from the manufacturer or prepared as described in Rice *et al*. (2017) using the chlorine DPD colorimetric method.
- 0.1 N sodium hydroxide
- 0.1 N sulphuric acid.

8.6.7.5.4 Required apparatus and instruments

- Spectrophotometer
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipette and pipette tips
- Sample cell (supplied by the manufacturer)
- Volumetric flask (1 L)
- Glass beakers (50 or 100 mL)
- Glass storage bottle
- pH test strips
- Blue micro spoon (supplied by the manufacturer)
- Laboratory cleaning tissues
- Filter paper (adequate for removing solids from the sample, for example a 0.45µm filter for liquid samples).

8.6.7.5.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with chlorine standard solution with every test batch (or on a daily or weekly basis depending on the testing load).
- For spectrophotometric measurements, the sample cells must be clean. Before analysis, wipe with a laboratory cleaning tissue.
- Measurement of turbid solutions yields false high readings. For faecal sludge, samples should be diluted with an appropriate dilution factor through serial dilutions and filtered for accurate measurements. Recommended dilution factors and filtration protocols will need to be developed based on the characteristics of the specific sludge.
- The pH of the measurement solution must be within the range 4.5-5.5.
- The colour of the measurement solution remains stable for up to 30 min after the end of the reaction time; thus the spectrophotometric measurement should be conducted within that timeframe.
- Common interferences in chlorine measurement include bromine and iodine. For specific concentrations, refer to the manufacturer's instructions.

8.6.7.5.6 Sample preservation

- Samples for chlorine analysis must not be stored. Samples must be analysed immediately after sampling without exposure to excessive light and agitation. In aqueous solutions, chlorine is unstable and its concentration decreases with storage, agitation and exposure to light (Rice *et al.,* 2017).

8.6.7.5.7 Sample preparation

For liquid samples:

- Samples containing more than 6 mgCl_2/L must be diluted with distilled water to within the range of 0.03-6.0 mgCl_2/L
- Turbid solutions falsely increase spectrophotometric readings. Therefore, turbid samples should be filtered with a 0.45 µm filter paper to prevent false high measurements.

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For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of the thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper.
- The sample pH must be within the range of 4-8; if necessary, adjust with sulphuric acid or sodium hydroxide solution.
- Total solids analysis should be performed on the samples so that the results of the chlorine measurement can be expressed as gCl_2/gTS.

8.6.7.5.1 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary. For this method, calibration can be performed as follows:

- Prepare a series of at least four different concentrations of a standard solution making sure to include the lowest and highest concentration of the kit testing range. It is typical to perform serial dilutions or dilutions with a uniform interval including the lowest and highest concentrations.
- Determine the chlorine concentration of the standard solutions.
- Multiply the spectrophotometric reading by the dilution factor and report the results in $mgCl_2/L$.
- Prepare a calibration curve by plotting the instrument response against the standard concentration.

Procedure

For measuring range of 0.03-6 mg/L free chlorine

- Pipette 5 mL of the sample into a sample cell.
- Add 1 level blue micro spoon of reagent Cl_2-1.
- Shake vigorously until the reagent is completely dissolved.
- Leave to stand for 1 min (reaction time).
- Wipe the sample cell with a laboratory tissue to clean it and then measure in a spectrophotometer.

8.6.7.5.9 Calculations

Liquid and slurry samples:

Result of analysis (mg/L)

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

8.6.7.6 Chloride – colorimetric (test strip) method[45]

8.6.7.6.1 Introduction

Chloride is classified as a pollutant because of the impact on organisms and plants in aquatic ecosystems. In faecal sludge, high levels of chloride can influence microbial cells activity and the characteristics of the

[45] This method should be cited as the specific manufacturer's method along with any modifications. The example used here is the Merck MQuant Chloride Test Kit (Merck, 2020k)[D].

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sludge such as dewatering and settling. In this method, chloride ions react with silver ions, decolorising red-brown silver chromate. The chloride concentration is measured semi-quantitatively by visual comparison of the reaction zones of the test strip with the colour rows of a colour scale provided by manufacturers. The example provided here is the MQuant Chloride -Test[D], one of many chloride colorimetric test methods for samples with concentrations of 500-3,000 mgCl^-/L. It is recommended for determination of the chloride content and interference level in conjunction with COD analysis.

8.6.7.6.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.7.6.3 Required chemicals

- Distilled water
- 0.1 M sodium hydroxide or 0.1 M nitric acid
- Chloride standard solution (typically supplied with a test strip kit)

8.6.7.6.4 Apparatus and instruments

- Test strips
- Glass beakers (50 or 100 mL)
- pH strips (0-14)
- Balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Volumetric flask (250 mL)
- Filter paper (adequate for removing solids from a sample, for example 0.45µm filter for liquid samples)

8.6.7.6.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- To make a standard solution, dilute the 1,000 mg/L Cl^- chloride standard solution with distilled water to 10-100 mg/L Cl^-.
- Perform quality control on a daily or weekly basis (depending on the testing load).
- The test strips are stable up to the date stated on the pack when stored closed at +2 to +8 °C.
- The colour of the reaction zones can continue to change after the specified reaction time has elapsed. This must not be considered in the measurement. The colour after the specified reaction time is the correct reading.
- Common interferences in faecal sludge include calcium, potassium, magnesium, sodium, ammonium, nitrates and nitrites.
- For faecal sludge, samples should be diluted with an appropriate dilution factor, based on the type of sludge, to prevent interference with the colour of the test strips. Always use serial dilutions.
- The chloride test strip method is used for qualitative to semi-quantitative measurement. For a quantitative measurement, refer to Method 8.6.7.7.
- The colorimetric test kit must be selected based on the expected range of chlorine concentration in the sample.

8.6.7.6.6 Sample preservation

- Samples should be analysed as soon as possible after sampling. For analysis within 24 hours of collection, sample must be refrigerated at 4 °C. Samples should be analysed immediately to prevent the reduction of residual chlorine to chloride, depending on the redox potential.

8.6.7.6.7 Sample preparation

For liquid, slurry, semi-solid or solid samples:

- Solid samples must be dissolved in water before further dilution is performed.
- Samples containing more than 3,000 mg/L Cl must be diluted with distilled water. The pH must be within the range 5-8. Adjust, if necessary, with 0.1 N sodium hydroxide solution or nitric acid.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of the thoroughly-mixed faecal sludge sample into a beaker.

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- Dilute the sample gravimetrically and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper.
- The sample pH must be within the range of 4-8; if necessary, adjust with sulphuric acid or sodium hydroxide solution.
- Total solids analysis should be performed on the samples so that the results of the chlorine measurement can be expressed as gCl^-/gTS.

8.6.7.6.8 Analysis protocol

For measuring range of 500-3,000 $mgCl^-/L$

- Immerse all the reaction zones of the test strip in the sample (15-25 °C) for 1 second. Shake off the excess liquid from the strip and after 1 min determine with which colour row on the label the colours of the reaction zones coincide most closely. Read off the corresponding result in $mgCl^-/L$.

8.6.7.6.9 Calculation

Result of analysis ($mgCl^-/L$) = measurement value × dilution factor

Liquid samples:

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
DF = Dilution factor (F/I)
F = Final diluted volume (L)
I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Colorimetric measurement value (mg/L)
V = Volume of dilution (L)
M = Wet weight of sludge used in sample preparation (g)

8.6.7.7 Chloride – spectrophotometric method[46]

8.6.7.7.1 Introduction

This method for quantifying chloride in faecal sludge is based on the principle that chloride ions react with mercury (II) thiocyanate to form slightly dissociated mercury (II) chloride. The thiocyanate released reacts with iron (III) ions to form red iron (III) thiocyanate which is quantified spectrophotometrically. Commercially available test kits for measuring chloride based on the standard methods are available with pre-packaged individual aliquots of the necessary chemicals in pillows (dry chemicals) and vials (liquid chemicals). Commonly used test kits from manufacturers such as Hach, Merck, and Hanna vary slightly in the methods they use for chloride measurement. The example provided here is the Merck Spectroquant® Chloride Cell Test (Merck, 2020c)[D] for samples with concentrations of 0.5-15.0 $mgCl^-/L$, and it is based on the manufacturer's protocol for water and wastewater using the standard method 4500 (Rice *et al*., 2017). For faecal sludge, samples must be diluted and filtered to prevent false high readings associated with turbid solutions.

8.6.7.7.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.

[46] This method should be cited as: Method 4500 CL-E (Rice *et al*., 2017) and, if test kits are used, also as per the manufacturer's directions including any modifications.

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- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.7.7.3 Required chemicals

- Distilled water (free from chloride)
- Bottle of reagent Cl-1K (supplied by the manufacturer)
- Chloride standard solution (2.50 mg/L Cl) can be purchased from the manufacturer or prepared as described in Rice *et al.* (2017) using the chloride automated ferricyanide method.

8.6.7.7.4 Required apparatus and instruments

- Spectrophotometer
- Analytical balance with weighing boats (slurry to solid samples)
- Blender (slurry to solid samples)
- Pipette and pipette tips
- Sample cell (supplied by the manufacturer)
- Volumetric flask (1 L)
- Glass beakers (50 or 100 mL)
- Glass storage bottle
- Laboratory cleaning tissues
- Filter paper (adequate for removing solids from sample, for example a 0.45 µm filter for liquid samples).

8.6.7.7.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Perform quality control with chloride standard solution with every test batch (or on a daily or weekly basis depending on the testing load).
- For spectrophotometric measurements, the sample cells must be clean. Before analysis, wipe with a laboratory cleaning tissue.
- Measurement of turbid solutions yields false high readings. For faecal sludge, samples should be diluted with an appropriate dilution factor through serial dilutions and filtered based on the type of sludge for accurate measurements.
- The colour of the measurement solution remains stable for up to 60 min after the end of the reaction time; thus the spectrophotometric measurement should be conducted within that timeframe.
- Common interferences in chloride measurement include calcium, potassium, magnesium, sodium, ammonium, nitrates and nitrites. For specific concentrations refer to the manufacturer's instructions.

8.6.7.7.6 Sample preservation

- Samples for chloride analysis must not be stored. Chloride samples must be analysed immediately to prevent reduction of residual chloride to chloride depending on the redox potential (Rice *et al.,* 2017).

8.6.7.7.7 Sample preparation

Liquid samples:

- Samples containing more than 15 mgCl/L must be diluted with distilled water to within the range of 0.5-15.0 mgCl^-/L.
- Turbid solutions falsely increase the spectrophotometric reading. Therefore, turbid samples should be filtered with a 0.45 µm filter paper to prevent false high measurements.

Slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of the thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Quantitatively transfer the contents of the blender into a 1 L volumetric flask, rinse the blender twice with 250 mL distilled water and dilute to the mark with distilled water.
- Transfer this 1 L solution into a plastic bottle and store at 4 °C until ready for testing.
- Filter the samples through a 0.45 µm filter paper.
- Total solids analysis should be performed on the samples so that the results of the chloride measurement can be expressed as mgCl/gTS.

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8.6.7.7.8 Analysis protocol

Calibration

Follow the spectrophotometer manufacturer's instructions for calibration. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary. For this method, calibration can be performed as follows:

- Prepare a series of at least four different concentrations of a standard solution making sure to include the lowest and highest concentration of the kit testing range. It is typical to do serial dilutions or dilutions with a uniform interval including the lowest and highest concentrations.
- Determine the chloride concentration of the standard solutions.
- Multiply the answer by the dilution factor and report the results in mgCl/L.
- Prepare a calibration curve by plotting the instrument response against the standard concentration.

Procedure

For measuring range of 0.5-15.0 mg/L chloride

- Pipette 10 mL of the sample into a reaction cell.
- Pipette 0.25 mL of reagent Cl-1K and add to the sample in the reaction cell and mix.
- Leave to stand for 10 min (reaction time).
- Wipe the sample cell with a laboratory tissue to clean it and then measure in a spectrophotometer.

8.6.7.7.9 Calculations

Result of analysis (mg/L)

Liquid and slurry samples:

$$\text{Final concentration } \left(\frac{mg}{L}\right) = A\left(\frac{mg}{L}\right) \times DF$$

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{\text{Final concentration } \left(\frac{mg}{L}\right)}{\text{Total solids concentration } \left(\frac{mg}{L}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

DF = Dilution factor (F/I)

F = Final diluted volume (L)

I = Initial sample volume (L)

Slurry, semi-solid and solid samples:

$$\text{Dry basis } \left(\frac{g}{g}\right) = \frac{A\left(\frac{mg}{L}\right)}{1{,}000} \times \frac{V}{M}\left(\frac{L}{g}\right)}{\text{Total solids content } \left(\frac{g}{g}\right)}$$

Where:

A = Spectrophotometric measurement value (mg/L)

V = Volume of dilution (L)

M = Wet weight of sludge used in sample preparation (g)

8.6.8 Colour and turbidity

Colour and turbidity in faecal sludge are related to concentrations of suspended particles, dissolved organic matter, and inorganic compounds. Colour imparted by dissolved compounds is referred to as 'true colour', whereas the 'apparent colour' of a sample refers to the colour contributed from soluble and suspended material. Turbidity is the cloudiness or haziness of a fluid resulting from suspended and colloidal material. Colour and turbidity can be used as indicators of concentration of particulate matter in faecal sludge, and colour can further indicate concentrations of soluble material. Colour and turbidity can be used to characterise both untreated faecal sludge and effluent from a treatment process. Colour can also be used as an indicator for the level of stabilisation.

Colour can be measured by visual comparison with standards, which works for both apparent and true colour. Other standard methods for colour analysis are spectrophotometric, and are only applicable for filtered samples (2120C, D, E, F in Rice *et al.,* 2017). A method using digital image analysis of colour-corrected photographs for monitoring colour and texture of faecal sludge is also in development by Eawag (Ward *et al.,* 2021). The preferred method for measuring turbidity in faecal sludge, water, and wastewater samples is the nephelometric method, due to its precision and sensitivity over a wide range of values of turbidity.

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8.6.8.1 Colour – visual comparison method[47]

8.6.8.1.1 Introduction

In a visual comparison method, the colour of a sample is determined by comparison to a set of colour standards. The standards are typically either a set of calibrated concentrations of coloured solutions or coloured glass discs. A comparison between the colour of a sample and the colour standards are made under identical conditions of illumination. This method can be used to measure true colour and apparent colour.

Standard methods exist for quantifying the colour of water and wastewater samples, which are typically much more dilute and lower in particulate matter than faecal sludge. Effluent from faecal sludge treatment processes will likely be relatively uncomplicated to characterise using existing methods for water and wastewater; however, untreated faecal sludge may be outside the range of the platinum-cobalt standard colour scale (*e.g.* untreated faecal sludge can be green or light grey as well as shades of brown, and higher TS samples may be heterogeneous in colour). Existing standard methods may not be applicable for semi-solid or solid sludges, as they were designed for liquid samples. Another method under development by Eawag is the digital image analysis of colour-corrected photographs of 10 mL aliquots of faecal sludge (Ward *et al*., 2021). The method presented in Ward *et al*. (2021) does not rely on the platinum-cobalt colour scale, but instead uses a colour checker chart to standardise colours for image analysis, as shown in Figure 8.11. This method enables the characterisation of faecal sludge with any TS, and is able to accommodate heterogeneous colours along with texture analysis.

Step-by-step procedures for colour characterisation will vary depending on the laboratory, the available equipment, and the characteristics of the incoming faecal sludge samples. One example is the visual colour comparison method used by the UKZN PRG laboratory in Durban, South Africa that is described here, which is based on the Hach colour test field method (Hach, 2016)[D]. In this method, the colour is determined by visual comparison of the sample with calibrated glass discs, which represent the colours of specific concentrations of platinum-cobalt standard solutions. The results are reported in PtCo units.

8.6.8.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.8.1.3 Required chemicals

- Deionised water.

8.6.8.1.4 Required apparatus and instruments

- Colour disc, 0-100 colour units, platinum-cobalt scale
- Colour comparator box
- Long path adapter
- Glass viewing tubes, 18 mm.

8.6.8.1.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- The colour value of water can be pH-dependent. Some laboratories measure the colour value at the natural pH of the sample, while some laboratories adjust samples to neutral pH before measuring colour. In cases where pH is adjusted before colour measurement, pH adjustment and the original sample pH should be reported along with the colour value. In every case, the pH of the

[47] This method should be cited as the specific method that is carried out in each laboratory, including the type of standards, the make and model of equipment, and the exact method of sample preparation. Existing standard methods for the measurement of colour in liquid samples can be found in the methods 2120B-2120E from the Standard Methods for the Examination of Water and Wastewater (Rice *et al.,* 2017).

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sample during colour measurement should be reported with the colour value.

- When measuring true colour, pre-treatment has to be carried out to remove turbidity. When reporting the true colour value, specify the details of the pre-treatment method to ensure comparability with other results.
- In practice, an apparent colour test will be applied prior to filtration/centrifugation and a true colour test will be applied after filtration/centrifugation. In this way 'apparent colour' tests measure all the colour in a sample, irrespective of how it is caused. Even slight turbidity causes the measured colour to be noticeably higher (or different) to the same sample without turbidity.
- Samples are typically contained in some type of vessel and the perception of colour may be influenced by the background colour of the containing surface. The perception of colour against a white or light background can be quite different from a black or dark background. These issues should be minimised by always characterising colour with a consistent background (*e.g.* using a colour comparator box) and consistent lighting conditions. As the light source can affect the perception of colour, information about the light source should be reported with the results. Important information to include could be time of day and season if using sunlight, and colour temperature and light intensity if using a lamp.

8.6.8.1.6 Sample preservation

It is recommended that colour is measured immediately after sample collection. If immediate measurement is not possible, the samples should be stored at 4 °C for no longer than 24 hr before analysis, and storage time and temperature noted and reported. The pH of the samples should not be altered for preservation, as this may affect the colour.

8.6.8.1.7 Sample preparation

If measuring apparent colour:

- Homogenise the sample thoroughly (see Section 8.4.2).

If measuring true colour:

- Homogenise the sample thoroughly (see Section 8.4.2).
- Filter (using a 0.45 µm filter) or centrifuge the sample to remove suspended matter.
- Note: centrifugation and filtration affect the colour, so it is important to report the sample preparation procedures along with the results.

8.6.8.1.8 Analysis protocol

- Measure and record the pH of the sample. Report the pH at which the colour was determined with the results.
- Load the colour disc and long path adapter into the colour comparator box.
- Fill a glass viewing tube to the top line with deionised water as a blank, and load the blank into the left opening of the colour comparator box.
- Fill a second glass viewing tube to the top line with the homogenised sample, and load the sample tube into the second opening in the colour comparator box.
- Hold the colour comparator box up to a light source (*e.g.* sunlight or lamp for a consistent light source). Turn the colour disc to find the colour match.
- Read and record the result displayed in platinum cobalt colour units in the scale window.

8.6.8.1.9 Calculation

No calculation required – direct reading.

8.6.8.1.10 Data set example

Meng *et al*. (2020) measured the colour intensity of effluent from anaerobic digesters by comparison with platinum-cobalt standard solutions within the range of 5-300 PtCo units, following Method 2120B from Rice *et al*. (2017). The effluent colour was influenced by the treatment technology employed. In this case, the effluent was slightly darker if a free nitrous acid pre-treatment was used before anaerobic digestion (1,667 ± 27 mg PtCo/L for pre-treated sludge, compared with 1,433 ± 27 mg PtCo/L for a control). A darker colour corresponded to higher concentrations of soluble COD in the effluent.

Ward *et al*. (2021) presented a new method for characterising the colour and texture of faecal sludge using a standard colour checker chart with subsequent colour correction assuming standard natural lighting (CIE Standard Illuminant D65) before digital image

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analysis. The average RGB (red, green, blue) colour of a faecal sludge sample in a colour-corrected image was calculated by averaging the R, G, and B values in a selected sample. RGB colours were then converted into HSV (hue, saturation, value). Examples of colour-corrected images are shown in Figure 8.11. In general, samples from pit latrines were more saturated in colour than samples from septic tanks. More saturated colour corresponded to higher COD concentrations, and colour and turbidity measurements were strongly correlated. The entire raw data set will be included with publication.

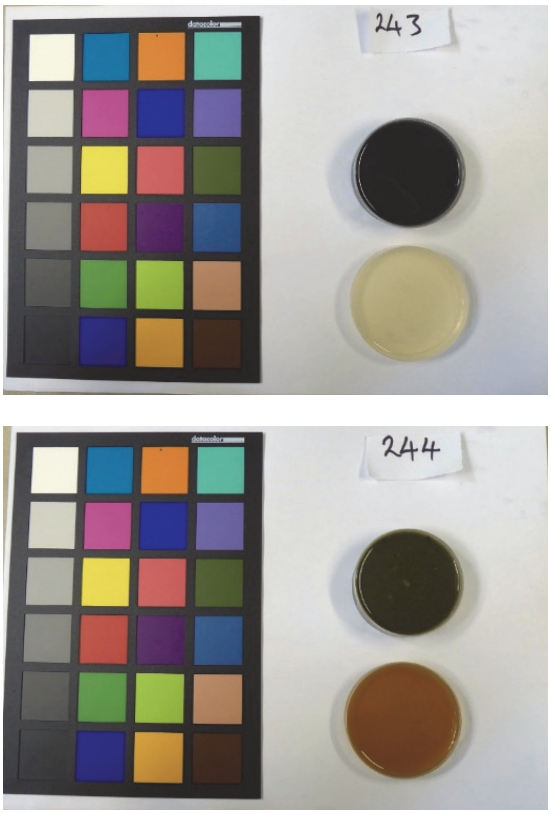

Figure 8.11 Example of photographs with standard colour checker chart used for colour characterisation (Ward *et al.*, 2021).

8.6.8.2 Turbidity – nephelometric method[48]

8.6.8.2.1 Introduction

Turbidity is used in faecal sludge management as an indicator of suspended solids concentration, for example in liquid fractions after solid-liquid separation processes (*e.g.* filtrate from drying beds, supernatant from a settling tank). Turbidity of supernatant or filtrate is sometimes used as a metric to indicate how well a treatment process is removing solids from the liquid stream. Examples of evaluation of supernatant turbidity are given in Section 2.3.4.1. Turbidity measured using the nephelometric method should be reported in nephelometric turbidity units (NTU). A consideration for measuring the turbidity of faecal sludge samples is that there are upper limits to accurate turbidity measurements. If the sample is too concentrated, turbidity readings may not be accurate. More concentrated samples with turbidity exceeding the measurement range of the instrument can be measured, but they must first be diluted. Dilution is generally not recommended, as this can affect the behaviour of the suspended solids in unanticipated ways, and introduce error to the measurement. For this reason, turbidity is mostly relevant for samples that have already undergone an initial solid-liquid separation step.

