SCOPING PAPER

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Centre for Science and Environment

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Contents

ABBREVIATIONS AND ACRONYMS

BOD	:	Biochemical oxygen demand
Cd	:	Cadmium
COD	:	Chemical oxygen demand
CSE	:	Centre for Science and Environment
DO	:	Dissolved oxygen
DWWT	:	Decentralized wastewater treatment system
E. coli	:	Escherichia coli
Fe	:	Iron
FS	:	Faecal sludge
FSM	:	Faecal sludge management
H. eggs	:	Helminth eggs
$NH4^+$	:	Ammonium
Ni	:	Nickel
Pb	:	Lead
PO_4	:	Phosphate
QA/QC	:	Quality assurance/quality control
rpm	:	Revolutions per minute
Std	:	Standard
TN	:	Total nitrogen
TP	:	Total phosphorus
TS	:	Total solid
TSS	:	Total suspended solid
VFA	:	Volatile fatty acid
VS	:	Volatile solid
VSS	:	Volatile suspended solid
WWTP	:	Wastewater treatment plant
Zn	:	Zinc

Chapter 1

1. Introduction

Faecal sludge management is a management system that safely collects, transports and treats faecal sludge (also called septage) from pit latrines, septic tanks or other on-site sanitation facilities. Selecting the most suitable faecal sludge treatment technology is not only about providing the best technology at the lowest cost, but also about sustainability, including social and environmental acceptance, and institutional feasibility. Faecal sludge is an offensive material that contains pathogens, can generate odours and cause surface-water as well as groundwater pollution. It is raw or partially digested, slurry or semisolid form, the collection, storage or treatment of combinations of excreta and black water, with or without grey water. It is the solid or settled contents of pit latrines and septic tanks. It differs from sludge produced in municipal waste water treatment plants.

Faecal sludge characteristics can differ widely from household to household, from city to city, and from country to country. The physical, chemical and biological qualities of faecal sludge are influenced by the duration of storage, temperature, soil condition, and intrusion of groundwater or surface water in septic tanks or pits, performance of septic tanks, and tank emptying technology and pattern. Characteristics of faecal sludge may vary widely due to climate, toilet type, diet and other variables. Performing a waste characterization study to understand local conditions provides data that factor into treatment plant sizing, as well as estimating the value of the products that can be derived from the treatment process.

1.1 BACKGROUND

The Centre for Science and Environment (CSE) is a public-interest research and advocacy organization based in New Delhi. CSE researches into, lobbies for and communicates the urgency of development that is both sustainable and equitable.

CSE has for the past few years advocated septage and decentralized wastewater treatment that can be undertaken by institutions and individuals for sustainable water and wastewater management. With the aim to push forward viable solutions, CSE is setting up a state-of-the-art referral laboratory for Faecal Sludge Management (FSM). The lab will characterize wastewater and faecal sludge—it will be the first in India to do so—to compare the performance (in terms of effective treatment) of various available on-site natural sanitation technologies.

This scoping paper documents protocols for various parameters for wastewater and faecal sludge characterization followed by labs in different parts of the world. It has also helped CSE's new FSM lab select the protocol for different parameters it will be follow for wastewater and faecal sludge characterization. The selected protocols will be validated experimentally in the FSM lab.

1.2 GLOBAL RELEVANCE OF ON-SITE SANITATION TECHNOLOGIES

The sanitation needs of 2.7 billion people worldwide are served by on-site sanitation technologies; this number is expected to grow to 5 billion by 2030. Only limited sections of urban business centres are linked to sewers. In Latin America, however, over 50 per cent of

the houses in cities are connected to a sewerage system, and most houses in medium-sized and small towns are served by on-site sanitation systems (OSS), notably septic tanks. OSS are also common in peri-urban areas of high-income countries. In the US, for example, 25 per cent of the houses are served by septic tanks.

1.3 TYPES OF TECHNOLOGY

There are a variety of on-site wastewater and faecal sludge treatment technologies. The treatment efficiency of the different technologies depends upon various external factors. The geography, food culture of the society and climatic conditions are important factors that affect treatment efficiency.

Taking into consideration Indian conditions, CSE has selected the following eight on-site sanitation technologies for performance assessment:

1. Decentralized wastewater treatment system (DWWTs) with constructed wetland
2. Soil biotechnology (SBT)
3. Phytorid wastewater treatment technology
4. Sintex-packaged sewerage treatment plant (PSTP)
5. Anaerobic process-based faecal sludge treatment (co-composting)
6. DEWATs with vortex system
7. Bio toilet
8. Bio digester

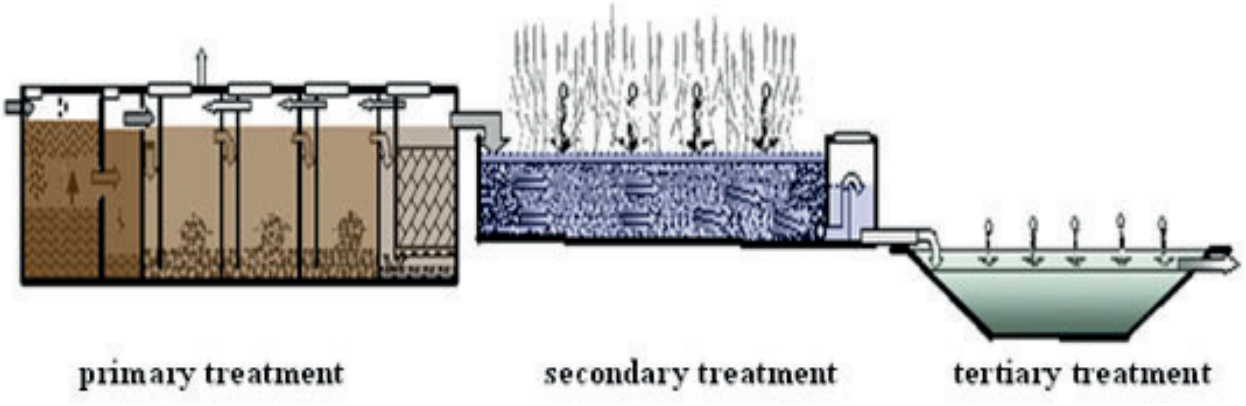
1.3.1 Decentralized wastewater treatment system (DWWT)

The wastewater enters a two-chambered settler for removal of suspended solids which is followed by its flow into an anaerobic baffled reactor (ABR). The ABR at the site is five chambered which facilitates anaerobic degradation of the organic impurities and reduces about 60–70 per cent of the BOD and COD levels of the wastewater. The treated water is further improved as it passes through the planted gravel filter (PGF) bed. The PGF at the site has Canna and Typha plantations. The treated water is stored in a collection tank.

1.3.2 Soil biotechnology (SBT)

Soil biotechnology is a terrestrial system for wastewater treatment based on the principle of trickling filter. It was developed by IIT Bombay.

Figure 1: A schematic of decentralized wastewater treatment system



SBT engages three fundamental processes of nature—photosynthesis, respiration and mineral weathering. Suitable mineral constitution, culture containing native micro-flora and bio-indicator plants are the key components of the system. It is also known as constructed soil filter (CSF). SBT systems are constructed from RCC, stone-masonry or soil bunds. It consists of raw water tank, bioreactor containment, treated water tank, piping and pumps.

SBT systems are typically housed in RCC, stone-masonry or soil bunds and consists of an impervious containment. It starts with an under-drain layer, above which lies a layer of media housing culture and bio-indicator plants. Water first passes through the additive layer and subsequently passes through the media. The process can be run as single stage or multistage, depending on water quality desired. Recirculation is provided for further polishing if required.

Figure 2: A schematic of soil biotechnology system

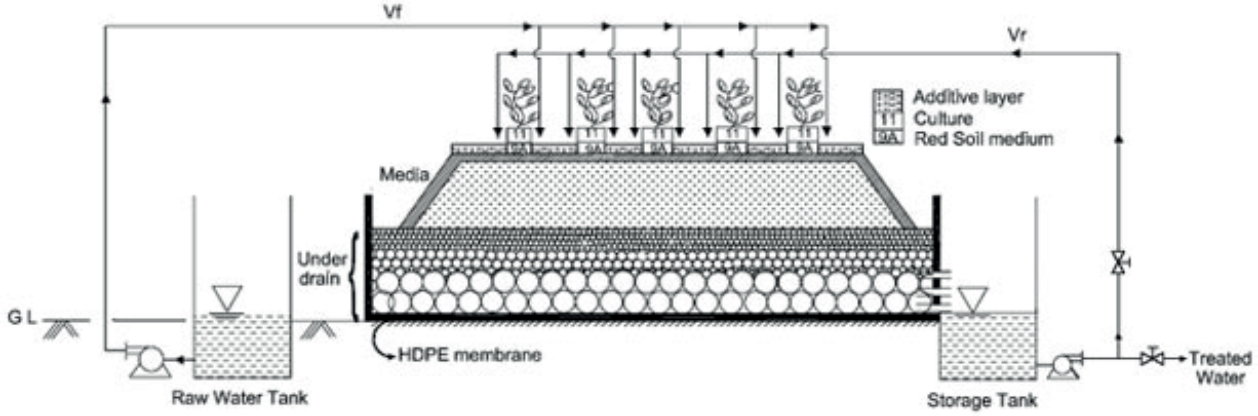
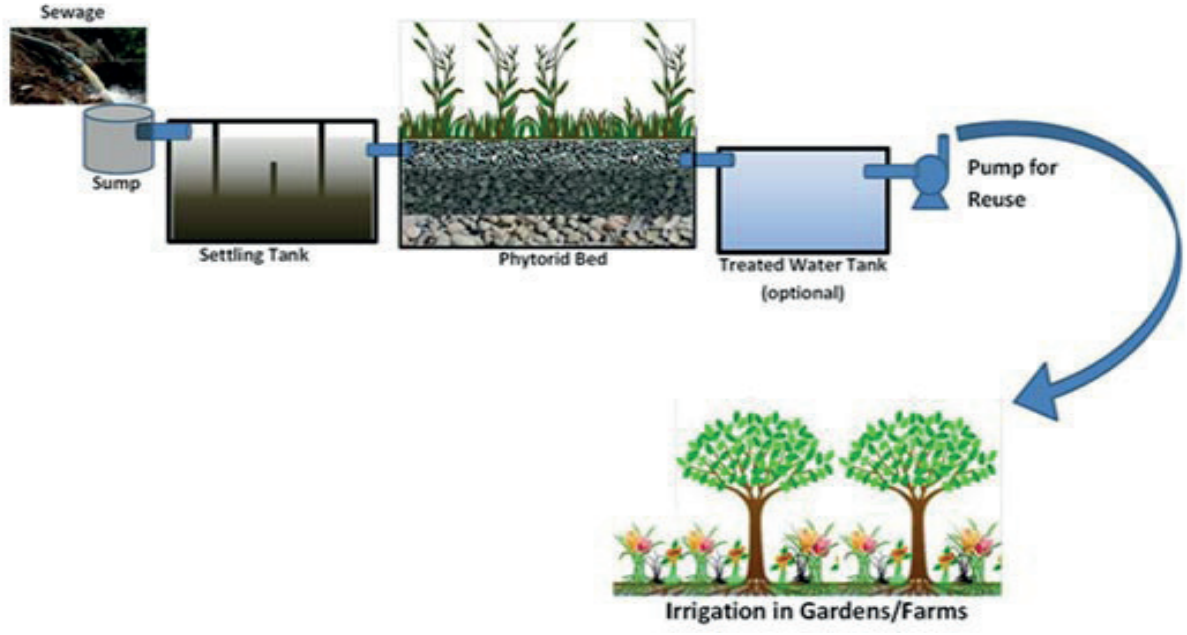