8.6.8.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- If using formazine calibration standards: exercise caution when working with hydrazine sulfate. It is carcinogenic. Do not inhale, ingest, or let it contact skin. Take care when handling formazine suspensions, as they may contain residual hydrazine sulfate.

[48] This method is based on Method 2130B from the Standard Methods for Examination of Water and Wastewater (Rice *et al.*, 2017). This method should be cited as: Method 2130 (Rice *et al.*, 2017).

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8.6.8.2.3 Required chemicals

- Chemicals for calibration standards (0)
- Low-turbidity dilution water.

8.6.8.2.4 Required apparatus and instruments

- Nephelometer
- Sample cells (clear glass or plastic)
- Lint-free tissue
- Ultrasonic bath (not necessary, but helpful for dissipation of bubbles).

8.6.8.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on operating conditions and interferences that are specific to this method includes:

- The type, concentration and number of standards required to calibrate the nephelometer is dependent on the instrument, as is the frequency of calibration. General recommendations from USEPA Method 180.1 state that determination of the linear calibration range must use at least three standards and one blank. At a minimum, a calibration check with one standard and one blank should be analysed with every batch of samples. If a calibration check is not within ± 10% of the expected value, the instrument must be recalibrated. If a portion of the calibration range is nonlinear, a sufficient number of standards must be used to define the nonlinear relationship (USEPA, 1993).
- If a sample reading is outside of the calibration curve, it should be diluted 1:1 and re-characterised.
- Air bubbles in the sample will result in incorrectly high readings. Bubbles should be dissipated before measurement.
- Allowing particles to settle to the bottom will result in incorrectly low readings. Samples should be homogenised and particles resuspended directly before measurement.
- The presence of particles that absorb light (*e.g.* activated carbon) can result in low readings.
- Smudged, dirty, or scratched sample cells will result in incorrect measurements. To avoid damage, sample cells should not be cleaned with solvents. Cells must be discarded if scratched or not clear.
- Sample cells should not be touched where the light beam will pass through (to avoid getting dirty fingerprints on the sample cell).

8.6.8.2.6 Sample preservation

It is recommended that turbidity is measured immediately after sample collection. If immediate measurement is not possible, the samples should be stored at 4 °C for no longer than 24 hr before analysis, and the storage time and temperature noted and reported. The pH of the samples should not be altered for preservation, as this may affect turbidity.

8.6.8.2.7 Sample preparation

Before a measurement is taken, allow the sample to reach room temperature. Ensure the samples are thoroughly homogenised by stirring or shaking.

8.6.8.2.8 Analysis protocol

Preparation of standards

Standards used for calibration of the nephelometer can either be made in the laboratory, or procured from the manufacturer of the nephelometer or a manufacturer of chemical standards. The manufacturer's instructions should always be consulted as to which calibration standards to use for a specific instrument. See Rice *et al*. (2017) Section 2130B for a detailed explanation of the different types of turbidity standards available. The following instructions on preparation of low-turbidity dilution water and formazine standards are from Rice *et al*. (2017), Section 2130B:

Low-turbidity dilution water (≤ 0.02 NTU)

- Filter laboratory-grade distilled water through a filter capable of removing particles larger than 0.1 µm.
- Rinse a collection flask at least twice with filtered water and discard the next 200 mL.
- Alternatively, if low-turbidity dilution water cannot be produced in the laboratory, some commercial bottled demineralised waters can be used, provided the turbidity is ≤ 0.02 NTU.

Formazine standard stock suspension (4,000 NTU)

- Add 1.00 g hydrazine sulfate, (NH2)2 H2SO4 to low-turbidity dilution water and dilute to 100 mL.

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- Add 10.00 g hexamethylenetetramine (CH2)6N4 to low-turbidity dilution water and dilute to 100 mL.
- Mix 5.0 mL of the hydrazine sulfate solution + 5.0 mL of the hexamethylenetetramine solution in a flask. Let stand for 24 hr at 25 ± 3 °C. The resulting suspension has a turbidity of 4,000 NTU.
- Store 4,000 NTU stock solution in an amber or opaque bottle. Stock suspension will remain stable for up to 1 year if properly stored.

Dilute formazine standard suspensions

- Dilute 4,000 NTU formazine standard stock suspension with low-turbidity dilution water to produce standard suspensions of various turbidities. Prepare immediately before use and discard directly after use. Typical standard suspension values could be *e.g.* 1, 10, 100, 500, 1,000, 2,000 NTU, depending on the nephelometer.

Calibration

Follow the nephelometer manufacturer's instructions for calibration. Calibration procedures vary between instruments. Certain instruments will require calibration with specific standards provided by the manufacturer, with a self-prepared formazine standard used only when no other options are available. It may be necessary to calibrate the instrument before every reading, or it may only be necessary to perform periodic calibration checks to determine when calibration is necessary (Section 8.3.4.2). Depending on the instrument, it may automatically integrate the calibration measurements, or a calibration curve may need to be manually prepared.

Procedure

- Follow the nephelometer manufacturer's instructions for measurement.
- Fill a sample cell with a homogenised, representative sample. Take care not to touch parts of the sample cell through which light will pass (*e.g.* only handle the top of the cell). Ensure that no bubbles are suspended in the sample. If possible, sonicate the cell in an ultrasonic bath for 1-2 sec to dissipate bubbles.
- Wipe the cell with a soft, lint-free cloth or laboratory tissue to remove water spots and fingerprints.
- Follow the instrument instructions to measure turbidity. Allow time for the displayed turbidity value to stabilise. Record the turbidity value (NTU) from the instrument display.

8.6.8.2.9 Calculation

No calculation – direct reading.

If the sample was diluted, multiply the reading by a dilution factor to calculate the turbidity of the undiluted sample.

Report turbidity measurements with the following precision (from Rice *et al*., 2017 and USEPA, 1993):

Turbidity reading (NTU)	Record to the nearest (NTU)
0.0-1.0	0.05
1-10	0.1
10-40	1
40-100	5
100-400	10
400-1,000	50
> 1,000	100

8.6.8.2.10 Data set example

Ward *et al*. (2021) evaluated the turbidity of supernatant following the centrifugation of faecal sludge samples from septic tanks and pit latrines in Lusaka, Zambia. A Hach 2100N nephelometer with an upper limit of 2,000 NTU was used, following AWWA standard method 2130B (Rice *et al.,* 2017). Supernatant turbidity for 179 samples of faecal sludge from septic tanks had a median turbidity of 100 NTU, an average of 180 NTU, and a standard deviation of 230 NTU. Supernatant turbidity for 46 samples of faecal sludge from pit latrines had a median of 650 NTU, an average of 850 NTU, and a standard deviation of 800 NTU. Only three supernatant samples initially measured were above 2,000 NTU. These were diluted 1:1 and remeasured, but were ultimately excluded from the dataset due to concerns about error introduced by dilution. Examples of measurement error (average values ± standard deviation) of triplicate measurements of three individual samples are: 21 ± 1 NTU, 90 ± 0 NTU, and 340 ± 10 NTU. The entire raw data set is included with publication.

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Junglen *et al*. (2020) measured the turbidity of faecal sludge samples from pit latrines in Nairobi, Kenya following AWWA standard method 2130 (Rice *et al*., 2017). This study evaluated the use of turbidity as an indicator of TSS in influent sludge. Turbidity readings were highly variable and were very high, requiring dilution before measurement. The turbidity of six pit latrine sludge samples from different containments had a median value of 13,300 NTU, an average of 14,600 NTU, and a standard deviation of 11,000 NTU.

8.6.9 Settleability and dewaterability

Settleability and dewaterability describe different aspects of the process of separating liquids and solids during faecal sludge treatment. Settleability is a description of how well sludge settles, and can be described by multiple characteristics, including how fast it settles, how compact the settled sludge is, and how many unsettled solids remain. Dewaterability is a description of how well liquid can be removed from sludge, and includes characteristics such as how quickly it can be filtered, the characteristics of the filtrate, the moisture content of the sludge cake after it has been dewatered, and how strongly water is bound to the sludge solids. More information about why and when to characterise metrics of settleability and dewaterability is included in Chapter 4.

This section includes a method for evaluating the selection and dose of conditioners for improved settling and dewatering (the jar test method), two methods for characterising dewaterability (the capillary suction time method and the water activity method), and one method for characterising settleability (the sludge volume index method).

8.6.9.1 Jar test[49]

8.6.9.1.1 Introduction

The jar test method is used to test the efficacy of a treatment process for removal of suspended solids from faecal sludge. This method can be used to identify required mixing time and intensity, and optimal conditioner and dose. Conditioners can be inorganic chemicals such as lime, ferric chloride, or aluminium sulphate, or they can be polymers. The aim of conditioners is to improve settling and dewatering performance by destabilising suspended particles in faecal sludge to form larger aggregates. Objectives of conditioning can include: supernatant turbidity reduction, compact settled sludge cake formation, reduced filter clogging and faster filtration, and lower moisture content in dewatered sludge. Jar tests enable controlled testing of different types and doses of conditioners to evaluate which yields the optimal settling or dewatering performance.

8.6.9.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.9.1.3 Required chemicals

- Conditioner standard solution (also called 'makedown' solution). Follow the manufacturer's instructions for the dilution factor of the standard solution. If no manufacturer's recommendations are available, typically 0.1-1.0% dilutions work best.

8.6.9.1.4 Required apparatus and instruments

- Jar test apparatus with gang stirrers with variable rpm settings.
- 1,000 mL beakers (one for each stirrer in jar test apparatus).
- Syringes or pipettes (10 mL).
- Analytical balance (for weighing out conditioners for standard solutions).

[49] This method is expanded from the jar test procedure outlined in Chapter 5.2 Conditioning in Faecal Sludge Management: Highlights and Exercises (Ward and Strande, 2019), and includes input from several industrial jar testing protocols for water and wastewater (SNF (2015), Microdyn Nadir (2020), and Christophersen (2000). This method should be cited as: Ward and Strande, 2019, as described in Velkushanova *et al*. (2021).

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- Blender/homogeniser, or bottle with secure cap (for mixing polymer standard solutions).
- Timer.

8.6.9.1.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Always include a blank faecal sludge sample with no added conditioner.
- Store the conditioners in accordance with the manufacturer's instructions. Polymer conditioners are often sold as concentrated polymer emulsions that are subsequently diluted to make conditioner standard solutions. Concentrated polymer emulsions may separate over time and will need to be re-mixed before making standard solutions.
- Cationic polymer emulsions are unstable in water with high levels of hardness or alkalinity. Standard solutions may need to be re-made if they are more than a few hours old.
- Before addition to the sample, ensure thorough mixing of conditioner standard solutions, especially polymer conditioners. This can be done using a blender or homogeniser, or by shaking thoroughly inside a capped bottle. Keep homogenising until all the visible droplets of polymer are gone.
- In cases when both a coagulant and a flocculant are being tested for use together, determine the correct selection and optimal dose of coagulant first. Dose the faecal sludge with the optimal dose of coagulant before evaluating flocculants, then repeat the test procedure with different flocculant doses.
- Avoid homogenising a faecal sludge sample in a way that would destroy particles (*e.g.* with a blender or homogeniser), instead mix with a spoon or stirrer, or pour back and forth between beakers to combine. Good practice is to not mix any faster than the highest rpm setting of the jar test.
- Ensure that the jar tests are performed on faecal sludge samples that are at the same temperature. Allow the samples to reach room temperature before proceeding with the jar test.
- Jar tests are only appropriate for use with liquid and slurry samples.

8.6.9.1.6 Sample preservation

It is recommended that jar tests are performed immediately after sample collection. If this is not possible then samples should be stored at 4 °C for no longer than 7 days before analysis, and storage time noted and reported.

8.6.9.1.7 Sample preparation

- Allow all the samples to reach room temperature.
- Homogenise the faecal sludge sample well by carefully stirring or pouring back and forth between beakers.
- Characterise TS (Method 8.6.1.1) or TSS (Method 8.6.1.3) of the faecal sludge sample, as these values will be needed to calculate conditioner doses.

8.6.9.1.8 Analysis protocol

- Fill each beaker with 1 L (or consistent volume) of the faecal sludge to be evaluated. Record the volume.
- Set the gang stirrers to 100 rpm.
- Add conditioner doses to each beaker, leaving one beaker unconditioned as a blank (example: from left to right, 0, 20, 40, 60 mL). If working with polymer solutions, ensure complete mixing by using a syringe or pipette submerged in the liquid sample to inject the polymer solution about halfway between the beaker wall and the stirrer.
- High-speed mixing: mix at 100 rpm for 2 min. This is a suggestion based on typical operation: the mixing speed and duration can be adjusted to mimic the conditions in a specific treatment process.
- Observe the jars during the high-speed mixing. Note which dose formed the first flocs. Note also which dose produces the clearest supernatant and/or largest floc size.
- After the high-speed mixing time has elapsed, reduce the stirring speed to 30-40 rpm (slow-speed mixing). Continue mixing for 5 to 20 min. It is recommended to adjust the mixing speed and duration in this step to better match the treatment process configuration.
- After the slow speed mixing time has elapsed, turn off the mixer. Let the conditioned samples settle for 15-20 min, or select an amount of time to

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reflect the settling time in a specific treatment process.

- After the settling time has elapsed, note the supernatant and floc appearance. Taking pictures may be helpful. If further characterisation is desired, now is the time to sample the supernatant for turbidity (Method 8.6.8.2), TSS (Method 8.6.1.3), or COD measurements (Method 8.6.2.1 or 8.6.2.2), and/or to conduct further quantification of the SVI (Method 8.6.9.4), CST (Method 8.6.9.2), or specific dewaterability of the conditioned sludge.

8.6.9.1.9 Calculation

Conditioner dose $\left(\frac{g}{kg\ TS}\right) =$

$$\frac{V_c\,(mL) \times C_c\left(\frac{g}{mL}\right)}{TS_{FS}\left(\frac{g}{L}\right) \times V_{FS}(L)} \times \frac{1{,}000\ g}{kg}$$

Where:

V_c = Volume of conditioner solution added to beaker (mL)

Cc = Concentration of conditioner solution (g/mL)

TS_{FS} = Total solids in the faecal sludge sample (g/L)

V_{FS} = Volume of the faecal sludge sample in the beaker (L)

8.6.9.1.10 Data set example

Gold *et al*. (2016) used jar tests to evaluate the performance of different conditioners at a range of doses for faecal sludge samples from Dakar, Senegal. A Velp Scientifica FC6S^D jar test apparatus was used. A high-speed mixing step at 200 rpm for 2 min was selected, with no follow-up slow-speed mixing step.

Moto *et al*. (2018) used jar tests to identify the optimal doses of chitosan and Moringa seed powder for faecal sludge from Dar es Salaam, Tanzania. A high-speed mixing step at 100 rpm for 2 hr was selected to mimic mixing conditions at a pilot scale treatment facility. More details about this study can be found in Case Study 4.1 in Chapter 4.

8.6.9.2 Capillary suction time[50]

8.6.9.2.1 Introduction

Capillary suction time (CST) is a measure of the rate of water release from sludge. This measurement is used as an indicator for the performance of many faecal sludge dewatering processes, for example dewatering time on drying beds and performance with geotextile filtration and mechanical presses. CST measurements can be used to evaluate different doses and types of conditioners, and are often used in combination with jar testing (Method 8.6.9.1). CST is measured by pouring sludge into a small reservoir placed on top of a sheet of chromatography paper. The water in the sludge is drawn into the chromatography paper via capillary action. The time it takes the water to travel a certain distance along the chromatography paper is recorded by a set of electrodes in contact with the paper. CST is reported in seconds or normalised by the sample solids content and reported as s L/gTS or s L/g TSS.

8.6.9.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.9.2.3 Required chemicals

- Distilled water.

8.6.9.2.4 Required apparatus and instruments

- CST Apparatus with 18 mm reservoir (s) (Figure 8.12). Commercially available from Triton Electronics Ltd., Essex, England.
- CST paper (supplied by the manufacturer CST, or Whatman No. 17 chromatography paper cut into 7 × 9 cm sections, with grain parallel to 9 cm side).
- Thermometer (accuracy of ±0.5 °C).
- Beaker with pourable spout.

[50] This method is based on Method 2710 G from the Standard Methods for the Examination of Water and Wastewater (Rice *et al*., 2017) with adaptations based on specific experience with faecal sludge. This method should be cited as: Method 2710 G (Rice *et al.,* 2017), as adapted in Velkushanova *et al.* (2021).

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8.6.9.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on operating conditions and interferences that are specific to this method includes:

- Measure the CST of distilled water, and record the measurement. Repeat this with every new box of filter paper. CST results are normalised by subtracting the CST of distilled water, which should account for the differences in performance due to variability in the batches of filter paper.
- Avoid homogenising the sample in a way that would destroy flocs (*e.g.* with a blender or homogenizer), instead mix with a spoon or stirrer, or pour back and forth between beakers to combine.
- Variations in sludge temperature can affect CST results. Ensure that CST tests are performed on faecal sludge samples that are at the same temperature. Allow the samples to reach room temperature before proceeding with the CST measurement.
- Sludge suspended solids concentration has a significant effect on the test results. This effect can be mitigated by homogenising well when performing replicates of the same sample. When comparing different samples, a rough correction can be made for sludges with different solid concentrations by dividing the CST value by the TS (Method 8.6.1.1) or TSS (Method 8.6.1.3) concentration of the sludge.
- CST values typically have a high variability for replicates of the same faecal sludge samples. It is suggested to perform a minimum of 5 replicates for each faecal sludge sample characterised. Up to a 10% relative standard error between replicates is considered acceptable.
- CST apparatus typically comes with reservoirs of two different diameters (10 mm and 18 mm). It is conventional to use the 18 mm diameter reservoir with faecal sludges. Reservoir diameter has a significant impact on the CST result, which is important to note when comparing results from other studies. Reservoir diameter should always be reported with experimental results.
- CST analysis is only appropriate for use with liquid and slurry samples.

8.6.9.2.6 Sample preservation

It is recommended that CST measurements are performed immediately after sample collection. If this is not possible then samples should be stored at 4 °C for no longer than 7 days before analysis, and the storage time noted and reported.

8.6.9.2.7 Sample preparation

- Allow the sample to reach room temperature.
- Homogenise the faecal sludge sample well by stirring or pouring back and forth between the beakers.
- Characterise the TS (Method 8.6.1.1) or the TSS (Method 8.6.1.3) of the faecal sludge sample, as these values will be used to normalise the effect of the solid concentration on the CST.

8.6.9.2.8 Analysis protocol

- Turn on and reset the CST apparatus. Plug the test block(s) into the CST apparatus (See Figure 8.12).
- For each test block: place new filter paper on the lower test block, with the rough side up. Add the upper test block, then insert an 18-mm funnel into the test block and seat it using light pressure and a quarter turn to prevent leaks where it meets the CST paper.
- Measure and record the temperature of the faecal sludge.
- Ensure the sludge is homogenised by stirring or pouring back and forth. Then pour a representative sludge sample into the reservoir until the liquid level reaches the top (approximately 7 mL). The CST apparatus will begin time measurement when the liquid being drawn into the CST paper reaches the inner pair of electrical contact points. Timing ends when the liquid reaches the outer contact points.
- When timer on digital display stops, record the CST value (in seconds).
- Empty the remaining sludge from the reservoir and remove and discard the used CST paper. Rinse and dry the test block and reservoir.

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8.6.9.2.9 Calculations

$$CST_{measured}(s) - CST_{Distilled\ water}(s) = CST_{adjusted}(s)$$

$$\frac{CST_{adjusted}(s)}{\text{TS of faecal sludge } (\frac{g}{L})} = CST_{normalized}(\frac{s \cdot L}{gTS})$$

$$\frac{CST_{adjusted}(s)}{\text{TSS of faecal sludge } (\frac{g}{L})} = CST_{normalized}(\frac{s \cdot L}{gTSS})$$

8.6.9.2.10 Data set example

Ward *et al*. (2021) measured the CST of 217 faecal sludge samples from septic tanks and pit latrines in Lusaka, Zambia using a Triton 319 Multi-CST apparatus with 18-mm funnels. CST values were adjusted by subtracting the CST of distilled water. As the objective of this study was to compare the time it took different samples to filter, CST was reported in seconds and not normalised by TS or TSS. Four replicates were performed for each sample, and the average relative standard error of the replicate CST measurements was 5%. The entire raw data set is included with publication[51].

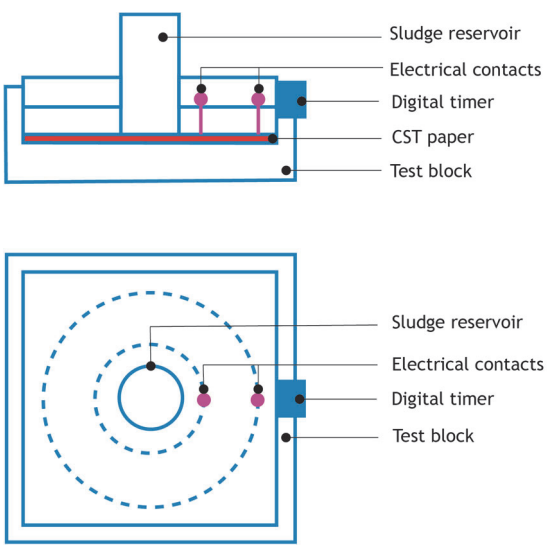

Figure 8.12 CST apparatus in side view (above) and top-down view (below).

8.6.9.3 Water activity[52]

8.6.9.3.1 Introduction

Water activity is a thermodynamic parameter that is defined as the vapour pressure of water in a sample divided by the vapour pressure of pure water in the same conditions. Water activity is an indicator of the binding strength of moisture within a faecal sludge sample. Water activity (often referred to as a_w) is unitless, and values can range from 0 to 1. A water activity of 1 would indicate that none of the water in the sample is bound to the solids, and is easily removable, whereas a water activity of 0 would mean that all of the water in the sample is strongly bound to the solids (Stringel, 2020). Water activity is also an indicator of microbial activity within the sample, as microorganisms cannot survive in environments with low levels of free water (typically no growth occurs at water activities less than 0.62, and most pathogenic organisms are inactivated at water activities less than 0.86) (Barbosa-Cánovas *et al*., 2003). Measuring water activity can provide information for the design of faecal sludge and faeces treatment technologies that incorporates drying and dewatering processes, and can provide information on the required level of dryness in order to limit microbial activity during storage of dried solids.