Figure 3: A schematic of phytorid technology



1.3.3 Phytorid wastewater treatment technology

The phytorid technology involves constructed wetland technology based on specific plants, such as elephant grass (*Pennisetum purpurem*), cattails (*Typha sp.*), reeds (*Phragmites sp.*), Cannas pp. and yellow flag iris (*Iris pseudocorus*), normally found in natural wetlands with filtration and treatment capability.

The phytorid technology treatment system is a subsurface flow-type constructed wetland system with a cell that has baffles in which wastewater is applied to the cell/system filled with porous media such as crushed bricks, gravel and stones. The hydraulics is maintained in such a manner that wastewater does not rise to the surface retaining a free board at the top of the filled media.

The system consists of the following three zones:

(i) Inlet zone comprising crushed bricks and different sizes of stones
(ii) Treatment zone comprising the same media as the inlet zone with plant species
(iii) Outlet zone

1.3.4 Sintex-Packaged Sewerage Treatment Plant (PSTP)

Deep-row entrenchment (land application)

Deep-row entrenchment is a method of disposal where the disposed of materials are often used in soil amendment. It requires careful design and operation to not contaminate groundwater. Deep-row entrenchment is done in batches. Untreated faecal sludge is placed in trenches and covered with soil. After three months the dried sludge can be taken out and used as manure, or trees can be planted on top or next to the trench. The length and the depth of the trench depend on the highest groundwater level and the quantity of faecal sludge. The trench can be lined, for example, with a layer of clay to reduce the risk of groundwater contamination. Deep-row entrenchment needs little energy. The packaged sewerage system is based on anaerobic and aerobic processes designed to have dual functions for efficient treatment of domestic wastewater from toilets bathrooms, kitchen and washing area.

1.3.5 Anaerobic process-based faecal sludge treatment (co-composting)

The main treatment steps followed in this FSTP are solid–liquid separation, stabilization and dewatering of sludge and pathogen removal. The separated liquid component is also treated to meet discharge standards. The faecal sludge is conveyed to the FSTP through a cesspool vehicle. The treatment modules for solid components are the feeding tank (FT) with screen chamber, biogas digester (BGD), stabilization tank (ST), sludge drying bed (SDB) and percolation pit. The treatment module for liquid components are integrated settler, anaerobic baffled reactor with filter chambers, planted gravel filter (PGF) and collection tank (CT). The treatment system also consists of a co-composting unit where the dried sludge from SDB is composted with municipal solid waste.

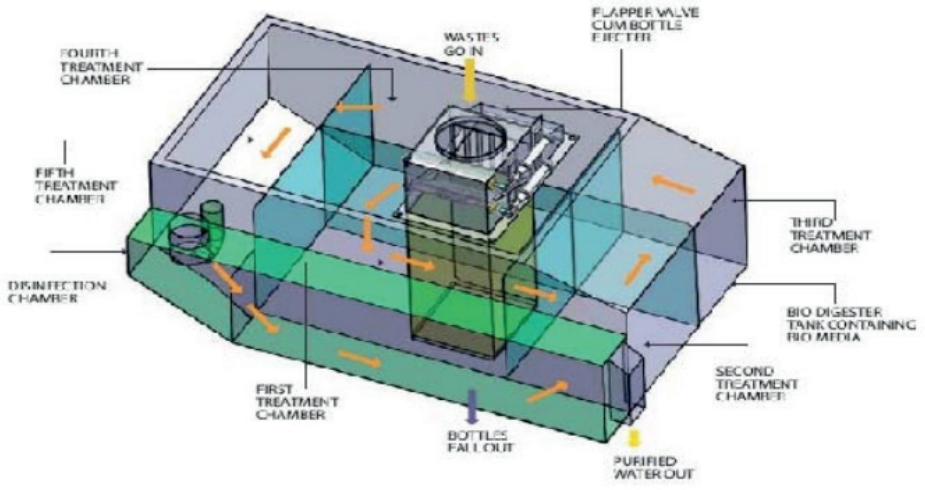
1.3.6 DEWATs with vortex system

The wastewater passes through three modules:

1) Settler
2) ABR and anaerobic filter (AF) and
3) Vortex system

   Sedimentation of settleable solids occur in settler with a BOD reduction of around 30 per cent, followed by digestion and filtration in ABR and AF with BOD reduction of around 80 per cent. The treated water is further polished as it passes through the vortex

Figure 4: A schematic of a bio toilet



system. The vortex system helps remove the odour accumulated because of anaerobic treatment in the first two modules. The treated water is stored in a collection tank and used for horticulture.