There are a number of methods for measuring water activity; however, only two have been reported in the literature for the characterisation of faecal sludge: 1. the static gravimetric saturated salts method; and 2. the chilled mirror dew point hygrometer method using a water activity meter. For the static gravimetric saturated salts method, sludge samples are sealed in containers with selected saturated salt solutions to produce a range of relative humidity conditions (Bourgault *et al*., 2019). Samples are stored for several weeks at a constant temperature to reach equilibrium conditions. Once the samples have equilibrated, the equilibrium moisture content is determined by measuring TS. Equilibrium moisture content values are used to calculate water activity, and can be plotted with relative humidity to produce a sorption isotherm. This method can be challenging

[51] https://doi.org/10.25678/00037X
[52] This method should be cited as adapted in Velkushanova *et al.* (2021), together with the specific analytical equipment (if different), and any manufacturer's modifications

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due to the time it takes to reach equilibrium and special precautions that need to be taken to ensure that bacterial growth does not occur. An alternative method for measuring water activity is the use of an automated water activity meter (Stringel, 2020; Getahun *et al*., 2020). In contrast to the saturated salts method, water activity can be quantified in several minutes. One of the most popular water activity meters is the AquaLab Series produced by Meter, because of its relative accuracy, precision, rapidity and ease of use. AquaLab water activity meters use the chilled mirror dew point hygrometer method to characterise water activity (Barbosa-Cánovas *et al*., 2003). This method works by sealing a sample inside an equilibration chamber and equilibrating the liquid phase water in the sample with the vapour phase water in the headspace and calculating the relative humidity in the headspace (using the dew point temperature of the air and sample temperature). When the sample is at equilibrium moisture content, the relative humidity of the headspace is equal to the water activity of the sample.

For a description of the saturated salts method for faecal sludge and faeces, see Bourgault *et al*. (2019) and Remington *et al*. (2020) (available open access). In this section, the example of a water activity meter with a dew point hygrometer method (AquaLab TDL)[D] is provided. The step-by-step procedure will vary depending on the laboratory and available equipment.

8.6.9.3.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.9.3.3 Required chemicals

- Standard salt solutions (LiCl, NaCl, KCl) with a specific molality and water activity constant (provided by manufacturer).
- Distilled water or USP purified water.

8.6.9.3.4 Required apparatus and instruments

- Water activity meter (e.g, Meter AquaLab, Rotronic AwTherm, or Novasina Lab).
- Sample cup with lid (comes with a water activity meter).

8.6.9.3.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on operating conditions and interferences that are specific to this method includes:

- Calibration with standard salt solutions of specific molality and water activity constant is necessary. Frequency of calibration is dependent on the water activity meter and the manufacturer's instructions should be followed. A general guideline is to check the readings daily with a standard (or before each use if the meter is not used daily) to ensure measurement falls within an acceptable range.
- It is recommended to use calibration standards provided by the instrument manufacturer to reduce preparation error and ensure the highest accuracy. Calibration standards should span the range of water activities to be measured.
- Temperature heavily affects measurements of water activity.
  - Ensure that the water activity meter is located in a laboratory where the temperature is relatively stable (*e.g.* away from air conditioning, heating vents or open windows).
  - Allow sufficient time for the water activity meter to warm up and reach a stabile temperature before commencing with the measurements.
  - Samples that are more than 4 °C colder or warmer than the instrument chamber temperature need to equilibrate to the instrument temperature for accurate reading. Previously refrigerated samples should be allowed to reach room temperature in a sealed container before analysis.
- Proper cleaning and maintenance of the instrument is crucial for obtaining accurate and repeatable measurements. Follow the manufacturer's instructions.
- It is possible to measure water activity for liquid, slurry, semi-solid, and solid faecal sludge samples.

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8.6.9.3.6 Sample preservation

It is recommended that water activity measurements are performed immediately after the sample collection. If this is not possible then samples should be stored at 4 °C for no longer than 7 days before analysis, and the storage time noted and reported.

8.6.9.3.7 Sample preparation

- Uniformly mix the sample using a stainless steel rod (or other appropriate tool) in order to have a thoroughly-mixed representative sample.
- Fill the sample cup about halfway full with the thoroughly-mixed sample. Ensure that the bottom of the cup is completely covered by the sample (this increases the efficiency of the instrument). Do not fill the sample cup more than halfway to prevent contamination of the sensors in the sampling chamber.
- Ensure the rim and outside walls of the sample cup are clean.
- Cover the sample cup immediately as evaporation can affect the characteristics of the sample. Analyse the sample directly after adding to the sample cup, or cover the sample cup if it must stand on the counter prior to analysis.

8.6.9.3.8 Analysis protocol

Calibration

Calibrate the water activity meter according to the manufacturer's instructions using standard salt solutions. Molalities and water activities of calibration standards used for the AquaLab TDL are displayed in Table 8.14.

Table 8.14 Water activities of calibration standards for the AquaLab TDL water activity meter.

Verification Standard at 25 °C	Water Activity
17.18 mol/kg LiCl	0.150 ±0:005
13.41 mol/kg LiCl	0.250 ±0:005
8.57 mol/kg LiCl	0.500 ±0:005
6.00 mol/kg NaCl	0.760 ±0:005
2.33 mol/kg NaCl	0.920 ±0:005
0.50 mol/kg KCl	0.984 ±0:005
USP purified water	1.000 ±0:005

Measurement

- Place the prepared sample cup in the instrument chamber.
- Carefully close the chamber to avoid spills and contamination of the chamber.
- Seal the chamber and start the reading. The instrument will take some time (several minutes) to take the measurement.
- At the end of the measurement, the instrument will display the water activity reading.
- Write down the reading.
- It is advisable to conduct at least two replicate measurements of each sample. Some extremely dry, dehydrated, highly viscous, high fat, or glassy samples may require multiple measurements before readings stabilise due to their slow water-emitting properties.

8.6.9.3.9 Calculation

No calculation is required, as the water activity meter reports the water activity directly.

8.6.9.3.10 Data set example

Practical example of the moisture content *versus* water activity for faecal sludge is presented in Figure 8.13.

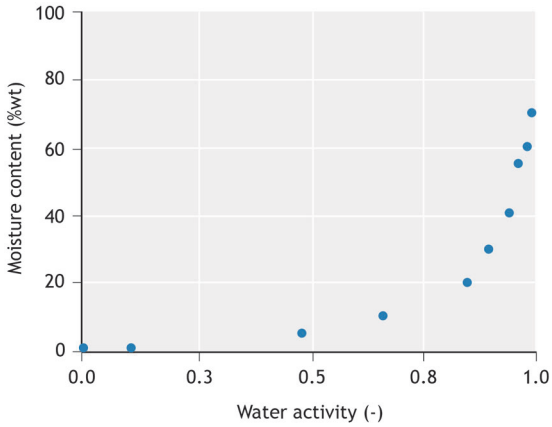

Figure 8.13 Moisture content *versus* water activity for faecal sludge from urine-diverting dry toilets dried to different final moisture contents (source: UKZN PRG).

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8.6.9.4 Sludge volume index[53]

8.6.9.4.1 Introduction

The sludge volume index (SVI) is a measurement of sludge settling performance, based on the amount of suspended solids that settle within a specified amount of time. SVI measurements can be used to monitor settling performance at faecal sludge treatment plants, to observe changes in settling that could lead to process upsets, such as sludge bulking, or to assess settling performance to aid in the design of new settling-thickening tanks. By definition, the SVI is the volume (mL) of settled sludge occupied by 1 g of sludge after 30 min of settling in a 1,000 mL graduated cylinder or an Imhoff cone. The reading is expressed in terms of mL/g.

Modifications to these methods for adaption to faecal sludge include using either a graduated cylinder or an Imhoff cone, depending on what equipment is available and the anticipated settled sludge volume, as described below. The settling time has also been modified from 30 minutes (specified in Rice *et al*., 2017) to 30-60 minutes, depending on the study objective (for example, mimicking the residence time of a specific treatment facility). This method is for liquid and slurry samples, with adaptations depending on the settleability.

8.6.9.4.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.6.9.4.3 Apparatus and instruments

- 1 L graduated Imhoff cone or 1 L Class A graduated cylinder
- Timer
- TSS and VSS analytical equipment, see sections 8.6.1.3.3 and 8.6.1.4.3.
- Standard laboratory glassware and utensils

8.6.9.4.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on operating conditions and interferences that are specific to this method includes:

- Settling behaviour is influenced by temperature. For comparable results, SVI should be measured on sludge that is at room temperature. To ensure a consistent temperature, sludge should be kept out of direct sunlight during the course of settling tests.
- Jostling the settling vessel during the settling period can affect the results. Sludge should be allowed to settle in a place where it will not get bumped or disturbed.
- Imhoff cones should be used for more dilute sludge, while graduated cylinders should be used for sludge with higher solids content. Following the recommendations from Rice *et al*. (2017), Imhoff cones should be used for samples with less than approximately 100 mL settled sludge per L. For samples more than approximately 100 mL settled sludge per L, it is recommended to use a Class A graduated cylinder. This is because Imhoff cones are typically only graduated at the bottom, so if settled sludge volumes are higher than 100 mL, graduated cylinders will offer better resolution.
- It is advised to always state whether a graduated cylinder or Imhoff cone was used in determining SVI, as results can be affected by the choice of settling vessel.

[53] This method is based on methods 2540 F, 2710C, and 2710 D from Rice *et al*. (2017), with minor changes to adapt to use with faecal sludge. The first steps of adapting this method to faecal sludge are described in Chapter 6 of Faecal Sludge Management: Systems Approach for Implementation and Operation (Dodane and Bassan, 2014), and further adaptations are presented here. This method should be cited as Method 2710 D (Rice *et al*., 2017) as adapted in Velkushanova *et al*. (2021).

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- The use of graduated cylinders with a high aspect ratio (height to diameter ratio) should be avoided. The friction created by the walls can reduce settling velocities, which can cause discrepancies in SVI results.
- If the settled sludge contains large pockets of liquid within the settled layer, the volume of trapped liquid should be estimated and subtracted from the volume of settled sludge, or the test should be repeated. If this is a recurring problem, it should be reported with the results.
- Floating material should not be included as part of the settled sludge.
- A settling time should be selected based on the objectives of the study (for example, to mimic the conditions of a specific treatment process). Typical settling times in SVI tests of faecal sludge range from 30-60 minutes. Longer settling times have also been selected in the past for studies investigating settling performance after long residence times, designed to mimic residence times at a specific treatment facility (*e.g.* 100 minutes, Dodane and Bassan, 2014). Because the duration of settling affects the volume of settled sludge, the settling time should always be reported with the SVI results. SVI results obtained using different settling times might not be comparable.

8.6.9.4.5 Sample preservation

- It is recommended that the sludge volume index is measured immediately after the sample collection; if not possible then samples should be stored at 4 °C for no longer than 7 days before analysis, and the storage time noted and reported.

8.6.9.4.6 Sample preparation

- Allow the sample to reach room temperature.
- Determine the total suspended solids concentration of a thoroughly-mixed sample (see Method 8.6.1.3).

8.6.9.4.7 Analysis protocol

- Uniformly mix the sample.
- Fill the Imhoff cone or graduated cylinder to the 1,000 mL mark.
- Allow the sludge to settle for a predetermined settling time.
- After settling time is complete, record the volume of the settled sludge and settling time.

8.6.9.4.8 Calculation

Calculate the sludge volume index (SVI) as follows:

$$\text{SVI}\left(\frac{\text{mL}}{\text{g}}\right) = \frac{\text{Settled sludge volume}\left(\frac{\text{mL}}{\text{L}}\right)}{\text{Suspended solids}\left(\frac{\text{g}}{\text{L}}\right)}$$

8.6.9.4.9 Data set example

Ward *et al*. 2019 evaluated SVI in liquid faecal sludge samples collected from 20 septic tanks in Dakar, Senegal using Imhoff cones and a 30-minute settling time. The median SVI was 32 mL/g, and mean and standard deviation were 45 and 51 mL/g, respectively. Several samples underwent no visible settling during the settling period, thus the settled sludge volume was recorded as the total volume of sample. In wastewater sludge literature, an SVI less than or equal to 100 mL/g is designated as good settling performance (Dodane and Bassan, 2014). The publication and entire raw data set are available at the link below[54].

Gold *et al*. (2016) evaluated the settling performance of liquid faecal sludge samples from Dakar, Senegal using Imhoff cones before and after the addition of conditioners. The settling time was 60 minutes. The SVI results were highly variable, so the researchers chose to report the volume of settled sludge, along with the TSS of the supernatant after settling, as metrics of the settling performance.

8.7 PHYSICAL PROPERTIES

8.7.1 Physical and mechanical properties

Physical properties are characteristics that do not change the chemical composition of a material. Examples of physical properties are density, particle size, and mechanical properties. Mechanical properties are the physical properties of a material that are measured by the application of force. These can include, for example, shear strength, viscosity,

[54] https://doi.org/10.1016/j.watres.2019.115101

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plasticity, etc. The physical and mechanical properties of faecal sludge are important for developing emptying and treatment technologies, especially when these technologies involve the application of force on the sludge; for example, in the case of pumping, compressing, or extruding faecal sludge or solid end products from its treatment (see Chapter 4). Methods included in this section include density, particle size, rheology, liquid limit, plastic limit, and texture analysis.

8.7.1.1 Density – mass and volume measurement method[55]

8.7.1.1.1 Introduction

Density is the relationship between the mass and volume of a substance. As explained in Chapter 2, density is important to convert concentrations between weight/volume and weight/weight. It is therefore recommended to measure density when possible, but especially when the faecal sludge to be analysed spans a range of sludge types. In the following method, the mass of a known volume of faecal sludge is measured and density is determined by direct calculation. This method measures wet bulk density, which is a commonly used parameter for faecal sludge, for example to convert between units. If dry bulk density and/or particle density or pore space are required, Method 8.7.1.2 should be used. This method provides sufficient accuracy for most of the applications where density for faecal sludge is needed. However, because the risk of potential (human) error is relatively high, it should not be used for measurements that require an accuracy of several decimal places. If a higher level of accuracy is required, a digital density meter can be used. A useful guide with more information on how to make accurate density measurements can be found on the Anton Paar website[56].

8.7.1.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3. to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.7.1.1.3 Required apparatus and instruments

- 5 mL measuring spoon or scoop (or other appropriate volume)
- Knife, to trim excess sludge from the measuring spoon
- Analytical balance
- Glass weighing dish
- Distilled water (for the quality control procedure).

8.7.1.1.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- The analytical balance must be calibrated regularly, following the method outlined in Method 8.6.1.1.
- Inaccuracies may arise for the following reasons:
  - If the measuring spoon is not completely filled with the sample.
  - If the sample is compressed in the process of filling the spoon.
  - If the sample is not levelled completely.

  These manual errors should be reduced as much as possible, to increase the accuracy of the measurement.
- Before every series of density measurements, do a check with distilled water. Follow the measuring procedure with distilled water, and compare the density with the density of water: $\rho_{water} = 0.998203$ g/cm³ for T = 20 °C. A common tolerance limit is 0.0001 g/cm³. If the results are not within the tolerance limit, clean the spoon thoroughly and try again.

8.7.1.1.5 Sample preparation

- Homogenise the faecal sludge sample thoroughly by stirring with a spoon or stirring rod.
- It is important to prepare the sample for density in the same way as other analysis that is being conducted, especially if the results will be used to

[55] This method should be cited as described in Velkushanova *et al.* (2021).

[56] https://wiki.anton-paar.com/en/density-and-density-measurement/

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convert between weight/weight or weight/volume concentrations (*e.g.* if a blended sample is used for TS measurement and that is the parameter of interest for the density measurement, then density should be measured on the blended sample).
- Exclude larger, inconsistent or floating particles from the sample if it is determined that their inclusion may affect the final result (*e.g.* hair, stones, glass, and maggots).

8.7.1.1.6 Analysis protocol

Liquid, slurry, semi-solid and solid samples:
- Place the measuring spoon and the glass dish on the balance, and tare the balance.
- Use the spoon to scoop a sample of faecal sludge, such that the sample completely fills the spoon. Avoid compressing the sample as much as possible.
- Wipe the bottom of the spoon with a laboratory tissue, removing any excess sample.
- Level the sample by removing any excess above the surface of the edge of the spoon with a knife, to leave a flat surface that is flush with the top of the spoon.
- Place the measuring spoon on the glass dish on the scale, and record the mass of the sample contained in the spoon.

8.7.1.1.7 Calculation

$$\text{Density}\ (\frac{\text{g}}{\text{cm}^3}) = \frac{\text{Mass (g)}}{\text{Volume (mL)}}$$

8.7.1.2 Density – volume displacement method[57]

Bulk density is a measure of mass per unit volume. It is used as a measure of wetness, volumetric water content, and porosity. Factors that influence the measurement include the organic matter content, porosity, and material structure. Particle density, or solid density, represents only the weight of dry material per unit volume of the material solids; the pore space is not included in the volume measurement. The porosity of a material is the pore space portion of the material volume occupied by air and water. Both density parameters, bulk and particle (solid), are commonly used, depending on the purpose of the measurement. For example, particle density might be more suitable for calculations on drying beds, while the bulk density will have more relevance for emptying and transportation.

8.7.1.2.1 Introduction

Wet bulk density is determined using the same techniques as presented in Method 8.7.1.1. Dry bulk density is determined by oven-drying a known volume of sample and measuring the mass of the dry sample. Particle density is determined using the volume displacement technique. Pore space is then calculated from these values.

8.7.1.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Wear gloves suitable for withstanding high temperatures when placing and removing crucibles from the oven.
- Use appropriate mechanical tools, such as metal tongs, to remove crucibles and trays after drying in the oven to avoid direct contact with hot surfaces.

8.7.1.2.3 Required apparatus and instruments

- Porcelain crucibles
- Desiccator with dry desiccant
- Drying oven
- Analytical balance with four decimal places
- 100 mL measuring cylinder
- 7.5 mL measuring scoop or 10 mL measuring cylinder (depending on sludge type)
- Tube to hold the sample that fits inside the 100 mL measuring cylinder
- Glass weighing dish
- Laboratory tissue

[57] This method follows British Standard 812-2:1995, determination of density for testing aggregates (1995), and should be cited as BS 812-2 (1995) as adapted in Velkushanova *et al.* (2021).

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- Knife, to trim excess sludge from the measuring scoop
- Heat-resistant gloves
- Thermometer (for the quality control procedure)
- Set of standard calibration weights (for the quality control procedure)
- Distilled water (for the quality control procedure).

8.7.1.2.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- The analytical balance and oven must be checked and calibrated weekly.
  - Check the temperature throughout the oven area by placing a calibrated thermometer on each shelf. After 30 min, check the temperature at each level against the oven setting. Using the same method, also check for temperature differences between the front and back of the oven. Adjust the oven setting if necessary. If temperatures are uneven on the shelves, check the insulation.
  - To calibrate the analytical balance, place a standard calibration weight on the balance and weigh. Adjust the balance manually if necessary. Do this with the whole range of weights from the calibration set. Make sure to include a standard weight of a mass similar to the mass of the expected sample + crucible.
- Make sure the desiccant in the desiccator is not saturated, otherwise samples may absorb water while cooling down in the desiccator. Routinely dry the desiccant in the oven at 105 °C (or at a different temperature, depending on the manufacturer's instructions), prior to the colour indicating that the desiccant is nearly saturated.
- Always keep the lid of the desiccator on and use a lubricant on the rim to ensure airtight sealing. Do not overload the desiccator.
- Before every series of density measurements, do a check with distilled water. Follow the measuring procedure with distilled water, and compare the density with the density of water: $\rho_{water} = 0.998203$ g/cm³ for T = 20 °C. A common tolerance limit is 0.0001 g/cm³.

8.7.1.2.5 Sample preparation

- Homogenise the faecal sludge sample thoroughly by stirring with a spoon or stirring rod.
- It is important to prepare the sample for density in the same way as other analysis that is being conducted, especially if the results will be used to convert between weight/weight or weight/volume concentrations (*e.g.* if a blended sample is used for TS measurement and that is the parameter of interest for the density measurement, then density should be measured on the blended sample).
- Exclude larger, inconsistent or floating particles from the sample if it is determined that their inclusion may affect the final result (*e.g.* hair, stones, glass, and maggots).

8.7.1.2.6 Analysis protocol

- Pre-heat the oven to 103-105 °C.
- Place a clean crucible in the oven at a temperature of 103-105 °C for 1 hr prior to use (to remove any moisture). After drying, place the crucible in the desiccator and allow it to cool down to room temperature. Keep the crucible in the desiccator until the next step.
- Weigh the crucible and record the mass (W_1).
- Place the measuring scoop and the glass dish on the balance, and tare the balance. If required, for liquid and slurry sludge types a measuring cylinder might also be used.
- Use the scoop to measure 7.5 mL of the sample, such that the sample completely fills the scoop. Avoid compressing the sample as much as possible.
- Wipe the bottom of the scoop with a laboratory tissue, removing any excess sample.
- Trim any sample from the top of the scoop with the knife, to leave a flat surface flush with the top of the scoop.
- Place the measuring scoop on the glass dish on the scale, and record the mass of the sample contained in the scoop (W_2).
- Transfer all the sample from the scoop into a dried crucible. Rinse the scoop with small volumes of distilled water to dislodge heavy particles. Make sure that all the particles are transferred to the crucible. Add the washings to the crucible.
- Oven-dry the sample at 103-105 °C for at least 24 hr.

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- Take the sample out of the oven, and place it in the desiccator to reach room temperature.
- Weigh the dry mass of the sample + crucible using an analytical balance and record the weight (W_3).
- Fill the 100 mL measuring cylinder with 50 mL water.
- Suspend an empty sample-holding tube inside the 100 mL measuring cylinder filled with 50 mL water and record the volume level of water (V_1).
- Carefully transfer all the dry sample from the crucible into the holding tube, ensuring that all the particles are transferred.
- Suspend the tube with the sample in the measuring cylinder with water and record the new level of the water (V_2).

8.7.1.2.7 Calculation

Bulk density

$$\text{Bulk density (wet)} \left(\frac{g}{mL}\right) = \frac{(W_2\text{-}W_1)\ (g)}{Vt\ (mL)}$$

Where:

W_1 = Mass of the crucible (g)
W_2 = Wet mass of sample
V_t = Total volume of sample (7.5 mL).

$$\text{Bulk density (dry)} \left(\frac{g}{mL}\right) = \frac{(W_3\text{-}W_1)\ (g)}{Vt\ (mL)}$$

Where:

W_1 = Mass of the dried crucible (g)
W_3= Dry residue + crucible after drying at 103-105 ºC (g)
V_t = Total volume of the sample, pore volume + solid volume (7.5 mL).