Note: There is no planted filter bed in this system.

1.3.7 Bio toilet

Bio toilets work by a process called 'bacterial digestion' of the faecal waste. Bacterial digestion is the process of bacteria consuming organic matter. Bacteria feed on organic waste, deriving nutrition from faecal waste for growth and reproduction. Bacterial digestants (containing combinations of bacteria cultures, enzymes and essential nutrients) that promote quick decomposition are added to the tank near or below the toilet. The bacteria used are aerobic in nature; each component of the digestants plays an important role in promoting fast, efficient elimination of unwanted organic waste. The digestants speed up the natural decaying process and eliminate clogs, pollution and odours by breaking down and converting the organic material into its two most basic components, carbon dioxide and water.

The combination of bacteria, enzymes and essential nutrients are necessary for quick decomposition of organic waste. The digestants include free enzymes to help jump start the bacteria into digestion. In general, the digester tank is designed such that the movement of the waste takes place in a clockwise direction creating various treatment chambers and eventually the treated water falls out of the tank.

1.3.8 Bio digester

Bio-digester technology degrades and converts the human waste into usable water and gasses using anaerobic bacteria. The process involves the bacteria which feed upon the faecal matter inside the tank/ septic tank, through anaerobic process which finally degrades the matter and releases methane gas. The bio-digester tank can be manufactured and customized as per the requirement to which an initial feed of anaerobic microbes is added.

1.4 AIM AND OBJECTIVES OF THE TESTING PROTOCOL

There is currently in India standard protocol for the sample collection and analysis of water and wastewater. Also, while Indian regulatory authorities have prescribed the discharge or reuse standard for water and wastewater there is no standard protocol or discharge or reuse standard for faecal sludge. We know little about the quality or characteristics of faecal sludge in various containment systems or septic tanks. While several types of on-site sanitation technologies are available in the Indian market no one knows the actual performance efficiency of the various on-site sanitation technologies. There is neither any standard protocol for testing the characterization of faecal sludge nor any institutional mechanism for testing faecal sludge in India. There is also no accredited laboratory that undertakes faecal sludge analysis as most labs are confined to wastewater characterization. India lacks science-based evidence to help make appropriate public investment decisions to address the sanitation challenges of non sewered area/on-site sanitation. Other regulatory measures are also non-existent or are ineffectively enforced, resulting in poor system performance, public health threats and degradation of surface- and groundwater. There is hence a direct need to build knowledge and practice decision making and analysis of cost-effective and sustainable DWWTs and FS treatment technologies.

Aim

The aim of this scoping paper is to develop and validate protocols for planning, designing and implementing sustainable and affordable wastewater treatment as well as faecal sludge/septage management solutions.

Objectives

- To enable the reader to identify suitable treatment options for various waste management technologies and faecal sludge management practices.
- To develop testing protocols, input–output analysis and method validation to test wastewater and faecal sludge
- To test the effectiveness of technologies in terms of performance and feasibility
- To understand how operational factors impact the variability of faecal sludge
- To understand the difficulties in obtaining reliable data on the quality of faecal sludge and wastewater

Chapter 2

2.0 Sampling

Sampling is the first and perhaps the most critical area of the entire process of obtaining sludge quality information. Standardized methods for sampling faecal sludge do not exist. This has likely contributed to the high variability that has been observed in previously conducted characterization studies. It also reduces the comparability of results, limits the understanding of the faecal sludge that will be delivered to treatment facilities and prevents the optimized design of treatment plants. Proper sampling is an integral part of monitoring the quality of sludge being removed for use or disposal.

2.1 PREPARE A SAMPLING PLAN

Consistency involves the assurance that samples are taken the same way from the same location every sampling event. Communication involves making sure the lab understands the proper methods to run, target reporting level and key details regarding the facility. Proper sampling activity documentation includes proper sample labelling, chain-of-custody procedures and a log book of sampling activities. Data handling means that after all aspects of the sampling event are documented, the data is reviewed before it is submitted.

2.2 REPRESENTATIVE SAMPLES

A sample that is representative of the sludge being removed must be acquired in a manner that will not compromise its subsequent analysis. Sampling needs may vary depending upon site location, sample composition, logistics, time of collection and analytes to be measured. A single approach to sampling is neither possible nor appropriate. Grab or composite samples may be appropriate depending on what the sample is being analysed for and what the operator thinks is representative. A grab sample is a specific quantity of sludge collected at a specific time and location. A single grab sample can represent sludge quality at the time and place it was collected. A composite sample is many grab samples that have been collected and mixed together to form a single sample. Generally, composite sampling is accomplished by collecting samples of equal size. In the case of continuous processes, the time interval between grab samples is typically kept constant. In composite sampling, the grab samples that comprise the composite should be completely and thoroughly mixed.

2.3 SAMPLE SIZE

Analytical protocols require minimum sample sizes to ensure analytical accuracy and precision. Laboratories should be consulted well in advance of any actual sample collection activities to ascertain the minimum sample size needed for each analytical method.

2.4 DOCUMENTATION OF SAMPLING ACTIVITIES

It is required that all sampling procedures be documented in a sampling plan. Some elements that should be documented in a sampling plan include sampling points, volumes to be drawn, days and times of collection, required equipment, instructions for labelling samples and ensuring chain of custody, and a list of contact persons and telephone numbers in case unexpected difficulties arise during sampling.

2.5 CONDUCTING PROPER ANALYSIS AND QA/QC

In order to demonstrate that a laboratory is producing data of adequate precision, accuracy and sensitivity it is necessary to assess all laboratory procedures at all stages from sampling to reporting. Analytical quality assurance procedures should be based on a system of traceability and feedback. Analytical QA will start with the examination and documentation of all aspects of laboratory management. This will include clearly identifying lines of communication and responsibility, description and documentation of all procedures carried out, and documentation of instrumental and analytical checks. Within this there should be specific control and assessment procedures designed to monitor quantitatively the accuracy and precision of specific assays.

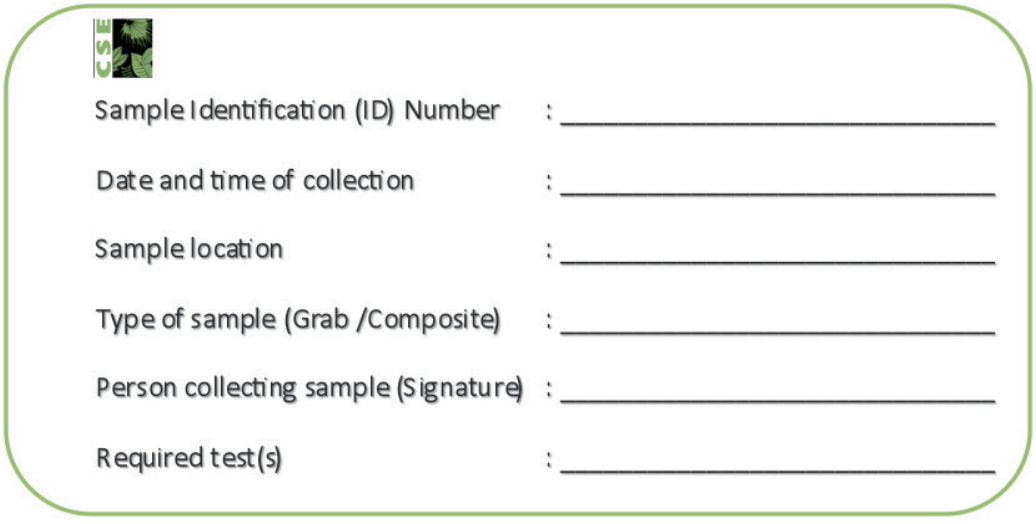
2.6 SAMPLING STAFF AND TRAINING

Staff for collecting samples needs to be adequately trained as handling faecal sludge and wastewater involves health risks from pathogens and other potentially harmful components. In addition, they should be informed about the aim of the sampling and types of sanitation systems. The number of people required for sampling events depends on the sampling method and strategy. Regular health checkups of sampling staff are necessary. Vaccination is required for the sampling staff as well as the analysts working on feacal sludge. Hepatitis A and Salmonella vaccines (e.g. Hepatyrix) are a must for sampling staff.

2.7 GENERAL SAMPLING PROCEDURE

- ✓ A week to several days prior to the proposed sampling, confirm or schedule sludge processing to ensure that sludge in the appropriate form is available for sampling at the proposed date, time and sampling point.
- ✓ A week to several days prior to the proposed sampling date, schedule/confirm that contract lab performing the analyses is ready and willing to accept samples on the proposed sampling date.
- ✓ At least one day before collecting samples, assemble the equipment necessary to accomplish the proposed sampling. Ensure that all equipment is clean and in good working order.
- ✓ On the day of sampling, obtain ice for sample storage and transportation and place in sample coolers.
- ✓ Put on nitrile gloves and other required/desired personal safety measures like mask, lab coat, boot, goggles etc.
- ✓ Collect the sample using a sampling device. A composite sampling takes the first of twenty-four grab samples from site to the container. All grab samples should be collected into a 1000 ml Teflon beaker and should be approximately 500 ml in volume. After collecting each grab sample, place the sample in the stainless steel bucket and record the time of collection. Wait one hour and collect the next grab sample. Repeat the process until all twenty-four grab samples are collected. Between collection of grab samples, the previously collected material should be kept cool (4–8°C).
- ✓ Once the last grab sample has been collected, thoroughly mix all material accumulated in the stainless steel bucket using a stainless steel rod. After the material is completely mixed, record the current time as the composite sample collection time.
- ✓ For pathogens and indicator organism testing, samples must be collected in sterilized containers. All samples for microbial analyses should be cooled to water-ice temperatures (4–8°C) when collected or very soon thereafter.
- ✓ After mixing, label all sample containers with the minimum information (see Fig. 5: Label format for sampling).
- ✓ After labelling, fill each individual sample container with portions of the homogenized

Figure 5: Label format for sampling



sample within the stainless steel bucket/plastic bucket.