Particle density

Particle density values represents only the weight of dry sample per unit volume of the sample solids; the pore space is not included in the volume measurement.

$$\text{Particle density} \left(\frac{g}{mL}\right) = \frac{(W_3\text{-}W_1)\ (g)}{Vs\ (mL)}$$

Where:

W_1 = Mass of the dried crucible (g)
W_3 = Dry residue + crucible after drying at 103-105 ºC (g)
V_1 = Volume in measuring cylinder with holding tube (mL)
V_s = Volume of the solids (ONLY) = V_t-V_1 (mL).

Pore space

Pore space (g/mL) =

Bulk density (g/mL) – Particle density (g/mL)

8.7.1.3 Particle size - laser light scattering method[58]

8.7.1.3.1 Introduction

Characterising particle size distribution can help in designing treatment processes and monitoring process effectiveness. Particle size influences how much organic material is available organic material is for degradation by microorganisms, and how the particle size distribution changes over the course of stabilisation and treatment. Particle size distribution affects settling and dewatering performance, and is also an important characteristic of end products from faecal sludge treatment (*e.g.* dried sludge solid fuels or feedstock for larvae rearing).

Several standard methods for characterising the particle size of water and wastewater exist, and these are discussed in Method 2560 Particle Counting and Size Distribution in the Standard Methods for the Examination of Water and Wastewater (Rice *et al.*, 2017). These include manual sequential sieving and filtration, the use of electronic measurement devices (including electronic sensing zone instruments, light-blockage instruments, and light-scattering instruments), and direct sizing and counting using microscopy. Manual sieving and filtration are slow, labour-intensive, and has a lower level of accuracy, but does not require expensive instrumentation. Electronic measurement of particle size is typically the method of choice if instruments are accessible. However, when large aggregates of particles (> 500 µm) are to be analysed, direct microscopic methods are advised (Rice *et al.*, 2017).

[58] This method should be cited as adapted in Velkushanova *et al.* (2021), together with the specific analytical equipment (if different), and any manufacturer's modifications.

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Step-by-step procedures for measuring particle size will vary depending on the selected method, the available equipment, and the characteristics of the incoming faecal sludge samples. One example of electronic measurement of particle size is the laser light scattering method used by the UKZN PRG laboratory in Durban, South Africa that is described here. This method is specifically written to be used with a Malvern Mastersizer 3000 particle size analyser[D], and follows the Malvern Mastersizer 3000 User Manual (Malvern Instruments, 2017) and Method 2560D in Rice *et al*. (2017). The Malvern Mastersizer 3000 measures particle size by shooting a laser beam through a dispersed sample, and measuring the angle and intensity of light scattered off the particles. Mie and Frauhofer theories are used to calculate the particle sizes based on the scattering pattern. A wet dispersion unit is used with faecal sludge samples to circulate samples through the measurement cell. The size range of the Mastersizer 3000 is 0.01-3,500 µm.

8.7.1.3.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.7.1.3.3 Required chemicals

- Particle size standards (for the Mastersizer 3000, Malvern recommends the Malvern QAS3002 Quality Audit Standard).

8.7.1.3.4 Required apparatus and instruments

- Mastersizer 3000
- Mastersizer wet dispersion unit
- Beaker.

8.7.1.3.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3 .Information on standards, operating conditions, and interferences that are specific to this method include:

- Calibration is performed using standard suspensions or dry powders of spherical particles of known size (*e.g.* standards provided by the manufacturer or NIST standard particles). Rice *et al*. (2017) recommend using at least three different-sized particle standards to calibrate a particle sensor. Follow the manufacturer's instructions to set up a calibration strategy.
- Sample blanks, handled identically to the faecal sludge samples, should be analysed daily. Generally, blanks should not show more than 5% of the counts in any size channel compared to the samples. See Section 7 Quality Control in 2560A (Rice *et al.,* 2017) for a detailed discussion of quality control for particle size analysis, or refer to the manufacturer's instructions.
- Large particles, solid waste, stones, and hair should be removed before testing, as they can harm the instrument. This can be achieved by passing the sample through a sieve before analysis. Sieve size and other pre-treatment steps should be selected based on the upper measurement limit of the specific instrument and the manufacturer's instructions.
- Minimise particle contamination (*e.g.* from airborne particles, contaminated dilution water, or contaminated glassware). Keep the samples in a closed container, ensure the dilution water is particle-free and run blanks to ensure this, and ensure the glassware is thoroughly cleaned and particle-free before use. For information about producing particle-free dilution water, see 2560A in Rice *et al*. (2017).
- Faecal sludge samples may require dilution prior to analysis. It is important to avoid breaking up aggregates or flocs during the sample preparation, so dilutions should be made carefully using pipettes with wide openings. Wide openings can be made by cutting off the tips of the pipettes. The sample should be added to the dilution water (not water added to the sample) in order to reduce shear on the sample. Be careful to use slow, low-intensity mixing. Avoid mechanical stirring and ultrasonication. For more sample preparation tips, see Section 3 Sample collection and handling in 2560A, Rice *et al*. (2017).
- Minimise the time between sample collection and measurement, as particles may agglomerate over

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time, changing the particle size distribution. Dilution can also influence agglomeration – make dilutions immediately before analysis.
- If samples must be stored before analysis, refrigerate them (4 °C), but make sure that they are brought back to room temperature before analysis.

8.7.1.3.6 Sample preservation

Samples should be analysed as soon as possible after collection, to prevent changes in particle size distribution due to agglomeration. If the samples must be stored before analysis, store them in a refrigerator (4 °C) and do not dilute them before storage.

8.7.1.3.7 Sample preparation

- Remove all the particles larger than the upper limit of the instrument by sieving.
- If the sample is semi-solid or solid, dilute the sample in particle-free water and gently mix to produce a slurry. Add to a beaker.
- If the sample is liquid or slurry, dilution may not be necessary. Gently mix the sample to ensure homogeneity, then add a portion of the sample to a beaker.

8.7.1.3.8 Analysis protocol

Instrument setup

- Switch on the instrument.
- Switch on the computer and start the Mastersizer software.
- Wait 30 min for the instrument to stabilise before using the instrument.

Measurement

- Select the instrument protocol for measuring the specific sample type (*e.g.* faecal sludge from VIP latrines) and allow the instrument to initialise. A background light measurement will then be taken.
- When prompted, add the sample a small amount at a time until the obscuration is within the correct range (displayed on the computer screen). Note: if the sample is too concentrated, it will immediately exceed the obscuration range - if this happens, the sample will need to be diluted and measured again.
- Run the sample measurement protocol.
- After measurement is completed, clean the system by following the prompts on the user interface.

8.7.1.3.9 Calculation

No calculation required – direct reading is based on the overall percentage of particle volume and does not require adjustment based on dilution.

8.7.1.3.10 Data set example

Faecal sludge at UKZN PRG was analysed (unpublished data, Figure 8.14), with the results interpreted as follows:

- Weighted residual - an indication of how well the calculated data was fitted to the measurement data. A good fit is indicated by a residual of less than 1%, while a residual over 1% may indicate the use of an incorrect refractive index and adsorption values for the sample.
- Dv 50, Dv 10 and Dv 90 are standard percentile readings from the analysis.
  - Dv 50 - the particle diameter in µm at which 50% of the sample volume is smaller and 50% is larger. This value is also known as the Mass Median Diameter (MMD) or the median of the volume distribution. The v in the expression Dv 50 shows that this refers to the volume distribution. Following the same naming convention, Ds refers to the surface area distribution, Dl is the length distribution, and Dn is the number distribution.
  - Dv 10 - the particle diameter below which 10% of the sample volume lies.
  - Dv 90 - the particle diameter below which 90% of the sample volume lies.
- D [4, 3] - the volume-weighted mean or Mass Moment Mean Diameter.
- D [3, 2] - the surface-weighted mean, also known as the Surface Area Moment Mean Diameter.
- Span - is the measurement of the width of the distribution. The narrower the distribution, the smaller the span becomes.
- Concentration - the volume concentration. This is calculated using the Beer-Lambert law.
- Obscuration - an ideal range of obscuration is usually between 3 and 20%, depending on the sample and dispersion unit used.
- Distribution - shows the type of distribution the analysis has used. Options include volume, surface area, length, or number. The Mastersizer 3000 measurement is fundamentally a

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measurement of volume distribution; the transformation of the results into a surface area, length, or number distribution may amplify any error in the original result, especially at the fine end of the size distribution.

Analysis

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Malvern

Measurement Details

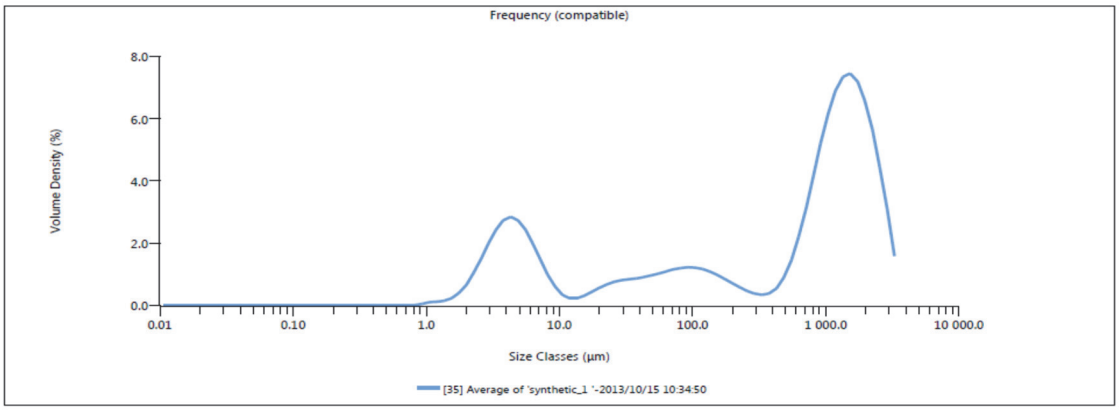
Sample Name	Average of 'synthetic_1 '	Measurement Date Time	2013/10/15 10:34:50 AM
Operator Name	MS3000	Analysis Date Time	2013/10/15 10:34:50 AM
SOP File Name	SOP_vip dry_example.msop	Result Source	Averaged

Analysis

Particle Name	Default	Particle Refractive Index	1.520
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.100	Laser Obscuration	19.01 %
Weighted Residual	0.75 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Normal

Result

Concentration	0.0431 %	Span	2.487
Uniformity	0.841	Result Units	Volume
Specific Surface Area	195.8 m^2/kg	Dv 10	4.16 µm
D [3,2]	17.0 µm	Dv 50	898 µm
D [4,3]	958 µm	Dv 90	2240 µm



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.00	0.0679	0.00	0.460	0.00	3.12	2.03	21.2	0.56	144	0.81	976	5.14
0.0114	0.00	0.0771	0.00	0.523	0.00	3.55	2.28	24.1	0.62	163	0.70	1110	5.77
0.0129	0.00	0.0876	0.00	0.594	0.00	4.03	2.38	27.4	0.67	186	0.59	1260	6.15
0.0147	0.00	0.0995	0.00	0.675	0.00	4.58	2.29	31.1	0.69	211	0.48	1430	6.24
0.0167	0.00	0.113	0.00	0.767	0.00	5.21	2.03	35.3	0.72	240	0.38	1630	6.02
0.0189	0.00	0.128	0.00	0.872	0.03	5.92	1.65	40.1	0.75	272	0.31	1850	5.50
0.0215	0.00	0.146	0.00	0.991	0.08	6.72	1.22	45.6	0.79	310	0.27	2100	4.72
0.0244	0.00	0.166	0.00	1.13	0.10	7.64	0.81	51.8	0.84	352	0.30	2390	3.73
0.0278	0.00	0.188	0.00	1.28	0.12	8.68	0.49	58.9	0.90	400	0.44	2710	2.57
0.0315	0.00	0.214	0.00	1.45	0.18	9.86	0.27	66.9	0.96	454	0.73	3080	1.32
0.0358	0.00	0.243	0.00	1.65	0.31	11.2	0.18	76.0	1.00	516	1.18	3500	
0.0407	0.00	0.276	0.00	1.88	0.54	12.7	0.18	86.4	1.02	586	1.81		
0.0463	0.00	0.314	0.00	2.13	0.86	14.5	0.26	98.1	1.01	666	2.59		
0.0526	0.00	0.357	0.00	2.42	1.25	16.4	0.36	111	0.97	756	3.46		
0.0597	0.00	0.405	0.00	2.75	1.66	18.7	0.47	127	0.90	859	4.34		

Figure 8.14 Example of data generated during the particle size analysis (source: unpublished data, UKZN PRG).

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8.7.1.4 Rheological properties – rheometer method[59]

8.7.1.4.1 Introduction

Rheology is related to the measurement of the response of soft solid materials or liquids to an applied force, such as sheering, where the deformation is a plastic flow in contrast to elastic deformation. Rheological properties for faecal sludge for example, give an estimation of the 'pumpability' of a sample by the change in viscosity under applied shear stress. A rheometer is used to carry out a number of rheological tests on faecal sludge and faeces samples. These include flow curves, amplitude and frequency sweeps, variable temperature tests and stress recovery tests. A number of different measuring systems exist (cone-cup, plate-plate, building material cell), and each is suited to different types of samples.

Step-by-step procedures for measuring rheological properties will vary depending on the available equipment and the characteristics of the incoming faecal sludge samples. One example of rheology measurement is the method used by the UKZN PRG laboratory in Durban, South Africa that is described here.

8.7.1.4.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.7.1.4.3 Required apparatus and instruments

- Rheometer
- 27 mm cone-cup attachment
- 32 mm cone-cup attachment
- Plate-to-plate attachment
- Building material cell
- Spatula.

8.7.1.4.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- A motor calibration and inertia calibration service must be performed every 90 days.
- Using a cone-cup attachment with a larger diameter (hence a larger surface area) produces more accurate results.
- Fluid samples with low viscosity tested at low shear rates may produce inaccurate results due to surface tension effects.
- For a long duration of testing (*e.g.* more than 1 hr), it is recommended to cover the cup containing the sample to avoid loss of moisture due to evaporation.
- Measurements should be conducted at a standard temperature of 25 °C. The temperature at which measurements were taken should be reported with the results.
- Blending or intensive mixing of the sample prior to measurement will change the structure of the faecal sludge material and affect the results. These homogenization techniques must never be applied for this method, and gentle mixing is preferable if homogenization is required.

8.7.1.4.5 Sample preservation

It is recommended that samples are analysed as soon as possible after collection. If immediate measurement is not possible, the samples should be stored at 4 °C for no longer than 14 days before analysis.

8.7.1.4.6 Sample preparation

- The sample should preferably not be homogenised as this will change its structural properties. The sample should be used as received and undisturbed. Only in case of differential moisture content within the sample, homogenise gently prior to the measurement. Never use a blender to homogenise.

[59] This method should be cited as adapted in Velkushanova *et al.* (2021), together with the specific analytical equipment (if different), and any manufacturer's modifications.

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8.7.1.4.7 Analysis protocol

Calibration

- The instrument must be switched on for at least an hour before analysis to perform motor adjustment (refer to specific manufacturer's information).
- For the motor adjustment procedure, connect the measuring system and leave a 1 mm gap from the top, then select the start service device, and then 'Start motor adjustment' from the software programme menu. This takes approximately 10 min to complete.
- Once the motor adjustment is completed, move the measuring system to the loading position.

Measurement

- Switch on the instrument.
- Remove the cap protecting the instrument's coupler.
- Open the rheometer software programme and control panel tab to initialise the instrument.
- Attach the cone cup to the rheometer plate.
- Manually enter 25 °C for the rheometer plate.
- Manually enter 23 °C for the VT-2 tower.
- Load the sample into the cone cup and then attach the cone cup to the base plate (Figure 8.15 A).
- Attach the measuring system to the adapter (Figure 8.15 B).
- Open the control panel and lower the measuring system to a gap of 0.00 mm.
- Close the control panel and select the measuring method (*e.g.* flowability: flow curve logarithmic).
- Select Start, enter the sample details, then select Continue.
- The test begins after reaching the set temperature (approximately 1 min).
- A generated report appears when testing is complete that can be saved as a PDF document.
- Once the test is complete, uncouple the measuring system, open the control panel and raise the measuring system.
- Remove the cone cup and discard the sample.
- Close the program and switch off the computer.

Note: these values are provided as an example, the actual measurement procedure will differ due to model type and software.

8.7.1.4.8 Calculation

No calculation required - direct reading.

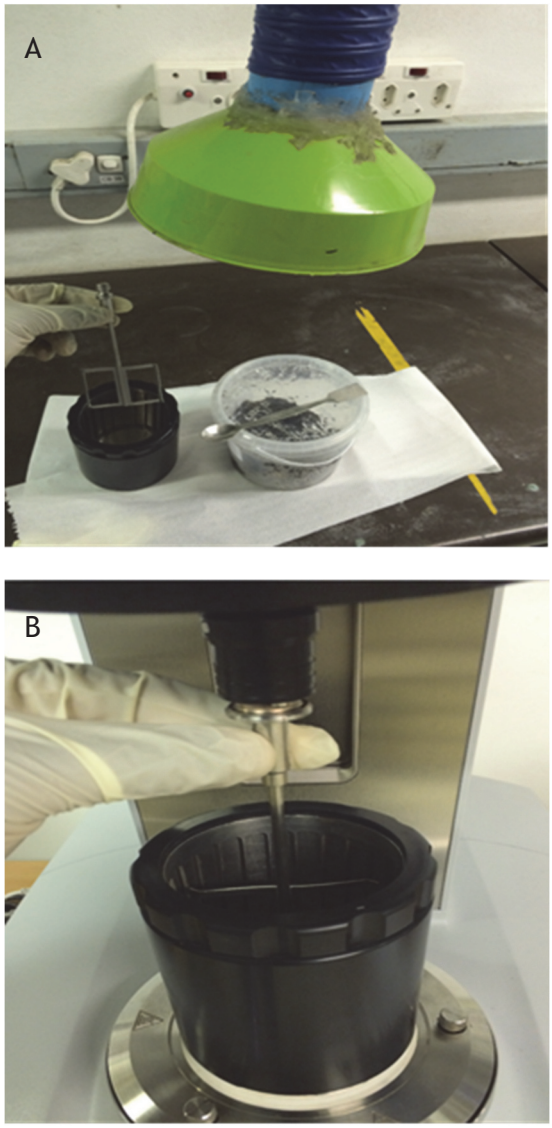

Figure 8.15 Loading the sample into the cone cup and then to the plate (A), and attaching the measuring system to the adapter (B) (source: UKZN PRG).

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8.7.1.5 Liquid limit – cone penetrometer method[60]

8.7.1.5.1 Introduction

The liquid limit is the moisture content at which a material passes from the liquid phase into the plastic phase, and is determined experimentally. The liquid limit is conventionally used to classify the consistency of the soil. Measurements of liquid limit in faecal sludge are useful when determining optimal methods of sludge emptying from onsite systems, or when determining the TS at which to pelletise dried sludge end products for resource recovery (Septien *et al*., 2018). The liquid limit and plastic limit (methods 0 and 0.) can be used to calculate the consistency index, liquidity index, and plasticity index, which are helpful in characterising the consistency of a material over a range of TS.

The cone penetrometer provides a static test using a material's resistance to penetration to determine its liquid limit. The cone is dropped from a specific height into the sample and the penetration into the sample is recorded and correlated to the moisture content of the sample. The moisture content is increased continuously and the test repeated, until an approximate linear graph can be produced. The water content corresponding to a penetration of 20 mm is the liquid limit of the sample.

8.7.1.5.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.7.1.5.3 Required chemicals

- Distilled water.

8.7.1.5.4 Required apparatus and instruments

- Cone penetrometer with a standard stainless steel cone (50 g), fitted with a 150 mm-diameter dial indicator for direct reading of the penetration
- Sample cup of 40 mm diameter and 55 mm deep (standard size)
- A stopwatch readable to 1 sec
- Two palette knives or spatulas
- Laboratory spoons for loading the penetrometer cup with the sample
- Rubber spatula to scrape any sample out of the cup
- A knife or flat sharp object to scrape excess material off the top of the cup.

8.7.1.5.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- Ensure that the penetrometer base level is horizontal using a bubble leveller.
- Measurements should be conducted at a standard temperature of 25 °C. The temperature at which measurements were taken should be reported with the results.

8.7.1.5.6 Sample preservation

It is recommended that samples are analysed as soon as possible after collection. If immediate measurement is not possible, the samples should be stored at 4 °C for no longer than 14 days before analysis.

8.7.1.5.7 Sample preparation

- Select a representative sample.
- Remove any foreign objects that might be mixed with the sludge (*e.g.* solid waste, stones, hair, and maggots).
- Do not allow the sample to dry before testing.

[60] This method is based on the British Standards Institution methods of test for soils for civil engineering purposes: Classification tests. This method should be cited as BS 1377:2, as adapted in Velkushanova *et al.* (2021).

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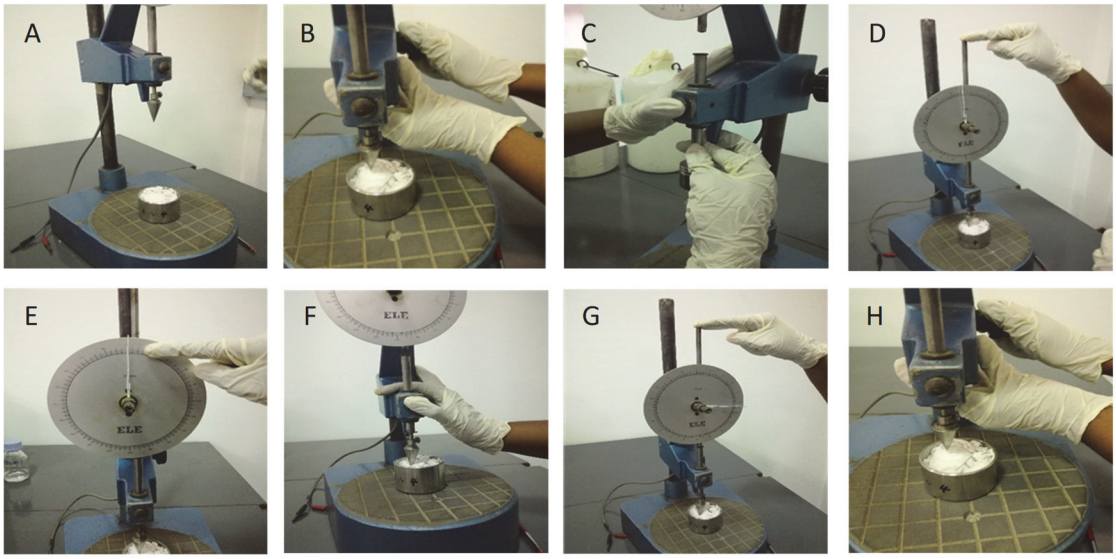

Figure 8.16 Illustration of testing stages (Source: UKZN PRG).