- ✓ After each sample container is filled, seal it with a signed custody seal and place on ice in a cooler for transportation to the laboratory.
- ✓ Prior to delivering the samples to the lab, complete a chain-of-custody sheet to document proper sample handling.
- ✓ After sample delivery, clean all equipment according to established procedures and store in a clean, dry area.

2.8 SAFETY PROCEDURE DURING SAMPLING

To ensure safety and health while sampling, it is important to avoid direct contact between sludge and skin. The following procedures need to be followed for this:

- Wear protective sampling clothes (pants, jacket and boots) that are only worn at the sampling site; store clean clothes and shoes in a closed plastic bag in a clean environment.
- Wear protective eyewear.
- Always wear two pairs of gloves (laboratory gloves covered by long rubber gloves).
- Once all samples are taken and on-site parameters analysed, clean all materials and devices with water, directly on the sampling site, taking care not to spread faecal sludge in the environment.
- Once all material and device are properly cleaned, and all containers are in the cold box, take off the long rubber gloves.
- Take off sampling shoes and your sampling clothes, put all sampling clothes and safety gears in a closed bag.
- Take off the laboratory gloves.
- Wash the sampling area with clean water and make sure to leave a clean environment around the sampling site before leaving.

2.9 SAMPLE TRANSPORTATION

Awareness of the properties of FS is necessary in order to understand the challenges faced in its collection and transport. These properties are primarily influenced by water content, sludge age, and presence of non-biodegradable material and organic material. The containers must have adequate wall thickness to withstand handling during sample collection and

transport to the laboratory. Containers with wide mouths are preferred to facilitate transfer of samples from samplers to containers. When samples are collected, they should be placed in clean bottles that are filled completely to the top and stored in an insulated box with cooled gel packs/ice during transportation to the laboratory. Conductivity, temperature and pH should be measured immediately on site, and transport to the laboratory should be as rapid as possible. The maximum transport time to the laboratory is six hours, and samples should be processed within two hours of receipt at the laboratory.

Chapter 3

3.0 Laboratory organization

Sample preservation and preparation will depend on the parameters to be analysed and available time. In order to ensure reliable and accurate results, the following aspects need to be planned in advance:

- Develop quality assurance and quality control procedures (QA/QC), including blanks, duplicates and calibration.
- Determine parameters and number of analyses.
- Determine required materials and laboratory equipments.
- Determine consumables, including laboratory supplies, chemical reagents and preservation methods.
- Outline laboratory safety measures for analysis.
- Outline time and logistics required to perform all analysis.
- Calibration of all laboratory instruments before and during use, including standards, dilution curves and blanks.
- Duplicate analysis every eight samples for all parameters to ensure reproducibility of analytical methods (i.e. larger sample volumes need to be taken, so that it is possible for each parameter to be analysed twice and results compared).
- It is recommended that the relative error does not exceed 10 per cent. If difference between duplicate analyses performed on the same sample is greater than 10 per cent, trouble shooting is required to determine the source of error in the analytical method.
- Develop standard operating procedures (SOP) for all instruments and ensure SOP training to all lab scientists.

3.1 QUALITY ASSURANCE AND QUALITY CONTROL

Quality control (QC) refers to steps taken to ensure and monitor precision and accuracy of test results. Quality control practices include analysis of quality control samples with each set of samples. They include calibration standards, certified reference materials, spiked samples, duplicate sample analysis and blanks. Additional QC measures include analysis of blind duplicate samples and participation in various proficiency testing programmes. Quality assurance (QA) refers to a completely separate and independent monitoring of laboratory studies and quality control activities. Quality assurance activities include the internal audit programme, review of data packages, evaluation of non-conformances and an annual management review of quality of data.

3.2 ANALYTICAL PARAMETERS

Analytical parameters are determined based on the objectives of the characterization study. Physical, chemical and biological parameters all influence the design and on-going operation of treatment plants. The major parameters that should be considered for the characterization of FS include solids concentration, chemical oxygen demand (COD), biochemical oxygen demand (BOD), nutrients, pathogens, and heavy metals. These parameters are the same as those considered for domestic wastewater analysis, however, it needs to be emphasized that the characteristics of domestic wastewater and FS are very different.

3.2.1 On-site measurements

It is important to take on-site measure rapidly on-site upon sampling.

Temperature is an important parameter in understanding and predicting rates of biological activity, treatment processes and pathogen die-off. Temperature is measured with a probe immediately after sampling.

pH (potential hydrogen) is a measure of the acidity (< 7) or alkalinity (> 7) of sludge based on the chemical activity of hydrogen ions in solution. pH also has a strong influence on biological processes, including pathogen inactivation. The pH is measured with a probe immediately at the sampling point.

EC (electrical conductivity) is one way to measure inorganic materials, including calcium, bicarbonate, nitrogen, phosphorus, iron, sulphur and other ions, dissolved in a waterbody. It is measured by placing a conductivity probe in the sample and measuring the flow of electricity between the electrodes.

3.2.2 Solid and organic content

Solid and organic content analyses are conducted in the laboratory.

Total solids concentration of FS comes from a variety of organic (volatile) and inorganic (fixed) matter, and is comprised of floating material, settleable matter, colloidal material, and matter in solution. Parameters that are typically measured include total solids, fractions of volatile or fixed solids, and settleable, suspended or dissolved solids.

Biochemical oxygen demand and chemical oxygen demand of FS are important parameters to monitor as the discharge of FS into the environment can deplete or decrease the oxygen content of waterbodies resulting in the possible death of aquatic fauna. The oxygen demand is reduced through stabilization and can be achieved by aerobic or anaerobic treatment. FS dewatering technologies do not necessarily decrease oxygen demand.

3.2.3 Nutrients content

Nutrients content analyses are conducted in the laboratory.

Total nitrogen (TN) is the sum of nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N) and organically bonded nitrogen and each form is generally analysed as a separate component. Total nitrogen is an essential nutrient for plants, animals and microorganisms.

Total nitrogen is quantified as the sum of total kjeldahl nitrogen (ammonia, organic and reduced nitrogen), NO_3 and NO_2. NO_3 and NO_2 are expected to be at very low concentrations in anaerobic conditions of septic tanks. Results are reported as mg/L or g/L of total N.

Ammoniacal nitrogen is a measure of the bio-available form of N in anaerobic sludge and is important for growth, but high concentrations can be toxic. It is quantified as nitrogen in ammonium ($N–NH^{4+}$) after a reaction in alkaline solution by titrimetric method.

Total phosphorus (TP) is a measure of the sum of all P (dissolved form—orthophosphate, inorganic and organic). Like nitrogen, phosphorus is an important source of nutrients. It is quantified by the spectrophotometric method after acid digestion.

Heavy metals (iron [Fe], zinc [Zn], nickel [Ni] and lead [Pb]) are important for microbial growth at low concentrations but can inhibit biological processes at high concentrations. They are quantified after a first step of digestion in acid that allow releasing all metals into measurable form.

3.2.4 Pathogenic organisms

Exposure to untreated FS should always be considered as a pathogenic health risk. Adequate reductions in pathogens need to be determined based on the intended end use or disposal option for treated sludge and liquid effluents.

Coliform bacteria

Coliform bacteria are bacteria that populate the intestinal tract and are pervasive in faeces. Their presence in the environment is therefore used as an indicator of faecal contamination. *Escherichia coli* (*E. coli*) is the target organism that has traditionally been used to identify faecal contamination in the environment.

Helminths

Helminths are most commonly used as an indicator of the effectiveness of pathogen reduction while analysing FS due to their prevalence in low- and middle-income countries and their persistence following treatment. Helminths (parasitic worms) are eukaryotic parasites, prevalent in about one third of the world's population. They include nematodes (roundworms), cestodes (flatworms) and trematodes (flukes). The eggs of these parasites are robust and persistent if present in the incoming faecal waste (sludge or sewage) from one infected person and can infect hundreds. Ascaris lumbricoides, a type of roundworm, is the most commonly used indicator for testing the performance of a treatment technology as its eggs are most resistant to inactivation and can be identified relatively easily.

3.3 LABORATORY TIMING

A laboratory study plan needs to be developed to optimize time during sampling, transportation, preservation and analyses (including availability of machines). It is essential to analyse samples in a timely fashion to ensure accurate results and prevent changes due to factors such as degradation or volatilization. When several people are involved in sampling and analysing samples, it is useful to have a table in the lab where all tasks are recorded, with the responsible person for each task and their contacts.

A standard operating procedure (SOP) should also include a log book with all analytical activities, responsible parties, use of machines and calibrations, including dates and comments. Each person working in the laboratory also needs to maintain their own laboratory log book to record all analyses, samples, experimental procedures, QA/QC, information on problems or uncertainties during the analyses, etc. Lab books need to be written in pen, should not have removable pages, and should be kept in the laboratory at all times.

3.4 LABORATORY SAFETY

In order to work in safe conditions, direct contact is to be avoided with sludge and reagents. Sludge should be assumed to contain pathogens such as virus, bacteria, protozoan, parasite, and some hazardous chemicals for human health might be used during analysis (e.g. acids). Therefore, the following minimum precautions are to be strictly adhered to.

- Read and follow safety-related indications on the chemical boxes and bottles prior to use.

- Wear laboratory jacket, closed shoes, long wears.
- Wear laboratory glasses when manipulating sludge and chemicals.
- Wear vinyl laboratory gloves when manipulating sludge and chemicals.
- Wear an active carbon mask when manipulating sludge and chemicals.
- If you get your skin in contact with chemicals, clean with detergent and rinse thoroughly with water.
- Always clean the working space and your hand with detergent and ethanol at the end of analysis.
- Ensure that chemicals and products are stored in a closed and safe location.
- Never eat, drink or smoke in the laboratory; never bring food or drink into the laboratory.
- Keep a first-aid box with essential medicines in the lab.

Chapter 4

4.0 Preparation and preservation of samples

4.1 COMPOSITE PREPARATION

Once the sample has been combined, then it needs to be homogenized and sub-samples prepared in volumes relevant to the selected analytical procedures. In order to ensure the representativeness of the sub-samples, be sure to include equal and representative quantities of liquid and solids in all jars. Leave approximately 2 cm of headspace at the top of the jar. All containers are to be labelled with the date and sample code and stored at 4°C in the fridge.