8.7.1.5.8 Analysis Protocol

1. Place 300 g of the prepared sample on the glass plate.
2. Mix the sample with the two palette knives for approximately 10 min and adjust with distilled water to achieve a first cone penetration reading of 15 mm.
3. Transfer the mixed sample to a metal cup using the palette knife and use a knife or long, straight edge to scrape the excess sludge from the top, creating a smooth, level surface flush with the top of the metal cup.
4. Place the metal cup in the designated position on the base of the instrument, ensuring the penetration cone is locked in a raised position (Figure 8.16 A).
5. Lower the penetration cone carefully until it just touches the surface of the sample; the correct position is indicated if the cone just scratches the surface when the cup is moved (Figure 8.16 B). Move the clamp to lower the cone.
6. When the cone has been placed in the correct position, lower the stem of the dial gauge until it just touches the cone shaft.
7. Set the dial gauge to zero (to the nearest 0.1 mm) (Figure 8.16 C).
8. Release the cone for 5 sec ± 1 sec. Lock the cone into position after the 5 sec have lapsed and lower the stem of the dial gauge again to touch the cone shaft. Read the dial gauge to the nearest 0.1 mm; this value is recorded as the cone penetration (Figure 8.16 G).
9. Lift the cone from the cup and clean it carefully.
10. Replace the wet sample in the cup, ensuring no air is trapped and repeat steps 3 through 9.
11. If the difference between the first and second penetrations is less than 0.5 mm, report the average value. If the difference is greater than 0.5 mm but less than 1 mm, repeat the test a third time and if the overall range is no greater than 1 mm, report the average of the three values. If the overall range is greater than 1 mm, remove the sample from the cup and repeat the procedure from step 2.
12. Take approximately 10 g of the sample where the cone penetrated the cup and measure the moisture content (see Method 8.6.1.1).
13. Repeat the entire procedure at least 3 times using the same sample to which increments of distilled water have been added.
14. Go from drier to wetter samples, until a cone penetration range of approximately 15 mm to 25 mm has been reached over the course of at least 4 test runs and the values are evenly distributed.

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15. Wash and dry the cup each time the sample is removed to facilitate the addition of water.
16. If the sample is left in the open for extended periods of time, cover with an evaporating dish or damp cloth to avoid drying.
17. Plot the moisture content against cone penetration to obtain a linear regression that best fits the plotted points.
18. The moisture content corresponding to a cone penetration of 20 mm is reported as the liquid limit, to the nearest whole number.

8.7.1.5.9 Calculation (if necessary)

Plot the water content (%) against penetration depth (mm). The water content corresponding to 20 mm penetration is the liquid limit (w_L). If the liquid limit cannot be measured, report the sample as non-plastic (see Figure 8.17).

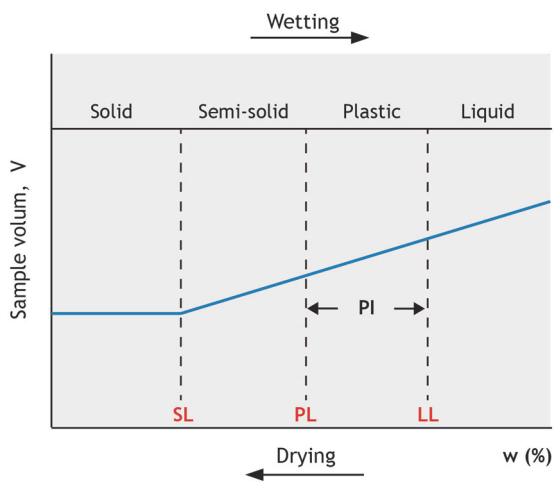

Figure 8.17 Relationship between liquid limit (w_L), plastic limit (w_P) and volume of the sample (adapted from Das and Sobhan, 2013).

The liquid limit can be used along with the plastic limit (Method 8.7.1.6) to determine the following indices:

$$\text{Consistency index [-]} = \frac{w_L - w}{w_L - w_P}$$

$$\text{Liquidity index [-]} = \frac{w - w_P}{w_L - w_P}$$

$$\text{Plasticity index [-]} = w_L - w_P$$

Where:

w_L = Liquid limit of a sample

w_P = Plastic limit of a sample (see Method 8.7.1.6)

w = Water content of a sample (see Method 8.6.1.1).

8.7.1.6 Plastic limit – thread-rolling method[61]

8.7.1.6.1 Introduction

The plastic limit of faecal sludge is the experimentally determined moisture content at which the sample is too dry to behave as a plastic. It indicates the plasticity of a material at a given moisture content. It is used in conjunction with the liquid limit to calculate the Plasticity Index in order to classify the consistency of faecal sludge. The sample is moulded from a ball shape into a thin thread of approximately 3 mm until cracks appear in the thread, both longitudinally and transversely. The moisture content at which the cracks appear and the thread cannot be rolled anymore without breaking is the plastic limit.

8.7.1.6.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

[61] This method is based on the British Standards Institution Methods of test for soils for civil engineering purposes: Classification tests. This method should be cited as BS 1377:2, as adapted in Velkushanova *et al.* (2021).

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8.7.1.6.3 Required apparatus and instruments

- Flat glass plate, to mix and roll samples (10 mm thick, 300 mm square)
- Two pallet knives or spatulas
- Rod (3 mm in diameter and 100 mm long).

8.7.1.6.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- This method assumes that heat from the hands contributes to drying out the faecal sludge during handling, contributing to transverse and longitudinal shearing. The length of time taken to dry out the sludge may be extended due to the necessity of wearing latex gloves.
- If the sample is too wet, a hair dryer can be used to evaporate extra moisture and save time on hand rolling.
- The results are subject to the interpretation of the person performing the test, leading to variations in the results. These differences should be quantified as part of a comprehensive quality control procedure (see Section 8.3).

8.7.1.6.5 Sample preservation

It is recommended that the samples are analysed as soon as possible after collection. If immediate measurement is not possible, the samples should be stored at 4 °C for no longer than 14 days before analysis.

8.7.1.6.6 Sample preparation

- Select a representative sample.
- Remove any foreign objects that might be mixed with the sludge (*e.g.* solid waste, stones, hair, and maggots).
- Ensure the sludge sample is thoroughly mixed.

8.7.1.6.7 Analysis protocol

1. Weigh approximately 20 g of the sample on to the glass plate for mixing.
2. Allow the sample to dry until it can be shaped into a ball.
3. Mould the sample into a ball between the fingers and then roll it between the palms until the heat of the hands has made it dry enough that small cracks appear on the surface.
4. Divide the sample into 2 subsamples of approximately 10 g, carrying out a separate determination for each subsample.
5. Divide each subsample into 4 more samples of approximately equal size and apply the following steps to each sample.
6. Mould the sample between the fingers to equally distribute the moisture and then roll the sample into a thread of approximately 6 mm between the thumb and first finger.
7. Roll the thread on the glass plate with the fingers, from their tip to the second knuckle using enough pressure to reduce the diameter to approximately 3 mm in 5 to 10 forward and backward rolls. It is important to maintain a constant rolling pressure.
8. Pick up the sample and mould between the fingers, reproduce a thread shape and repeat step 7.
9. Continue step 8 until the thread shears both longitudinally and transversely when it is rolled to a 3-mm diameter, which is determined using the rod. After the thread has crumbled, do not reproduce the thread, as the first crumbling point is the plastic limit.
10. Place the pieces of the thread in a container and seal with a lid.
11. Place the pieces of all four threads in the one container and determine the moisture content (Method 8.6.1.1).
12. Repeat steps 5 through 11 for the second set of 4 subsamples.

If the moisture content of the 2 sample replicates differs by more than 0.5% the entire test must be repeated.

8.7.1.6.8 Calculation (if necessary)

Calculate the average of the two moisture content values and round to the nearest whole number. This is the plastic limit (w_P).

Plastic limit can be used along with liquid limit (Method 8.7.1.5) to determine the following indices:

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$$\text{Consistency index [-]} = \frac{w_L - w}{w_L - w_P}$$

$$\text{Liquidity index [-]} = \frac{w - w_P}{w_L - w_P}$$

$$\text{Plasticity index [-]} = w_L - w_P$$

Where:

w_L = Liquid limit of a sample (see Method 8.7.1.5)

w_P = Plastic limit of a sample

w = Water content of a sample (see Method 8.6.1.1).

8.7.1.7 Compressibility and stickiness – texture analyser method[62]

8.7.1.7.1 Introduction

Texture analysis refers to a technique for evaluating the mechanical and physical properties of materials. A texture analyser can be used to carry out a variety of tests for characterising the properties of faecal sludge and treated end products. Different mechanical properties of the sample can be measured with a texture analyser, including: compression, tension, flexure, penetration, extrusion, and adhesion, which are used to measure hardness, crispiness, crunchiness, softness, springiness, stickiness, tackiness and other material properties. An assortment of probes is available, and changing the probe enables different properties to be characterised in a variety of samples. Compression tests, the focus of this method, are specifically used to measure compressibility, compactibility, springiness, stress relaxation, crust strength, firmness, and elastic recovery. Characterisation of these properties of solid and semi-solid faecal sludge and end products is informative for the design of new treatment and resource recovery technologies.

8.7.1.7.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.7.1.7.3 Required apparatus and instruments

- Texture Analyser (Stable Micro Systems TA.XT Express)
- Petri dish (> 75 mm diameter, glass)
- Probe - p/75 compression platen attachment.

8.7.1.7.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- Major calibration of the equipment should be carried out by the service company at regular intervals, according to the manufacturer's instructions.
- Routine calibration of the equipment should be performed when starting up the equipment, following the manufacturer's instructions.
- Do not use solvents or cleaners with the instrument or probes. Clean with a nonabrasive cloth using water.
- Do not apply excessive upward, downward or sideways force to the probe while connected to the texture analyser, as damage may occur to the load cell.
- Use a consistent mass for all the replicates.
- Remove any foreign objects that might be mixed with the sludge (*e.g.* solid waste, stones, hair, and maggots).

8.7.1.7.5 Sample preservation

It is recommended that the samples are analysed as soon as possible after collection. If immediate measurement is not possible, the samples should be stored at 4 °C for no longer than 14 days before analysis.

[62] This method should be cited as adapted in Velkushanova *et al.* (2021), together with the specific analytical equipment (if different), and any manufacturer's modifications.

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8.7.1.7.6 Sample preparation

- Ensure the sample is thoroughly mixed.
- After mixing, if the samples are not to be analysed immediately, cover them with foil or a lid to minimise water loss due to evaporation.

8.7.1.7.7 Analysis protocol

Start up

- Turn on the computer and texture analyser. Start the texture analysis software.
- Release the safety button on the texture analyser by pressing and rotating it clockwise.
- Attach the probe to the texture analyser.

Calibration

- Place the petri dish containing the sample in the designated place and secure its position with clamps.
- For height calibration before each set of experiments, select 'Calibrate' (set up for the specific petri dish in use) in the software programme and calibrate the height.
- In the software window, ensure the contact force is 1 g and the height is 25 mm return distance, and the return speed is 10.
- The probe should go down to touch the base of the petri dish and return to 25 mm above the surface.
- Click 'OK' after successfully calibrating the height.

Run a test

- Select 'Run a test' in the software programme.
- Set the test mode ('Compression'), pre-test speed (1 mm/sec), test speed (2 mm/sec), post-test speed (10 mm/sec), target mode ('Distance'), distance (5 mm), trigger type ('Auto-force') and 'Trigger force' (5 g) in Newtons, and click 'OK'. For tension experiments, set the test mode (tension), pre-test speed (3 mm/sec), post-test speed (10 mm/sec), target mode (distance), distance (10 mm) and trigger type (button).
- Do not change the parameters; these include sample shape which is undefined as default, and the data acquisition. Use the default settings. These have the sample width, sample length, sample height, temperature and stress area without any values as the sample is not rigid and the temperature is not automatically regulated. The data acquisition tab has the data acquisition rate (pps) as 200 and the typical test time (sec) at 150 as the default settings.
- Weigh 100 g of the thoroughly-mixed sample into the petri dish, and ensure the sample surface is level for optimal contact with the probe.
- Secure the petri dish with clamps.
- Lower the probe using the stylus or the software.
- Start the test by clicking 'Start'.
- The compression platen on the texture analyser moves until it reaches the top surface of the sludge sample then it exerts a force of 0.098N (10 g) on the sample and compresses the sludge at a speed of 2 mm/s until the probe is in tight contact with the bottom of the petri dish. Afterwards, the probe is raised back up to its initial position at a speed of 10 mm/s, thus exerting pulling force on the faecal sludge.

Check results and save

- Once the analysis is done, click on the 'Results' tab to view the tabulated results such as peak positive and negative forces.
- To view the maximum peak negative and positive force, click on the 'Go to' tab then view and select the required force.
- To save data, export and save the raw data as an Excel spreadsheet to the required directory.
- After saving a graph and raw data (combined), close the software program.
- Move the probe to 25 cm above the platform.

Shut down

- Press the emergency stop button.
- Switch off the texture analyser.
- Remove the sample and clean the probe.

8.7.1.7.8 Calculation

No calculation required - direct reading. An example of a typical graphical test result is presented in Figure 8.18

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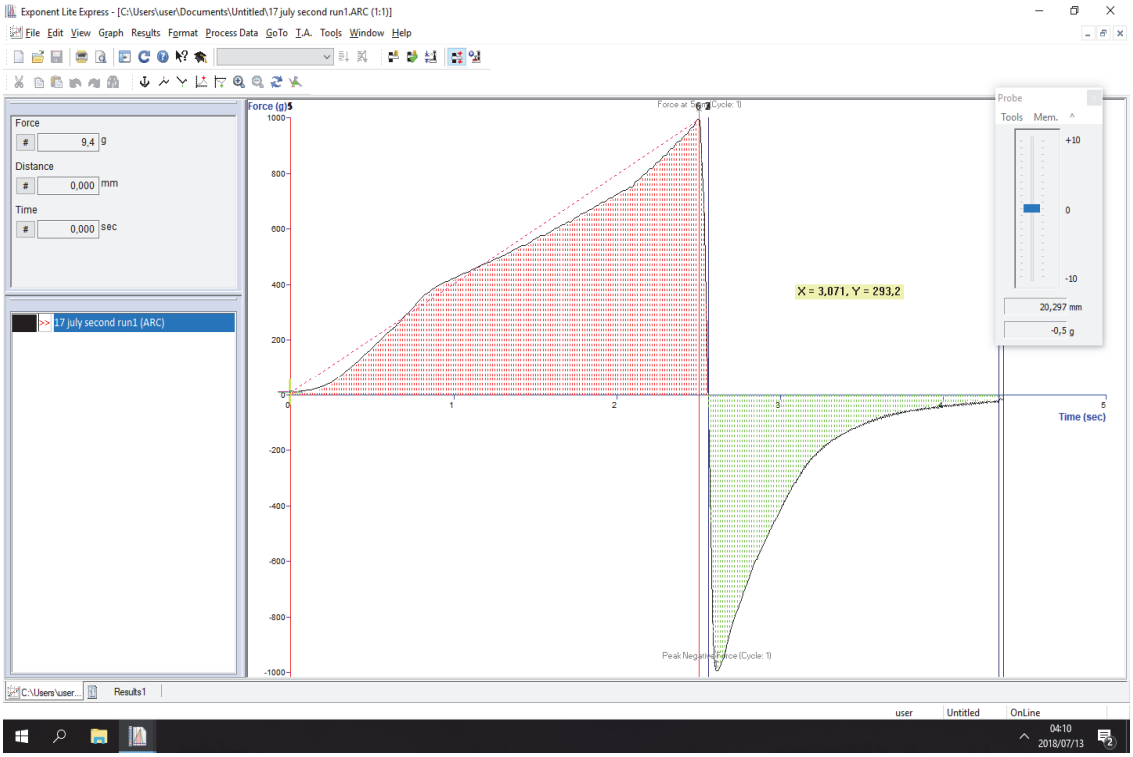

Figure 8.18 Typical graph of a test with the red part showing the compression forces and the green part showing the tension forces.

8.7.2 Physical and thermal properties

The evaluation of thermal properties of faecal sludge is important for treatment and resource recovery applications, such as the production of heat-treated pellets and the combustion of solid fuels or biofuels from faecal sludge. Thermal properties include thermal conductivity and diffusivity, specific heat, and calorific value. The calorific value of a material is the quantity of heat produced from its combustion, which is important in evaluating the suitability of faecal sludge end products as solid fuels. The thermal conductivity is the ability of a material to conduct heat, and heat capacity is the amount of heat energy required to change the temperature of an object by a certain amount. Thermal conductivity and heat capacity are important in the heat treatment and drying of faecal sludge for pathogen reduction and resource recovery.

8.7.2.1 Thermal conductivity – thermal conductivity analyser method[63]

8.7.2.1.1 Introduction

Thermal conductivity is the thermal property of a material that describes its ability to conduct heat. Thermal conductivity, along with several other thermal characteristics, can be measured using a thermal conductivity analyser. The method presented here is written for a C-Therm TCi Thermal conductivity analyser[D]. This instrument uses the modified transient plane source method, which involves monitoring the temperature increase in the

[63] This method should be cited as adapted in Velkushanova *et al.* (2021), together with the specific analytical equipment (if different), and any manufacturer's modifications.

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sample during and after exposure to a short heat stimulus. The thermal conductivity of the sample is inversely proportional to the rate of temperature increase in the sample. This method usually requires small amounts of sample and can accommodate solid, semi-solid, slurry, and liquid faecal sludge samples, as well as dried end products. In addition to thermal conductivity, this method can be used to determine thermal effusivity, thermal diffusivity, heat capacity, the R value, and depth of heat penetration. Characterisation of these thermal properties of faecal sludge and end products is helpful in the development and design of drying technologies.

8.7.2.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.7.2.1.3 Required apparatus and instruments

- Thermal conductivity analyser (C-Therm TCi)
- Small volume test kit (SVTK) to measure liquid and powder samples
- Measuring spoons (1/8 or 0.63 mL and ¼ or 1.25 mL).

8.7.2.1.4 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- The sensor is factory-calibrated. See the manufacturer's instructions for routine calibration and maintenance.
- Check the R2 value for each measurement. If the R2 value is less than 0.995, the measurement is not valid and should be repeated.

8.7.2.1.5 Sample preservation

It is recommended that samples are analysed as soon as possible after collection. If immediate measurement is not possible, the samples should be stored at 4 °C for no longer than 14 days before analysis.

8.7.2.1.6 Sample preparation

- Remove any foreign objects that might be mixed with the sludge (*e.g.* solid waste, stones, hair, and maggots).
- Ensure the sample is thoroughly mixed.
- After mixing, if samples are not to be analysed immediately, cover the samples with foil or a lid to minimise water loss due to evaporation.
- To perform this method, the density of the sample must be known. Before starting the analysis, measure the density of the sample using Method 8.7.1.1.

8.7.2.1.7 Analysis protocol

Measurement - solid and dry faecal sludge samples:

- In the TCi software, select 'New test'.
- Choose 'Liquids and powders' as the group/material.
- Wait for the instrument and sensor to be detected.
- Enter the density of the sample (m/v) alongside the material.
- Scoop 3 × 1/8 teaspoons, or 1.8 mL of the sample onto the test cell.
- Place the quick clamp cap on the test cell.
- Monitor the sensor temperature with the TCi software until it is stable and the sensor, sample and environment have all reached a state of thermal equilibrium.
- Initiate the test sequence with the TCi software.
- Alter 'Test method' to enter the number of measurements required (usually five measurements are required).

Measurement - liquid or slurry faecal sludge samples:

- In the TCi software, select 'New test'.
- Choose 'Liquids and powders' as the group/material.
- Wait for the instrument and sensor to be detected.
- Enter the density of the sample (m/v) alongside the material.

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- Measure 1.25 mL (1/4 teaspoon) of the total liquid volume of the specimen.
- Transfer this volume directly to the test cell.
- Place the quick clamp cap on the test cell.
- Monitor the sensor temperature with the TCi software until it is stable and the sensor, sample and environment have all reached a state of thermal equilibrium.
- Initiate the test sequence with the TCi software.
- Alter 'Test method' to enter the number of measurements required (usually five measurements are required).

Cleaning after testing

- Pour out the contents of the sample from the test cell or remove it with a paper towel.
- Place the sensor upside down and remove the test cell by gradually unfastening the three screws.
- Remove the sensor test and clean with either soap and water, water, or propyl alcohol.
- To test again, place the test cell on the sensor and place upside down in order to have easy access to the screws.
- Tighten gradually until the test cell seats perfectly flat against the sensor-housing surface.

8.7.2.1.8 Calculation (if necessary)

No calculation is required, as the analyser calculates and reports the thermal properties.

8.7.2.1.9 Data set example

Table 8.15 contains experimental data from five replicate measurements of thermal properties of a faecal sludge sample from VIP latrines in Durban, South Africa, using a C-Therm TCi thermal conductivity analyser. Effusivity, thermal conductivity, diffusivity, heat capacity, and the R value are thermal properties of the material. R2 is an indicator of smoothness of the curve generated for each measurement. Measurements are deemed valid when R2 > 0.995 (unpublished data, UKZN PRG).

Table 8.15 Experimental data for faecal sludge from VIP latrines in Durban, South Africa.

Replicate number	Sensor	Effusivity ($Ws^{1/2}/m^2K$)	Thermal conductivity (W/mK)	Diffusivity (m^2/s)	Heat capacity (J/kg.K)	R value (m^2K/W)	R2
1	T298	488.58	0.1816	0.0000	1,205,649.27	0.0037	0.9990
2	T298	489.81	0.1821	0.0000	1,208,939.75	0.0037	0.9988
3	T298	507.26	0.1880	0.0000	1,255,668.41	0.0035	0.9985
4	T298	517.42	0.1915	0.0000	1,282,799.11	0.0035	0.9986
5	T298	523.54	0.1936	0.0000	1,299,144.06	0.0034	0.9986

8.7.2.2 Calorific value – bomb calorimeter method[64]

8.7.2.2.1 Introduction

Calorific value is defined as the amount of heat energy released by the mass of a sample when combusted in an enclosure of constant volume. It is a measure of the energy content of a sample. Calorific value is an important metric for evaluating the suitability of faecal sludge end products as biofuels, for example, dried sludge, pellets, and char. The calorific value of faecal sludge is affected by multiple factors (*e.g.* type of onsite sanitation technology, level of stabilization, and sand content), and a range of variation in calorific values has been reported for faecal sludge end products worldwide, as summarised in Andriessen *et al*. (2019). Faecal sludge end products can have calorific values comparable to wood and waste biomass (Andriessen *et al*., 2019; Murray Muspratt *et al*., 2014; Diener *et al*., 2014). Calorific value is

[64] This method should be cited as adapted in Velkushanova *et al.* (2021), together with the specific analytical equipment (if different), and any manufacturer's modifications.

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expressed as energy/mass. Common units for calorific value of fuels are MJ/kg or BTU/lb.

The bomb calorimeter method is commonly used for measurement of calorific value. The calorific value obtained with a bomb calorimeter represents the higher heating value (HHV), or the gross heat of combustion per unit mass of sample. This is the heat produced when the sample burns, plus the heat given up when the newly formed water vapour condenses and cools to the temperature of the calorimeter. This method is intended for use with dry end products or oven-dried samples. Calorific value can be determined for oven-dried moisture-free samples, or for as-received samples containing some moisture. A thorough introduction to the theory and use of bomb calorimetry is available in Parr Instrument Company (2013).

Step-by-step procedures for measuring calorific value will vary depending on the available equipment and the characteristics of the incoming faecal sludge samples. One example of calorific value measurement using a bomb calorimeter is the method used by the UKZN PRG laboratory in Durban, South Africa using a Parr 6200 Oxygen Bomb Calorimeter[D], described here.

8.7.2.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.
- Exercise caution and follow the instrument's instructions carefully when charging and handling the oxygen bomb. Never over-pressurise the bomb. Maximum filling pressure may vary with the equipment being used, for example, for the Parr 6200 Oxygen Bomb Calorimeter the bomb must never be filled to more than 600 psi (40 atm).
- Work carefully when moving the pressurised bomb after filling with oxygen.
- During firing and for at least 15 sec after firing, stand back from the calorimeter and keep clear of the top of the calorimeter. If the bomb does explode, it is likely that the force of the explosion will be directly upward.

8.7.2.2.3 Required chemicals

- Oxygen cylinder (≥ 99.5% pure oxygen)
- Standard benzoic acid pellets
- Ethylene glycol as a combustion aid (for samples that are difficult to combust).

8.7.2.2.4 Required apparatus and instruments

- Oxygen bomb calorimeter, including an oxygen combustion bomb
- Bomb head support stand
- Analytical balance with four decimal places.

8.7.2.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- Benzoic acid is used as a calibration standard of known calorific value to determine the heat capacity of the calorimeter.
- The heat capacity of the calorimeter should be checked at least once a month, and also after changing any part on either the calorimeter or the oxygen canister. See ASTM D5865 (ASTM 2004) for a thorough description of the heat capacity checks, and refer to the manufacturer's instructions.
- The temperature measurement in the calorimeter needs to be accurate to 0.0001 °C, and should be calibrated by a recognised certifying agency (ASTM 2004).
- The analytical balance must be calibrated regularly, following the method outlined in Method 8.6.1.1.
- After filling the bomb with oxygen, check that the bomb is not leaking by submerging it in water. Do not fire the bomb if gas bubbles are leaking from the lid.
- If leaking, depressurise and then open it, then clean the seals and O rings, and then re-seal, re-fill, and re-check.

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- The fuse used to ignite the sample is made of cotton thread. Ensure the fuse thread stays dry, as a wet fuse will prevent the sample from igniting. The fuse should not be immersed in the sample; instead, it must be placed above the sample.
- The test should be operated at room temperature (20-25 °C).
- Regular maintenance must be carried out after every 30 tests, including replacement of the O ring and wire.
- Allow at least 20 min for the calorimeter to warm up and the jacket temperature to reach standard operational temperature.
- If an as-received sample containing some moisture is to be characterised, it may be difficult to achieve complete combustion of the sample. In this case, ethylene glycol or another combustion aid may be used. According to ASTM D5865, a minimum of 0.4 g of combustion aid is used, and its weight recorded to the nearest 0.0001 g. Calorific value results must be corrected for the use of the combustion aid by subtracting the heat of combustion of the aid multiplied by the mass of the combustion aid from the overall calorific value obtained.
- Other corrections to the measured calorific value may also be required, although this process is automated in more automated instruments. See the manufacturer's instructions for specific calibration calculations and protocols.
- Do not use too much sample. The standard bomb cannot withstand the effects of combustible charges which liberate more than 8,000 calories. This generally limits the total weight of combustible material (the sample plus the combustion aid) to no more than 1.1 g.

8.7.2.2.6 Sample preservation

When this analysis is to be performed on moisture-free samples, faecal sludge can be dried immediately after collection (in a drying oven at 105 °C), then ground to powder. Dried powder samples can be stored long-term prior to analysis, in a cool dry place. When samples are to be analysed as-received, still containing moisture, they should be refrigerated (4 °C) until analysis and stored for no longer than 30 days. The preparation steps and duration of storage should be reported with the results.

8.7.2.2.7 Sample preparation

- If the sample is to be analysed as a moisture-free sample, dry at 105 °C in a crucible for 24 hr or until completely dry, following the method outlined in Method 8.6.1.1 for assuring complete sample dryness. Even if the sample has been dried as a sample preservation step, it should be dried again immediately before characterisation to ensure it is entirely free of any moisture that could have been absorbed during storage.
- If the sample is to be analysed as-received, do not dry.
- Grind or pulverise moisture-free and as-received samples, and sieve to ensure the particle size of the sample is less than 250 µm (*e.g.* with a 250 µm sieve or No. 60 sieve).

8.7.2.2.8 Analysis protocol

Calibration

- Use a 1-g benzoic acid pellet for calibration. Always record the exact weight of benzoic acid (to 0.0001 g) used for the calibration even if using the pellets, as weight may vary.
- The heat of combustion of benzoic acid is ~26 MJ/kg, and the exact heat capacity will be listed on the certificate that comes with the benzoic acid standard.
- Run the benzoic acid standard using the same method used for a sample measurement in the following section.

Measurement

- Open the oxygen gas cylinder and set the flow rate to constant pressure (*e.g.* 400 psi, 3,000 kPa).
- Fill the water chamber with distilled water to the mark, and then turn on the calorimeter, pump and heater. When the calorimeter is ready the 'Start' key will appear and begin testing.
- Fill the calorimeter bucket with 2 L of distilled water and place the bucket in the calorimeter. It is important that the water level is exactly at the 2 L mark to maintain accuracy. It is recommended to replace the water every day in case there is ionization/adulteration of the distilled water during the experiments.
- Record the moisture content of the sample. If analysing a moisture-free sample, note 0% moisture. If analysing as-received samples,

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measure the moisture content of a representative sample (see Method 8.6.1.1).

- Weigh between 0.5 and 0.7 g of sample into a capsule, and record the sample weight to the nearest 0.0001g.
- If the moisture content of the sample is higher than 80%, add ethylene glycol to the sample before combustion to facilitate ignition. Adjust the sample mass accordingly so that the combined weight of ethylene glycol and sample is no more than 1 g. Record the mass of ethylene glycol added to the nearest 0.0001 g.
- Follow the manufacture's instruction to set up the bomb.
- The Parr 6200 calorimeter will conduct the test automatically.
- Read and record the calorific value from the screen.
- Remove the bomb from the chamber after 3 min and depressurise the bomb by opening the valve knob slowly. After all the pressure has been released, unscrew the cap and lift the head straight out.
- Remove the chamber containing the ash.
- Wipe the inside of the bomb with a clean laboratory tissue and proceed with the next sample.
- Clean the bomb and soak in a citric acid solution, either overnight or whenever the bomb is dirty.
- Aqua regia can also be used to clean the bomb; remove the wire if aqua regia is used.

8.7.2.2.9 Calculation

No calculation required - direct reading. If using a basic calorimeter instead of an automated calorimeter such as the Parr 6200, follow the instructions in the manual, or use the equations in ASTM D5865 to calculate the heat capacity of the calorimeter and the calorific value of the sample.

8.8 BIOLOGICAL PROPERTIES

8.8.1 Pathogens

The current methods presented in this section are for pathogens, specifically the detection of total and viable Helminth eggs, and the qualitative and quantitative enumeration of coliform bacteria and coliphage as indicators of pathogens. Biological activities related to the production and consumption of organic matter, or respiration, are included under Section 8.6. Further types of analytical methods for biological examinations include identifying specific pathogens (*e.g.* viruses, bacteria, protozoans, and helminths), metrics of toxicity (*e.g.* use of bioassays), enumeration (*e.g.* plate counts, flow cytometry, and MPN), and types and functions of organisms (*e.g.* DNA/RNA analysis).

The methods of detection for pathogens are important to ensure adequate protection of public health and are commonly used for compliance, evaluation of treatment performance, and research purposes. Isolating specific microorganisms can be tedious, so indicator organisms that send a signal that samples could be contaminated with pathogens are commonly used (Madigan *et al.,* 2018). Indicator organisms are selected based on their ease of detection, similar behaviour in the environment, and greater resistance to die off than other pathogens of concern. For a detailed overview of disease-causing organisms and enteric pathogens of concern in wastewater treatment, and the pathways of contamination, see Gerba (2020) and Cairncross and Feacham (2019).

Coliform bacteria are commonly used as indicators, as they live in the intestines of humans and other warm-blooded animals and so are considered to indicate faecal contamination. Coliforms are operationally defined as 'aerobic and facultatively aerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 hr at 35 °C' (Madigan *et al.,* 2018). In general, most coliforms are not harmful, and only some cause signs of infection. One caveat on using coliforms as indicators is that they are not specific to humans. *E coli* can be distinguished from

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other types of coliforms (*e.g.* the absence of urease and the presence of B-glucuronidase) and is more likely to indicate faecal pollution (Gerba, 2020).

The coliform test is commonly carried out with the most-probable-number (MPN) method or the membrane filter procedure. In the MPN method, samples are serially diluted in liquid culture medium in test tubes until no growth is observed. In the membrane filter procedure, samples are passed through a filter that captures all of the bacteria, and the filter is then incubated on a plate of eosin-methylene blue (EMB) culture media. However, microbial methods can be challenging when resources are limited, requiring a wide range of laboratory equipment and skilled personal. Presented in Bain *et al*. (2012) is a summary of commercially available test kits for coliform bacteria, including a cost breakdown, and which type of settings they are appropriate for (no laboratory, a basic laboratory, or a highly resourced laboratory). The summary covers the types of presence/absence tests, and quantitative tests using colony counts and MPN. Colony counts use plating, filtration, or immobilization of the bacteria within a media, whereas MPN tests rely on sample division or dilution and a statistical method for estimating the number of organisms (Bain *et al.,* 2012). This book presents examples of the 3M Petrifilm colony count method[D], and the Colilert presence/absence[D] and MPN method. For a more detailed review of available kits, refer to Bain *et al*. (2012). Reporting of MPN and CFU are equivalent, both are units to measure the estimated number of bacteria in a water sample. CFU/100 mL is based on counting colonies on a plate, whereas MPN/100 mL is the statistical probability of the number of organisms. The membrane filtration and the multi-enzyme substrate methods are included in the Standard Methods for the Examination of Water and Wastewater, Section 9060A (Rice *et al.,* 2017).

Bacteriophage are a type of virus that are able to infect and grow in bacteria. Coliphage are a type of bacteriophage that specifically infect *E. coli*, so their presence suggests the presence of *E. coli*. They are used as indicators due to their continual presence in wastewater. Coliphage are similar to many human viruses, they cannot replicate in the environment without a host, are relatively resistant to disinfection processes, and they can be detected with simple and inexpensive methods that yield results in 8-18 hr, which make them good candidates for indicators (Rice *et al.,* 2017; Gerba, 2020). Presented in this book is the example of a coliphage plaque assay. Step-by-step procedures for variants of the double-agar-layer method, single-agar-layer format, and filter-adsorption technique, are presented in Rice *et al*. (2017).

In faecal sludge, helminths are commonly used as an indicator of the effectiveness of pathogen reduction due to their prevalence in low- and middle-income countries, and their persistence following treatment. Helminths are important pathogens to monitor, especially *Ascaris lumbricoides*, whose eggs are one of the pathogens most resistant to inactivation in treatment processes. Presented in this chapter is a method developed by the UKZN PRG specifically for the detection of total and viable helminth eggs in faecal sludge.

8.8.1.1 *E. coli* and total coliforms – colony forming unit method[65]

8.8.1.1.1 Introduction

Coliform bacteria are present in the faeces of warm-blooded animals, and are used as common indicators of faecal pollution. Escherichia coli (*E. coli)* is one type of coliform bacteria; most *E. coli* and coliforms are harmless, but some cause signs of infection in humans. Coliform bacteria are relatively easy to culture, and are used as indicators of the possible contamination by or presence of faecal matter. Coliforms and *E. coli* can be measured by pour plate or spread plate counts, the membrane filtration method or the multi-well enzyme substrate method results. The plate count test for *E. coli* and total coliforms is based on the principle that microbial cells quickly grow into visible colonies when they are provided with a suitable growth media and growth

[65] This method should be cited as the specific method that is carried out in each laboratory, including the manufacturer's make and model (where necessary) for the total coliforms and *E. coli* test, and the exact method of sample preparation.

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conditions. This method has been used frequently to culture different microorganisms on a nutrient media by adding substances that enhance the growth of organisms of interest or inhibit unwanted species. Colony-forming methods for analysing *E. coli* and total coliforms give an estimate of the population density of *E. coli* and total coliforms in the sample. To ensure that plates with countable colonies are produced, diluted samples are used for this method. Decreasing concentrations of the original sample are made using serial dilutions to plate a range of dilutions. This ensures that the plates contain reduced numbers of *E. coli* and total coliform that are distinct and can be counted as individual colonies. The number of colonies counted on a plate gives the colony-forming units, which is divided by the volume of sample used to get the CFU/volume (CFU/mL).

The example provided here is the 3M Petrifilm *E. coli*/coliform test[D], which is based on the manufacturer's protocol and can be found on the 3M website[66]. For a more detailed review of available kits, refer to Bain *et al*. (2012). The 3M Petrifilm *E. coli*/coliform count plate is a sample-ready culture-medium system, which contains Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity (BCIG), and a tetrazolium indicator that facilitates colony enumeration in microbiological samples. These plates provide both *E. coli* and total coliform count information with confirmed results in 24-48 hr. UKZN PRG in Durban has adapted this method for analysis of total coliforms and *E. coli* in faecal sludge.

8.8.1.1.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.8.1.1.3 Required chemicals

- Ringer's solution
- Saline solution (0.85-0.90%)
- Phosphate buffer water or distilled water.
- 0.1 N NaOH
- 0.1 N HCl.

8.8.1.1.4 Required apparatus and instruments

- 3M Petrifilm *E. coli* /coliform count plates
- Laminar flow hood
- Autoclave
- 1 mL micropipette and tips
- 50 or 100 mL beaker
- Blender
- Analytical balance
- Incubator for temperatures up to 45 °C.

8.8.1.1.5 Quality Control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- Use appropriate sterile buffered dilution water, Ringer's solution, saline solution (0.85-0.90%), phosphate buffer or distilled water.
- Do not use diluents containing citrate, bisulphite or thiosulphate with 3M Petrifilm plates as these may inhibit the growth of organisms.
- The samples should be adjusted to a pH of 6.6-7.2 to ensure optimum growth and recovery of the organisms.
- Opened 3M Petrifilm should be sealed by folding the end of the pouch over and applying adhesive tape.
- Storage of 3M Petrifilm plates: store unopened pouches at temperatures lower than 8 °C or frozen. For opened pouches, store sealed pouches in a cool dry place for no longer than four weeks and prevent exposure to moisture.
- If the sample is too concentrated, serial dilutions can be done using distilled water or appropriate buffer solutions using sterile equipment to prevent errors when counting the colonies.
- The samples should be mixed thoroughly as analytical results depend on adequate sample

[66] https://multimedia.3m.com/mws/media/701951O/product-instructions-3m-petrifilm-e-coli-coliform-count-plate.pdf

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mixing. If the sample is not adequately shaken before the aliquots are removed, the actual bacterial density could be underestimated.

8.8.1.1.6 Sample preservation

- The samples should be analysed immediately after sampling if possible. Samples can be stored for up to 24 hr between 1-4 °C (Rice *et al.,* 2017). The samples should be allowed to reach room temperature before analysis.

8.8.1.1.7 Sample preparation

For liquid samples:

- In general, no preparation for liquid samples is required. Appropriate dilutions should be made using a serial dilution procedure. For faecal sludge samples, a minimum of four sample dilutions are required. However, five or more dilution are preferred (USEPA, 2006).

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of the thoroughly-mixed sample into a beaker.
- Dilute the sample gravimetrically and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec on the highest speed.
- Transfer this solution into a plastic bottle for testing.
- The sample pH must be within the range of 6.6-7.2. Adjust the pH with sulphuric acid or sodium hydroxide solution if necessary.
- Total solids analysis should be performed on the samples so that the results of the measurement can be expressed as CFU/gTS.

8.8.1.1.8 Analysis protocol

- Place the 3M Petrifilm *E. coli*/coliform count plate on a flat surface (Figure 8.19 A)
- Lift the top film of the plate and dispense 1 mL of the sample onto the centre of the bottom film (Figure 8.19 B).
- Slowly roll the top film down onto the sample, making sure there is no entrapment of air bubbles (Figure 8.19 C).
- Distribute the sample evenly within the circular well using a gentle downward pressure on the centre of the plastic spreader (flat side down) (Figure 8.19 D). Do not slide the spreader across the film.
- Remove the spreader and leave the plate undisturbed for one min to permit solidification of the gel.
- Incubate the plates in a horizontal position with the clear side up.
- Incubate the plates at 35 °C for 24 + 2 hr and examine for coliforms and *E. coli* growth. The incubation times and temperature can be selected based on the current local reference methods.
- Some *E. coli* colonies require additional time to form the blue precipitate. Re-incubate the plates an additional 24 + 2 hr to detect any additional *E. coli* growth.
- The count plates can be counted on a standard colony counter (Figure 8.19 E)
- Enumerate the total coliforms as the sum of the red colonies plus the blue colonies associated with entrapped gas. Enumerate *E. coli* as the sum of the blue colonies with entrapped gas.
- Report the results as a colony count cfu/mL.
- Figure 8.19 F and Figure 8.19 G display an example where colonies are too numerous to count.
- Colonies may be isolated for further culturing. Lift the top film and pick the colony from the gel (Figure 8.19 H).

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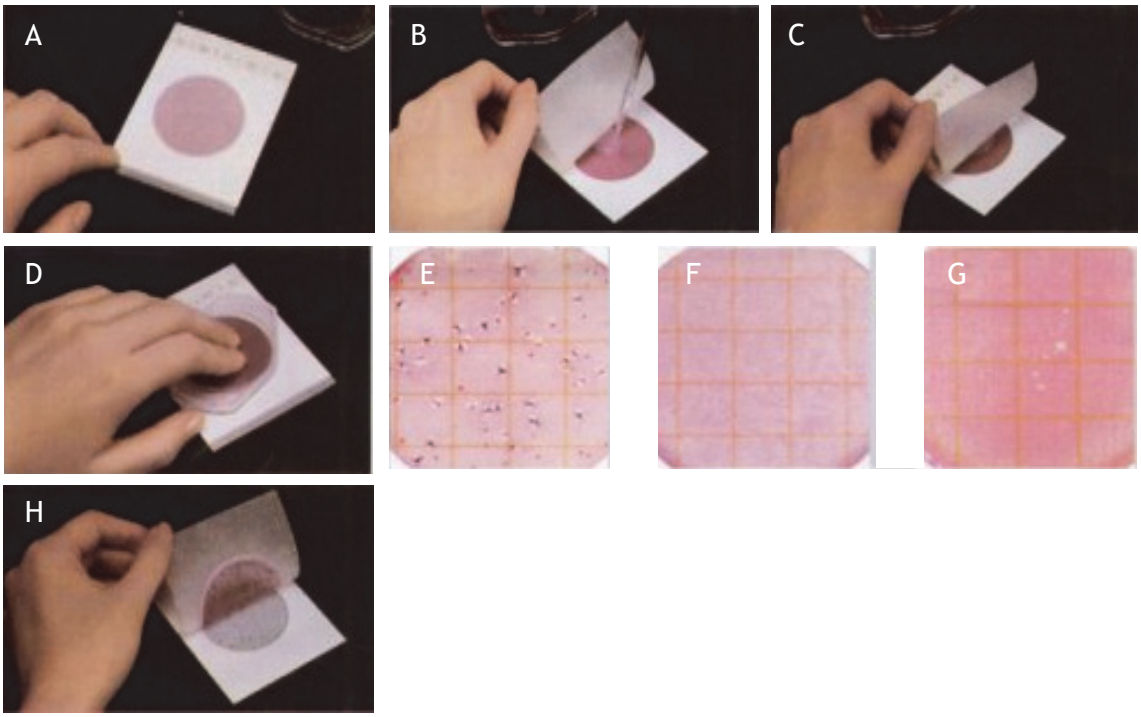

Figure 8.19 *E. coli* count – stages of the analysis protocol (www.multimedia.3M.com).

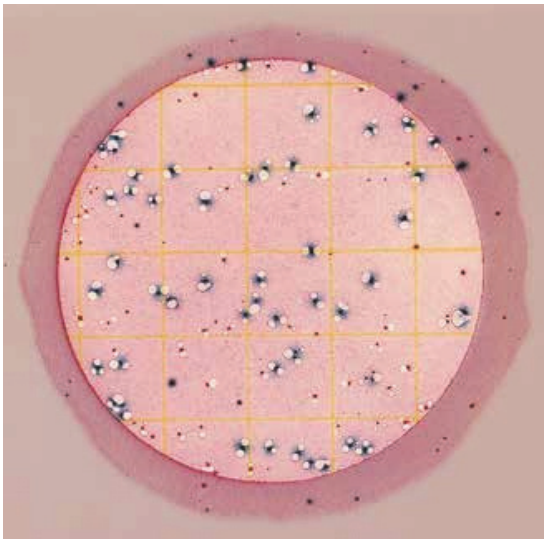

Figure 8.20 *E. coli* count = 49 (blue colonies with gas); Total Coliforms count = 87 (red colonies and blue colonies with gas). Source: UKZN PRG.

8.8.1.1.9 Calculation

$$\text{Coliform forming unit} = \frac{A}{V} \times DF$$

Where:

A = Number of counted colonies
V = Volume plated (mL)
DF = Dilution factor

8.8.1.2 *E. coli*, faecal coliforms, and total coliforms – the most probable number method[67]

8.8.1.2.1 Introduction

The most probable number (MPN) method is a very common quantitative technique, used to estimate numbers of viable cells in water, soils and sediment. The test is based on diluting the microbial content in the sample to a point where samples might not contain any microbial unit that can be cultured. In the MPN technique, replicate sample dilutions are made in an appropriate growth medium and incubated. In the

[67] This method is designed for water analysis and has been adapted by UKZN PRG in Durban for analysis of total coliform and *E. coli* in faecal sludge. This method should be cited as the specific method that is carried out in each laboratory, including the manufacturer's version and model for the total coliform and *E. coli* test, and the exact method of sample preparation.

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dilutions, some will contain a single viable cell (which will grow), whereas others will not. Usually, the growth of cells is indicated by changes in the medium. By counting the number of positive and negative tubes for each dilution, and referring to statistical tables, the MPN can be determined. The use of MPN in the quantification of cells is particularly useful for samples with low cell densities (Duncan and Horan, 2003; Oblinger and Koburger, 1975).

Presented here is the example of the Colilert 18®D commercially available test kit. For a more detailed review of available kits, refer to Bain *et al*. (2012). The Colilert 18® is a proprietary test designed for the qualitative presence/absence detection and/or quantification by MPN of total coliforms and *E. coli* in water, and faecal coliforms in wastewater. It uses what the manufacturer terms as 'Defined Substrate Technology (DST)'. The method is based on β-d-galactosidase activity, an essential enzyme that is possessed by both coliforms and *E. coli* and used for lactose fermentation. Two nutrient indicators, o-nitrophenyl-β-d-galactopyranoside (ONPG) and 4-methyl-umbellfieryl-β-d-glucuronide (MUG) are used to detect coliforms and *E. coli*, respectively. Coliforms use β-d-galactosidase enzyme to metabolise ONPG to o-nitrophenol, a yellow-coloured product. In addition, β-glucuronidase, expressed by the majority of *E. coli*, can hydrolyse MUG, forming the fluorescent product 4-methylumbelliferone. These enzymes do not usually occur in non-coliforms. Thus, the growth of non-coliforms is eliminated by this method. Nevertheless, the growth of non-coliforms that possess these enzymes is inhibited by the specially formulated Colilert 18 test matrix. The Colilert 18 test is therefore able to determine *E. coli* and single viable coliforms without interference from non-targeted organisms as compared to conventional media methods. The test can be qualitative following the presence/absence procedure or quantitative following the Quanti-tray® or Quanti-tray 2000® analysis procedure.

8.8.1.2.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.8.1.2.3 Required chemicals

- Colilert 18 reagent 'snap packs' for 18-22 hr incubation (supplied by the manufacturer)
- Colilert sterile trays (supplied by the manufacturer)
- Sterile deionised or distilled water (supplied by the manufacturer)
- Antifoam
- Thiosulphate.

8.8.1.2.4 Required apparatus and instruments

- Autoclave
- Incubator (35-37 °C)
- Pipette and pipette tips
- 50 or 100 mL beaker
- Test tube (sterile)
- Fridge or cold room (8-15 °C)
- Water bath (35-44.5 °C)
- Fluorescent UV lamp 6 watts, 365 nm
- Quanti-tray® sealer (supplied by the manufacturer)
- Quanti-tray/2,000® rubber inserts (supplied by the manufacturer)
- Sampling bottle, 100 mL (supplied by the manufacturer).

8.8.1.2.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- Detection limit: the detection limit for this analysis is 1 MPN per 100 mL of sample, and the maximum limit is 2,000 MPN per 100 mL of sample.
- If excess foam causes problems while using the Quanti-Tray®, IDEXX antifoam solution can be used.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert 18. If observed the sample should be considered invalid and discarded.

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8.8.1.2.6 Sample preservation

- The samples should be analysed immediately after sampling, if possible. Samples can be stored for up to 24 hr between 1-4 °C (Rice *et al.,* 2017). The samples should be thawed to room temperature before analysis.
- Store Colilert 18 reagent kits at room temperature (between 20-25 °C) and preferably in a cool dry place.

8.8.1.2.7 Sample preparation

For liquid samples:

- Collect the samples in 100 mL autoclaved sampling bottles.
- Ensure the sampling bottles have 1 mg thiosulphate to neutralise chlorine interferences.
- Dilute highly turbid, concentrated samples with sterile distilled water to concentrations within the range of the test.
- Test the samples at room temperature.
- For 10-fold serial dilutions, fill 9 mL of distilled water into ten tubes labelled as 10-1 through 10-10. Pipette 1 mL of the prepared sample to the first tube titled 10-1 and mix very well. Transfer 1 mL from the first tube labelled 10-1 to the next tube, labelled 10-2. Mix this tube as well. Continue this pattern to create a serial dilution series. You will end up with 9 tubes of 9 mL and 1 tube of 10 mL. Each tube represents a ten-fold dilution of the sample.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec in a blender on the highest speed.
- Transfer this solution into bottles with 1 mg thiosulphate to neutralise chlorine interferences.
- Dilute highly turbid, concentrated samples with sterile distilled water.
- Test the samples at room temperature.
- Do not dilute with buffers to avoid interferences with the Colilert reagent.
- Mix the sample by inverting 3 times.

8.8.1.2.8 Analysis protocol

Procedure - Quanti-Tray 2000® total coliforms and E. coli and faecal coliforms (quantitative):

- Switch on the Quanti-Tray® sealer and allow 15 min to warm up.
- Add a sachet of Colilert reagent to the 100 mL sample and mix. Shake the sample until the reagent is completely dissolved.
- Allow the sample to sit and the foam to settle. Add antifoam if necessary.
- Hold the tray upright with the well side facing the palm and bend the ends inwards to open up the tray.
- Pour the sample into the Quanti-tray® once the foam settles.
- Avoid contamination. Do not touch the foil.
- Hold the black rubber frame upright with one hand and place the tray against the frame to fit it into the slots.
- Feed the unit with tray side up into the sealer and collect the sealed tray at the bottom end.
- Press the reverse button on the sealer if jamming occurs.
- Incubate trays at 35 °C for 18 hr ± 4 hr for total coliforms and *E. coli.* Trays can be stacked during incubation.
- Incubate trays at 44 °C for 18 hr ± 4 hr for faecal coliforms (thermo-tolerant coliforms).

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Figure 8.21 Quanti-Tray 200 total coliforms and *E. coli* procedure (source: UKZN PRG).

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Reading

- Yellow wells = total coliforms.
- Yellow/fluorescent wells = *E. coli.*
- Multiply by the dilution factor for MPN/100 mL if necessary (IDEXX/MPN table is available at the link below[68])
- Enumerate the total coliform by counting the number of large yellow wells (including the single large well at the top) and the small wells on each tray.
- Record the numbers on the relevant data sheet and refer to the table for the total coliform MPN value.
- Enumerate *E. coli* by counting the number of large and small wells that fluoresce under UV illumination in a dark room (Figure 8.22).
- Count the positive wells and refer to the MPN table provided by the manufacturer using the link below.
- Multiply by the dilution factor for MPN/100 mL if necessary.
- Calculate and then record the MPN/100 mL and the upper and lower boundaries of the 95% confidence interval. Record the QA sample results (including zero) the same as the routine results (using IDEXX/ MPN table).

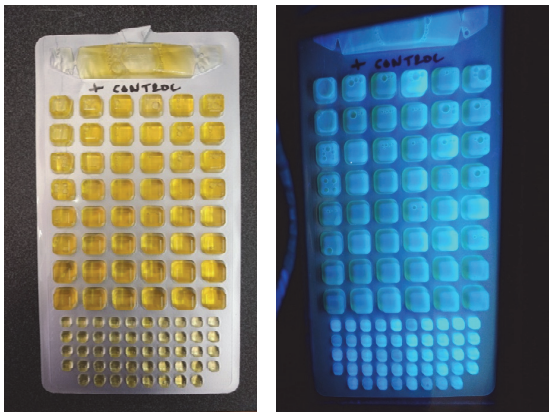

Figure 8.22 Control tray for Total Coliforms (left) and fluorescent control tray of *E. coli* (right) (source: UKZN PRG).

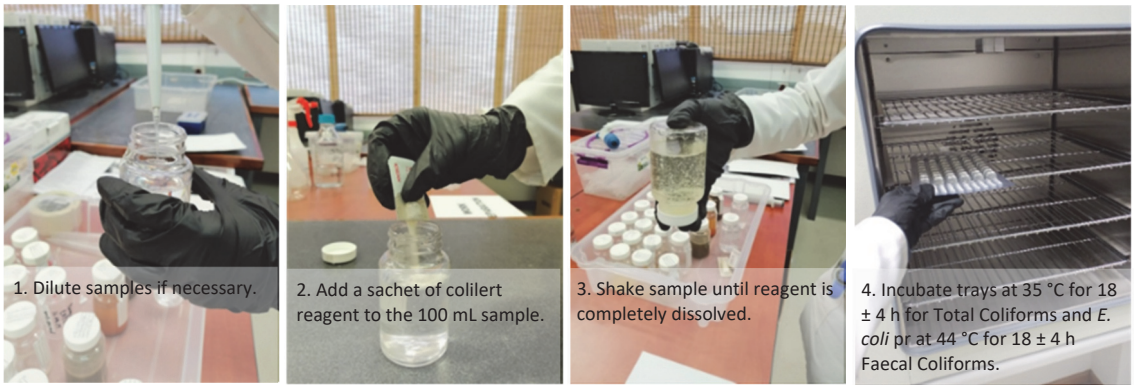

Figure 8.23 Procedure for Presence/Absence (P/A) test for total coliforms and *E. coli* and Faecal Coliforms (source: UKZN PRG).

[68] https://www.idexx.co.za/en-za/water/resources/mpn-generator/

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Procedure - presence/absence (P/A) test for total coliforms and E. coli and faecal coliforms (qualitative):

- Dilute the samples with distilled water if necessary.
- Add a sachet of Colilert 18 reagent to the 100 mL sample and mix. Shake the sample until the reagent is completely dissolved.
- Allow the sample to sit and the foam to settle. Add antifoam if necessary.
- Incubate the bottles at 35 °C for 18 hr ± 4 hr for total coliforms and *E. coli*. The bottles can be stacked during incubation.
- Incubate the trays at 44 °C for 18 hr ± 4 hr for faecal coliforms (thermo-tolerant coliforms).

Reading

- Total coliforms - samples turn yellow after incubation at 35 °C.
- *E. coli* - fluorescent.
- Faecal coliforms - samples turn yellow after incubation at 44 °C.

Disposal of used Quanti-trays

Dispose of the trays in a biohazard waste box and arrange collection by a relevant hazardous waste management company.

Table 8.16 Comparison of total coliforms, E. coli and faecal coliform procedure.

E. coli (positive control)			Yellow wells and fluorescence	
Blank sample (sterile water) (negative control)			Clear wells and no florescence	
	Total coliforms	*E. coli*	Faecal coliforms	P/A
Volume	100 mL	100 mL	100 mL	100 mL
Incubation temperature	35 °C	35 °C	44 °C	35 °C
Incubation time	18 hr	18 hr	18 hr	18 hr
Reading	Counting positive wells	UV light	UV light	UV light

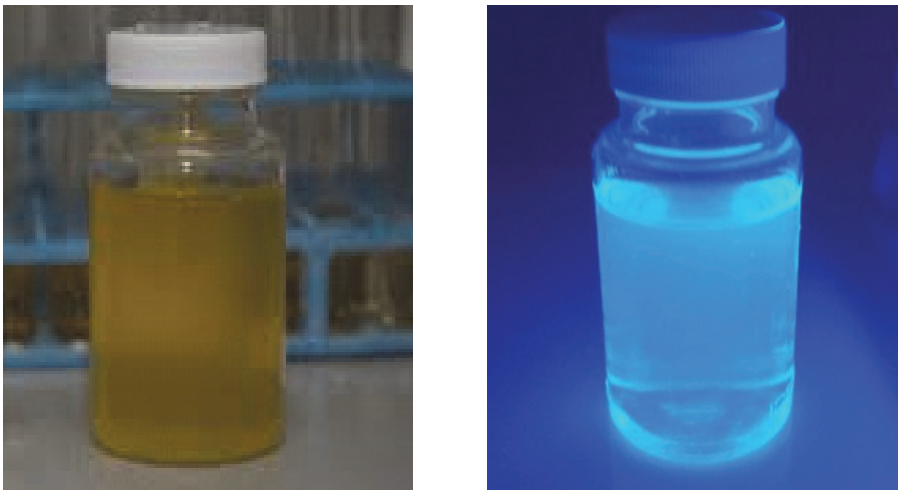

Figure 8.24 Control sample for Total Coliforms (left) and fluorescent control sample of *E. coli* (right) (source: UKZN PRG).

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8.8.1.3 Bacteriophage - plaque assay method[69]

8.8.1.3.1 Introduction

Bacteriophages are viruses that are able to infect and replicate inside of bacteria and archaea, and they are pervasive in the environment. They could potentially be an alternative to using faecal indicator bacteria, due to their close morphological and biological properties (McMinn *et al.,* 2017). The problems with the use of faecal coliforms as indicators are that they are present in many different types of animals and are not specific to humans, and they could also have different environmental fates than other pathogens (*e.g.* viruses and protozoa). Coliphage are specifically a type of bacteriophage that infect and replicate in *E. coli*. These organisms have been found to be persistent in sewage systems and resistant to treatment. Additionally, they are relatively easy to enumerate as compared to enteric viruses (Jofre *et al*., 2016). These characteristics make coliphages potentially significant in monitoring the effluent quality of wastewater and faecal sludge treatments. Coliphage can be used to track the origin of faecal contamination in the environment, and also in laboratory experiments, such as spiking into reactors to evaluate treatment performance.

Plaque assays are used to quantify bacteriophage. The theory behind the method is that a cloudy layer of bacterial cells called a 'lawn' grows across a petri dish. If a bacteriophage grows inside a bacterial cell and lyses it, then this results in a clear spot representing no growth, which is called a plaque. Since the cells are lysed, they no longer scatter light and therefore no longer look cloudy. Examples of step-by-step procedures for variants of the double-agar-layer method, single-agar-layer format, and filter-adsorption technique are presented in Rice *et al*. (2017).

8.8.1.3.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.8.1.3.3 Required chemicals

- Tryptone agar
- Tryptone broth
- Calcium chloride
- Phosphate buffer saline (PBS)
- Reagent grade water
- Yeast extract
- Glucose
- NaCl
- Beef extract
- Glycerol.

8.8.1.3.4 Required apparatus and instruments

- Laminar flow hood
- Autoclave
- Incubator (37 °C)
- Refrigerator
- Petri dishes (sterile)
- Water bath with adjustable temperature (45-100 °C)
- Pipettes and pipette tips
- 50 or 100 mL beaker
- 1 or 2 mL Eppendorf tubes
- Bunsen burner
- Shaker (optional)
- Magnetic stirrer and stirrer bars
- Mass balance
- Test tube racks and Eppendorf holders
- Thermometer

[69] This method is based on Method 9224 E of the Standard Methods for the Examination of Water and Wastewater and should be cited as Rice *et al.* (2017) as described in Velkushanova *et al*. (2021).

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8.8.1.3.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions, and interferences that are specific to this method include:

- Coliphage positive controls (Coliphage ΦX174) must be prepared according to the procedure described in the standard method 9224B (Rice *et al.,* 2017).
- Non-sterile/contaminated apparatus such as test tubes, pipette tips or pipette can result in false results.
- Molten agar should not be too hot (> 45 °C). Insufficient cooling can result in the death of host cells put on the top of the soft agar and growth of *E. coli* host cells and plaques will not occur. However, if the agar is cooled too much (< 45 °C) it will solidify before it is dispensed.
- If the sample is too diluted no plaques may appear. Ensure the appropriate dilution factor is used.

8.8.1.3.7 Sample preservation

- The samples should be analysed immediately after sampling if possible. The samples can be stored for up to 24 hr between 1-4 °C (Rice *et al.,* 2017). The samples should be thawed to room temperature before analysis.

8.8.1.3.8 Sample preparation

For liquid samples:

- No preparation for liquid samples is required. If necessary, appropriate dilutions should be made using the serial dilution procedure. For faecal sludge samples, a minimum of four sample dilutions is required. However, five or more dilution are preferred.

For slurry to solid samples:

- Weigh out between 1.8 g and 2.0 g of thoroughly-mixed faecal sludge sample into a beaker.
- Dilute the sample gravimetrically using PBS buffer and transfer to the blender, as described in Section 8.4.2.
- If necessary, adjust the dilution based on the specific sludge characteristics.
- Blend for 30 sec in a blender on the highest speed.
- Dilution of the sample: prepare the dilution of the sample in 9 mL PBS by taking 1 mL from the 50 mL diluted sample and add to 9 mL PBS. Vortex for 30 sec and repeat the process until 6 tenfold dilutions are made (10^{-1} to 10^{-6}). Since viruses can grow to incredibly high concentrations, they need to be diluted in order to count them effectively.
- The same sample preparation method applies to all replicate measurements (if applicable).

Serial dilution for host culture

- Put 9 mL of tryptone broth in each of ten culture tubes labelled as 10^{-1} through 10^{-10}. These tubes will be used for viral serial dilutions.
- Take 1 mL of the coliphage culture stock from the freezer and let it thaw in the laminar flow. Transfer 1 mL of it to the tube labelled 10^{-1} with a pipette. Mix the tube well. This is the first ten-fold dilution (*i.e.* a 1 in 10 dilution)
- Take 1 mL of the mixed culture from your tube labelled 10^{-1} and transfer it with a new pipette to the next tube, labelled 10^{-2}. Mix this tube as well.
- Continue this pattern to create a serial dilution series. You will end up with 9 tubes of 9 mL and 1 tube of 10 mL. The viral loads in your tubes will be diluted anywhere from 10 times (your first tube) or 100 times (your second tube) to ten billion times (your final tube).
- Label all the dilution tubes and media as follows. Each tube represents a ten-fold dilution of the virus.
  - Four tryptone soft agar tubes: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.
  - Four tryptone hard agar plates: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.
  - Six PBS tubes: 10^{-1} through 10^{-6}.

8.8.1.3.8 Analysis protocol

Preparation of media

- Tryptone top (soft) agar: add 10 g tryptone, 0.2 g calcium chloride, 5 g sodium chloride, 1 g yeast extract, 1 g glucose and 7 g agar in 1 L of water in a conical flask. Using a stirrer, mix until most of the solute is dissolved. Sterilise and dissolve by autoclave at 121 °C for 20 min. Let the agar cool while it is still molten and dispense 7 mL of the molten agar in the test tubes or 15 mL centrifuge tubes.

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- Tryptone bottom (hard) agar: add 10 g tryptone, 0.2 g calcium chloride, 5 g sodium chloride, 1 g yeast extract, 1 g glucose and 15 g agar in 1 L of water in a conical flask. Using a stirrer, mix until most of the solute is dissolved. Sterilise and dissolve by autoclave at 121 °C for 20 min. While the agar is cool pour it into bottom Petri dishes.
- Tryptone broth: add 10 g tryptone, 0.2 g calcium chloride, 5 g sodium chloride, 1 g yeast extract and 1 g glucose in 1 L of water in a conical flask. Using a stirrer, mix until the solute is dissolved. Sterilise and dissolve by autoclaving at 121 °C for 20 min. Store at room temperature.
- Phosphate buffer saline (PBS): add 5 mL of $MgCl_2 \cdot 6H20$ + 1.25 mL KH_2PO_4 into 1,000 mL dH_2O. Sterilise at 121 °C for 20 min. Store the sample in a cold room or refrigerator at 4 °C. The phosphate buffer will be used as diluent for the sludge sample.
- PBS dilution tubes: aseptically dispense 9 mL of PBS into sterile test tubes or 15 mL centrifuge tubes pre-sterilised by autoclaving at 121 °C for 15 min.

Storage of E. coli C host culture

- Grow *E. coli* overnight in 10 mL of tryptone broth in a centrifuge tube and incubate at 36.5 ± 2 °C. Once grown, add 10 mL of 50% glycerol and then vortex until thoroughly mixed. Once mixed, aliquot 200 µL into vials or 1 mL Eppendorf tubes. Store at -80 °C in the freezer. Autoclave at 121 °C for 10 min. Store at 4 °C.

Preparation of E. coli C host culture

- The day prior to the assay, inoculate 5ml of tryptone broth with the stored *E. coli* host culture using an inoculating loop and incubate at 36.5 ± 2 °C overnight. Transfer 1.5 mL of the incubated culture into 30 mL tryptone broth and incubate for 4 hr at 36.5 ± 2 °C with gentle shaking. Ensure that the lid is loosely closed to ensure enough oxygen for growth. Label the tube as *E. coli*/date/temperature.
- Prepare tryptone agar plates, tryptone soft agar tubes (7 mL/tube) and tryptone broth tubes (10-15 mL/tube). Label appropriately and store in the cold room for use the next day.

Assay procedure

- Warm 100 mL of the sample in a water bath at 44.5 ± 1 °C for 3 min.
- Add 5 mL of $CaCl_2$ solution and 5 mL of appropriate host bacterium (*E. coli* C) preparation to the warmed sample.
- Mix the inoculated sample with 100 mL melted tryptone agar at 44.5 ± 1 °C and distribute to eight 150 × 15 mm petri dishes.
- For the positive control, mix 1 mL of the appropriate positive control preparation (30-80 PFU/mL) and 1 mL host bacterium, *E. coli* C with 12.5 mL warmed agar that has been diluted with an equal volume of warm sterile water.
- Pour into a single 150 × 15 mm Petri dish.
- Repeat for a negative control without the 1 mL of phage preparation.
- Incubate the dishes at 36.5 ± 2 °C overnight and examine for plaques the following day.
- The petri dishes will be covered with a cloudy area of bacteria cells and clear spots indicating regions where plaques have formed.
- Count the number of plaques on the eight dishes that received the sample.

8.8.1.3.9 Counting and calculation

Plaque visualisation, counting and calculating of viral titer (pfu/mL):

- Count the plaques on each plate, taking the average for any technical replicates of the same dilution.
- Determine the viral titer of the stock sample by taking the average number of plaques for a dilution and the inverse of the total dilution factor.

  NOTE: As an example, 30 and 32 plaques counted for replicates of the 1×10^{-7} dilution [31 (average)/10^{-7} (dilution) × 0.4 mL (inoculum)] would yield a titer of 7.75 × 108 pfu/mL.

$$\text{PFU/mL} = \frac{\text{Average number of plaques}}{\text{DF} \times \text{V}}$$

DF = Dilution factor
V = Volume of diluted virus added to the plate.

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8.8.1.4 Helminth – microscopy method[70]

8.8.1.4.1 Introduction

The prevalence of helminth infections in people living with rudimentary water and sanitation in low-income countries is generally high. Due to the extreme hardiness of the eggs of the roundworm, *Ascaris lumbricoides*, they are used in the waste and sanitation field as a 'marker' or 'indicator' for the safe end-use of resource recovery products from faecal sludge. Since *Ascaris* eggs are so difficult to inactivate, if treatment of faecal sludge is successful in killing *Ascaris* eggs, then it is likely that other pathogens are also inactivated (viruses, bacteria, protozoa and helminths). Other commonly found helminths are *Trichuris trichiura*, *Taenia* spp. and in areas with very sandy soils, hookworm spp. (*Necator americanus* and *Ancylostoma duodenale*) and *Strongyloides stercoralis*. Various animal parasites are also commonly encountered. In countries where piped water is not chlorinated, the presence of free-living soil and water organisms are encountered and need to be differentiated from pathogens.

Helminth eggs are thought to adhere to soil particles, possibly as a result of charge interactions with, or adsorption of, eggs to the particles. Faecal sludge samples are often contaminated with silica particles, hence the use of ammonium bicarbonate as a wash solution. Liquid samples that have a high fat content need to be treated differently. Here, it is suggested that a surfactant such as Tween 20[D], TritonX-100[D], or 7X[D] is used to break up the fats, rather than ammonium bicarbonate.

Laboratory testing for helminths is based on four main principals: washing, filtration, centrifugation and flotation of the eggs to remove them from the various waste media:

1. Ammonium bicarbonate is used as both a wash solution and also to dissociate the eggs from the soil particles (surfactants should be used for fatty samples).
2. Filtration, using 100 µm and/or 20 µm sieves is used to separate larger and smaller particles from the eggs both after washing and after flotation.
3. Centrifugation is used to sediment the deposit so water can be discarded after washing, to aid the separation process during flotation, and to sediment the washed eggs after flotation.
4. Flotation, using a solution of zinc sulphate at a specific gravity (SG) of 1.3 is used to separate eggs (with a relative density of < 1.3) out of the matter retained (retentate) with them on the 20 µm sieve.

8.8.1.4.2 Safety precautions

- General health and safety (H&S) procedures specific for conducting the laboratory analysis of faecal sludge are presented in Section 8.2. Before conducting this method, it is important to be familiar with Section 8.2.3 to ensure safety measures are properly carried out.
- Appropriate personal protective equipment (PPE) should be used; specific details are covered in Section 8.2.3.1.

8.8.1.4.3 Required chemicals

- Physiological saline (8.5 g/L NaCl)
  Dissolve 8.5 g sodium chloride in 1,000 mL deionised water. If this amount will not be utilised in less than a week, it is preferable to decant it into smaller containers and autoclave for 15 min at 121 °C. Cool to room temperature and store.
- Ammonium bicarbonate (AmBic)
  Dissolve 119 g of ammonium bicarbonate in 1 L de-ionised water (use a magnetic stirrer and bar magnet) - store in a glass jar.
- Tween 80, TritonX100, or 7X
  Note: use neat - see 'Test procedure' below.
- Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)
  Dissolve 500 g zinc sulphate in approximately 800 mL deionised water (use the magnetic stirrer and bar magnet) and adjust SG using more of the chemical or water to raise or lower the SG to 1.3.
- 0.1 N sulphuric acid (H_2SO_4)
  Add 500 mL de-ionised water to a 1 L plastic bottle, pour 3 mL concentrated sulphuric acid into

[70] This method is based on Pebsworth *et al*. (2012), Belcher *et al.* (2015), Naidoo et al., (2016), Grego *et al.* (2018), Naidoo *et al.* (2019), and Naidoo *et al.* (2020), and should be cited as the UKZN PRG Helminth Method

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a 10 mL graduated cylinder, then pour the H_2SO_4 into the plastic bottle containing the water, then re-cap and shake. Uncap, add 497 mL of deionised water to the plastic bottle, recap and shake.

8.8.1.4.4 Required apparatus and instruments

- Compound microscope with 10× and 40× objectives (and preferably, a camera)
- Bench-top centrifuge with a swing-out rotor that can spin a minimum of 8 × 15 mL plastic conical test tubes (*e.g.* Falcon tubes) and, if possible, buckets that can also spin a minimum of 4 × 50 mL plastic conical test tubes
- 15 mL plastic conical test tubes (Falcon tubes)
- 50 mL plastic conical test tubes (Falcon tubes)
- Sink with hose attached to tap for washing using strong water pressure
- Top-pan balance (for weighing up to 1.20 g and accurate to 2 decimal places)
- Magnetic stirrer and bar magnets
- Vortex mixer
- Hydrometer that can measure SG between 1.2 and 1.3
- 100 µm mesh stainless steel pan sieve, diameter 200 mm, height 50 mm
- 20 µm mesh stainless steel pan sieve, diameter 200 mm, height 50 mm
- 20 µm mesh stainless steel pan sieve, diameter 100 mm, height 45 mm
- Plastic conical test tubes (Falcon tubes), 15 mL or 50 mL
- Plastic test tube racks to hold the 15 mL Falcon tubes (and if using 50 mL tubes, one for these)
- Plastic 200 mL beakers
- Plastic 'hockey-stick' shaped spreaders
- Plastic 3 mL Pasteur pipettes (non-sterile)
- Non-sterile gloves
- Applicator sticks and wooden tongue depressors
- Microscope slides (76 × 26 × 1.2 mm)
- Cover glasses (22 × 40 mm).

8.8.1.4.5 Quality control

General information on quality assurance and quality control (QA/QC) is provided in Section 8.3. Information on standards, operating conditions and interferences that are specific to this method includes:

- Source an uninfected slurry or semi-solid sludge sample (preferably of a consistency very similar to the samples being tested) and uninfected liquid sludge sample, to make up QA/QC samples. Add 10% formalin (in a 1:10 ratio of formalin to sludge) and store at 4 °C for up to 6 months.
- Make one NEGATIVE control per sample type being tested, *i.e.* one uninfected sample weighed (in grams) and one measured (in litres).
- Make up one POSITIVE control per sample type being tested, *i.e.* spike a known number of *A. suum* eggs into one weighed solids sample and another known number of *A. suum* eggs into a measured liquid sample (egg stocks for preparing the positive controls should be purchased from a reputable supplier).
- Run a negative and a positive sample in parallel with a batch of similar consistency samples per day.
- Once the technician has processed and examined the slides made from the controls, they should be re-examined by a senior, experienced analyst as a control for the microscopy part of the analysis.
- Most sludge and wastewater methods consider recovering > 80% of spiked eggs to be extremely satisfactory.

8.8.1.4.6 Sample preservation

After collection, the samples should be stored at approximately 4-10 °C. Processing is always best carried out as soon after sampling as possible, but providing that there is sufficient moisture and the samples are fairly large (≥ 100 mL/100 g), the eggs should be unharmed and development will be arrested at these low temperatures.

8.8.1.4.7 Analysis protocol

Procedure for slurry, semi-solid and solid faecal sludge samples (TS > 5%):

1. Place a 200 mL plastic beaker (labelled with the sample number) on the top-pan balance, zero the balance, and weigh 10 g or 20 g of the sample into the beaker.

   Note: if waste material is very dry (*e.g.* pelletised or completely desiccated), then soak the weighed sample for 12 – 24 hr in ± 80 mL physiological saline to soften. Next, break up and mix the sample well in the saline. Allow to stand to sediment the

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solids for 4 hr. Remove as much supernatant as possible without disturbing the deposit, and continue with the next step below.

2. Add 50-80 mL AmBic and a magnetic stirring bar, and mix on the magnetic stirrer for 10 min.
3. Pour this mixture over the 100 µm mesh sieve placed on top of the 20 µm sieve (wet the sieves with tap water first).
4. Rinse the beaker with tap water and pour over the sieves.
5. Wash the magnet over the sieves and remove, wash the 100 µm sieve well (using a 'hockey stick'-shaped spreader, or preferably, a gloved hand) over the 20 µm filter, regularly checking the bottom sieve for fluid build-up. Use the same hockey stick spreader to stir the sample on the 20 µm sieve while holding the 100 µm sieve directly above so as not to lose any sample. When the 20 µm sieve has drained sufficiently, place the 100 µm sieve back on top and continue washing. Repeat this until the sample on the 100 µm sieve is sufficiently well washed.
6. Separate the sieves and then rinse the 20 µm sieve well. Use water pressure to wash the material to one side of the sieve to make collection easier.
7. Rinse all the material off the 20 µm sieve into the original rinsed-out, labelled beaker.
8. Pour the beaker contents into 4 × 15 mL conical test tubes labelled with a sample number or if the retentate is large, use 50 mL labelled tubes. Rinse the beaker with a small volume of water and add this to the test tubes until all the sample is collected. (The aim after the next step is to have ≤1 mL deposit in a 15 mL tube and ≤5 mL in a 50 mL tube.)
9. Centrifuge at 3,000 rpm (1,512 g) in the centrifuge with a swing-out rotor for 10 min.
10. Pour off the supernatant, leaving deposits in the test tubes.
11. Place the test tubes in the rack with the applicator stick in each (as a stirring rod) and pipette in $ZnSO_4$, 3 mL at a time, vortexing in between the addition of the chemical, until the tubes are filled to the 14 mL mark for the 15 mL tubes/45 mL mark for the 50 mL tubes.
12. Centrifuge at 2,000 rpm (672 g) for 10 min.
13. Pour the supernatant flotation fluid over the 100 mm diameter 20 µm sieve. Wash the remaining deposits out of the test tubes and keep one aside for re-use.
14. Wash the material on the sieve well with tap water and rinse it down to one side of the sieve for collection. Using a 3 mL plastic pipette, transfer the material back into the test tube kept aside.
15. Centrifuge at 3,000 rpm (1,512 g) for 10 min to obtain the final deposit.
16. Pour off the supernatant water and pipette up the deposit, place it on one or more microscope slides (but make one slide at a time so they do not stand for long periods and dry out), place a 22 × 40 mm coverslip on top, examine and count every *Ascaris* egg, classifying them as viable, potentially viable or dead. *Trichuris*, *Taenia,* and hookworm spp. eggs must also be counted and assessed simply as potentially viable or dead (Figure 8.25).

Procedure for liquid faecal sludge samples (TS <5%):

1. Select an appropriate volume of the sample.
   Note: If the water is effluent from a wastewater treatment plant and is fairly clean with low suspended solids, then it is preferable to use a large sample of 5-10 L, measured out using a 1 L measuring cylinder. If the sample is dirty water, but with low to moderate suspended solids, use a 1-5 L sample.
   Note: If the sample is black water with a high concentration of solids (3-5%), then use amounts of 200-500 mL.
   Note: If the sample is fatty, then measure out a selected sample size (from 200-500 mL). For all types of liquid samples, pour into a plastic beaker large enough to contain the sample with at least 5-10 cm above the surface, so that it does not spill when mixing on the magnetic stirrer.
2. Add 1 mL per litre of neat Tween 80 or 7X directly into the sample (so as to make a ± 0.1% solution). Mix well using the magnetic stirrer and magnet in the beaker for 20 min.
3. The measured sample is poured slowly through a 100 µm sieve which fits on top of a 20 µm sieve and is well washed, regularly checking the bottom sieve for fluid build-up.
4. Separate the sieves and then rinse the 20 µm sieve well and wash the material to one side of the sieve for collection.

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5. Rinse all the material off the 20 µm filter into 2-4 × 15 mL or 50 mL conical test tubes.
6. Centrifuge at 3,000 rpm (1,512 g) in the centrifuge with a swing-out rotor for 10 min.
7. Step 7. Pour off the supernatant and retain the deposits left in 2-4 ×15 mL or 50 mL test tubes.
8. Place the test tubes in the rack with an applicator stick in each (as a stirring rod) and pipette in $ZnSO_4$, 3 mL at a time, vortexing in between the addition of the chemical, until the tubes are filled to the 14 mL mark.
9. Centrifuge at 2,000 rpm (672 g) for 10 min.
10. Pour the supernatant flotation fluid over the 100 mm diameter 20 µm sieve. Wash out the test tubes and keep one aside for re-use.
11. Wash the material on the sieve well with tap water and rinse down to one side of the sieve for collection. Using a 3 mL plastic pipette, transfer the material back into the test tube kept aside.
12. Centrifuge at 3,000 rpm (1,512 g) for 10 min to obtain the final deposit.
13. Pour off the supernatant water. There should be about 0.2-0.3 mL water left on top of the pellet. If the pellet is small, you may be able to pipette up everything and make one slide to examine. If the deposit is thick, dilute it with water (0.1 mL at a time) until it is of a thinner consistency, then pipette up enough to make one slide at a time, place it on a microscope slide, place a 22 × 40 mm coverslip on top, and examine and count every *Ascaris* egg, classifying them as viable, potentially viable or dead. *Trichuris*, *Taenia* and hookworm spp. eggs must also be counted and assessed simply as potentially viable or dead. Continue making more slides until the entire sample has been examined and all the helminth eggs have been counted and assessed.

8.8.1.4.8 Calculation

Once all the eggs have been counted, the results should be calculated to report the number of eggs per litre or per gram for each species of helminth and within each species, and those that are viable and non-viable.

Example 1

If 2.5 L of liquid sample was analysed and there were 500 *Ascaris* eggs found, then use simple proportions: 500/2.5 : X/1 = 500 × 1/2.5 = 200 eggs/L.

Example 2

If 15 g of solid sample was analysed and 3,450 *Ascaris* eggs were counted, then using proportions again:

3,450/15 : X/1 = 3,450 × 1/15 = 230 eggs/g of sample (wet mass) (abbreviated EPG).

Note: Adjust the egg counts to per gram dry mass (or per gram TS) if a sample of the sludge has been tested for moisture content.

8.8.1.4.9 Data set example

Table 8.171 Data set of faecal sludge samples.

ID No.	Sample type	Sample quantity	*Ascaris* - DEAD			*Ascaris* -p otentially VIABLE				Trich	Trich	Taen	Taen	Other
			Inf	Dead	Nec	Imm	Mot	Devel	Undev	Pot vi	Dead	Pot vi	Dead	
0.1	Effluent	10 L	1	261	12	3	9	15	8	2	26	1	7	0
	Results per litre		<1	26.1	1.2	<1	<1	1.5	<1	<1	2.6	<1	<1	0
0.2	Sludge	10 g	6	543	28	23	19	267	399	88	54	49	9	1 E v
	Results per gram		<1	54.3	2.8	2.3	1.9	26.7	39.9	8.8	5.4	4.9	<1	<1 E v

ID No. = Sample identification number
Inf = Infertile, *i.e.* eggs that were not fertilised
Nec = Necrotic, *i.e.* egg contains a dead, shrivelled larva
Imm = Immotile larva, healthy looking, but not moving
Mot = A motile larva
Devel = Embryo in egg in ≥ 2-cell stage of development
Undev = Embryo in single cell stage
Trich = *Trichuris* sp.
Taen = *Taenia* sp.
Pot vi = Potentially viable, i.e for the same developmental stages as described under "*Ascaris* - potentially viable": Imm, Mot, Devel, Undev
Dead = The developmental stages described under "*Ascaris* - DEAD": Inf, Dead, Nec
Other = Any other helminth eggs found (count and record number of eggs only)
E v = *Enterobius vermicularis*.

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Interpretation of results

The term helminths encompasses round worms (nematodes), tapeworms (cestodes) and flatworms (trematodes). The nematodes that are a concern in sanitation are those that lay eggs or produce larvae in an undeveloped stage and require time in the soil to develop into infective larvae (geohelminths) *e.g. Ascaris* spp., *Trichuris* spp., hookworm spp. and *Stongyloides stercoralis*. Other nematode eggs that rapidly develop larvae and are infective for humans in a few hours, *e.g. Enterobius vermicularis*, may also be a concern.

Geohelminth eggs are considered as potentially viable (and thus infective) if they are in the undeveloped stage, developing stages [a 2-, 4-, 8-, 16, 32-cell stage or more (blastula stages) to an immature larva (gastrula)], then to a developed larva (L1 and finally an infective L2 larva). If an L2 larva is moving in the egg, then it is viable and infective. When eggs that are undeveloped or in early cleavage (one of the blastula stages) die, they may become globular, have broken shells or collapsed walls, or appear empty inside - these are termed dead. If a formed larva dies inside the egg, it appears shrivelled and occupies much less space than a plump, healthy viable larva, and is termed necrotic. Eggs that have never been fertilised (infertile) cannot develop and are therefore classified under the NON-viable eggs.

Cestodes such as *Taenia* spp. contain an oncosphere within the egg that does not develop further, thus it is described as potentially viable if it looks in good condition and the hooklets are visible, and dead if the contents are globular or have no structure. Cestode eggs (except for *Hymenolepis nana*) require an intermediate host (such as the pig) to ingest them before that host passes on the infection to humans; however, ingesting *Taenia solium* eggs poses a serious risk for humans to become the intermediate host and develop cysts in the brain resulting in neurocysticercosis (*T. saginata* poses no risk). The eggs of these two species are indistinguishable and therefore all *Taenia* eggs are counted, assessed for potential viability and reported as *Taenia* sp.

Some trematode eggs are excreted in the undeveloped stage and a miracidium develops in approximately two weeks, while others contain a miracidium when laid - these eggs are only a concern if fresh sanitation waste is dumped into water bodies as they require aquatic plants to encyst on or an intermediate host to develop within, for transmission to occur. All trematode and cestode eggs (except for *Taenia* spp.) are thus counted and recorded, however recording their viability status is optional and not a requirement unless specifically requested by the client.

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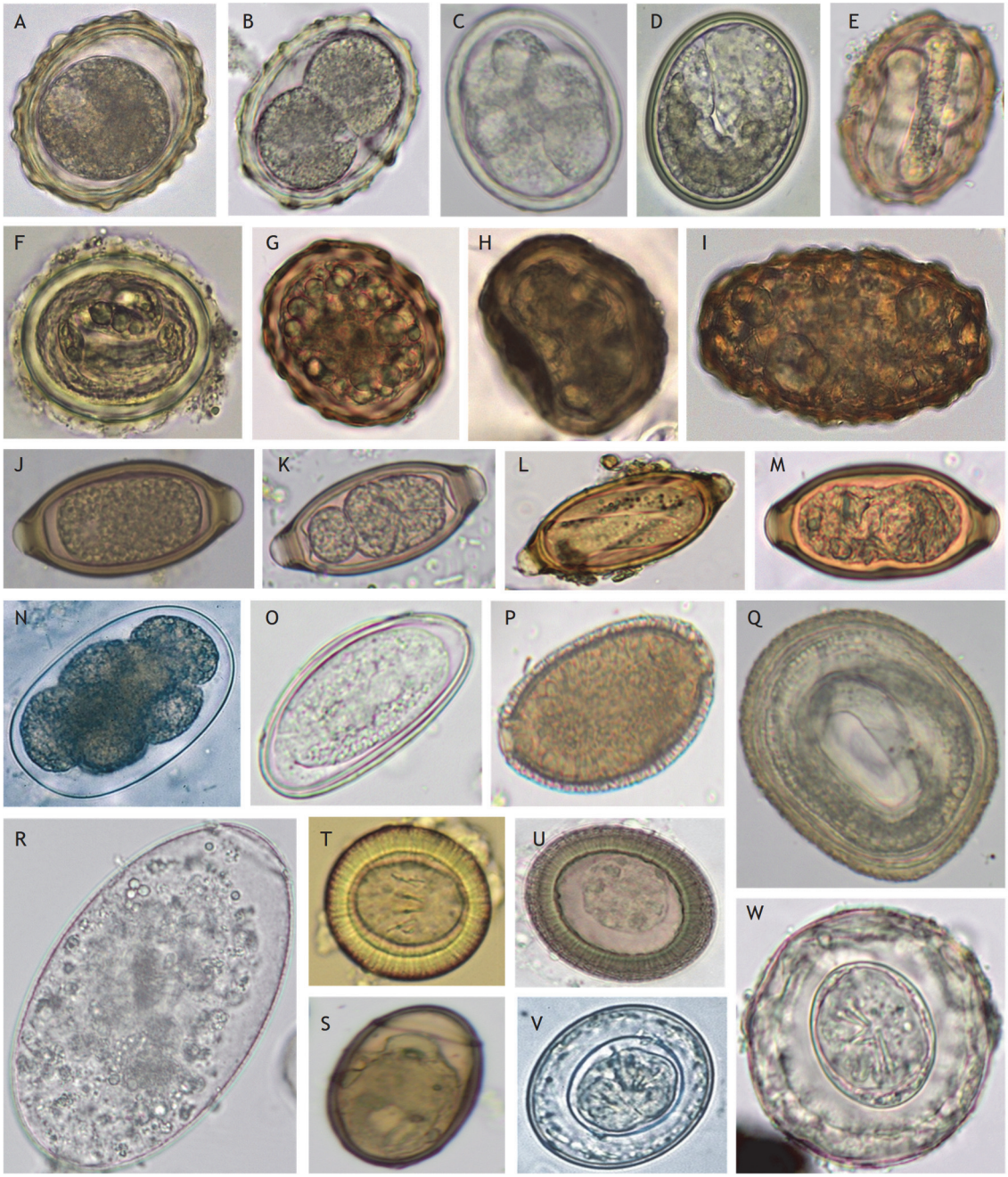

Figure 8.25 Photographs of some helminth eggs A) Undeveloped *Ascaris* egg, B) *Ascaris* 2-cell developing, C) *Ascaris* multiple cells developing, D: *Ascaris* gastrula, E) *Ascaris* viable larva, F) *Ascaris* egg containing necrotic (dead) larva, G) dead *Ascaris* egg containing globules, H) dead *Ascaris* egg, empty with collapsing wall, I) infertile *Ascaris* egg, J) undeveloped *Trichuris* egg, K) developing *Trichuris* egg, L) *Trichuris* egg containing a viable, motile larva, M) dead *Trichuris* egg, N) Hookworm sp. developing, O) *Enterobius vermicularis* dead, P) *Capillaria* sp. dead, Q) *Toxocara* sp. developed larva, R) *Fasciola* sp. dead, S) *Dicrocoelium dendriticum* possibly viable, T) *Taenia* sp. egg in good condition (probably viable), U) dead *Taenia* sp. egg, V) *Hymenolepis nana* possibly viable; and W) *Hymenolepis diminuta* possibly viable (source: UKZN PRG).

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