4.2 PRESERVATION PROCEDURE

Preservation refers to sample handling processes aimed at preventing or minimizing chemical or biological activity within the sample after it has been collected. Preservation techniques are different for sludge samples and liquid trip or equipment blank samples. Sludge samples are generally preserved by cooling and maintaining samples at 4°C. Depending on the analytical method, liquid samples may be preserved with the combination of a chemical preservative and chilling to 4°C.

To preserve field and laboratory bio solids samples, cooling to 4°C is (in most cases) the most appropriate method since high solids sewage sludge cannot be mixed with other preservatives. In addition, laboratory personnel must be notified if chemical preservation is to be done by laboratory staff.

4.3 SAMPLE HOLDING TIMES

For all environmental samples, the term 'holding time' refers to the maximum time that can elapse before a sample is analysed and valid results can still be obtained. Table 1: Preservation and hold times for analysis of bio-solids samples provides general examples of preservation temperatures and maximum holding times, from field collection to analysis, for typical bio-solids sample analyses.

Table 1: Preservation and hold times for analysis of bio-solids/ samples

Analyte	Preservation	Maximum hold time from field collection to analysis
Total solids	Store at 4^0C	7 days
Volatile solids	Store at 4^0C	7 days
Ammoniacal nitrogen	Store at 4^0C	28 days
Total kjeldahl nitrogen	Store at 4^0C	28 days
Total phosphorus	Store at 4^0C	28 days
Chloride	Store at 4^0C	28 days
Sulphate	Store at 4^0C	28 days
Total organic carbon	Store at 4^0C	28 days
Volatile and semi-volatile Organic compounds	Store at 4^0C	14 days
Faecal coliform	Store at 4^0C	24 hours
Helminth ova	Store at 0–10^0C	24 hours
Potassium	Store at 4^0C	6 months
Arsenic	Sstore at 4^0C	6 months
Cadmium	Store at 4^0C	6 months
Chromium	Store at 4^0C	6 months
Copper	Store at 4^0C	6 months
Lead	Store at 4^0C	6 months
Mercury	Store at 4^0C	28 days
Nickel	Store at 4^0C	6 months
Zinc	Store at 4^0C	6 months

Chapter 5

5.0 Analysis protocols

5.1 STANDARD OPERATING PROCEDURE FOR PH DETERMINATION

Scope and field of application

This method is an electrometric procedure for measuring pH in waste samples. Waste may be liquid, solid, sludge or non-aqueous liquid.

Interferences

Samples with very low or very high pH may give incorrect readings on the meter. For samples with a true pH of >10, the measured pH may be incorrectly low. This error can be minimized by using a low-sodium-error electrode. Strong acid solutions, with a true pH of <1, may give incorrectly high pH measurements. Errors will occur when the electrodes become coated. If an electrode becomes coated with an oily material that will not rinse free, the electrode can 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. It can also be cleaned as per the manufacturer's instructions.

Safety precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- After the analysis clean bottles and beakers with clear water and keep them for drying.
- Dispose of the used gloves after completion of analysis.
- Clean hands using antiseptic soap.
- Avoid spillage and contact with skin.
- Disinfect hands after washing with soap.

Apparatus

- pH meter with means for temperature compensation
- Glass electrode
- Reference electrode—a silver chloride or other reference electrode of constant potential may be used
- 50 ml beaker
- Thermometer and/or temperature sensor for automatic compensation
- Analytical balance capable of weighing 0.1 g

Procedure

Sample preparation

To 20 g of waste sample in a 50 ml beaker, add 20 ml of distilled water, cover, and continuously stir the suspension for 5 minutes. Additional dilutions are allowed if working with hygroscopic wastes and salts or other problematic matrices.

Let the waste suspension stand for about 15 minutes to allow most of the suspended waste to

settle out from the suspension or filter or centrifuge off aqueous phase for pH measurement. Note: If the waste is hygroscopic and absorbs all the reagent water, begin the experiment again using 20 g of waste and 40 ml of reagent water.

Note: If the supernatant is multiphasic, decant the oily phase and measure the pH of the aqueous phase. The electrode may need to be cleaned if it becomes coated with an oily material.

Measurement of pH
Adjust the electrodes in the clamps of the electrode holder so that upon lowering the electrodes into the beaker the glass electrode will be immersed just deep enough into the clear supernatant to establish good electrical contact through the ground glass joint or the fibre-capillary hole.

Insert the electrode into the sample solution in this manner. For combination electrodes, immerse just below the suspension. If the sample temperature differs by more than 2 °C from the buffer solution, the measured pH values must be corrected.

Results
Report the results as 'waste pH measured in water at __°C' where '__°C' is the temperature at which the test was conducted.

5.2 STANDARD OPERATING PROCEDURE FOR TOTAL SOLIDS (TS)

Scope and field of application
Total solids are determined in a wide variety of liquid and semi-liquid materials. These include portable waters, domestic and industrial waters, polluted waters and sludge produced from treatment processes. It is of particular importance for the efficient operation of a treatment plant.

Principle
An appropriate volume of well-mixed sample is evaporated to dryness in a weighed evaporating dish in a hot air oven at 103-105°C, the residue remaining are cooled and weighed. The residual material in the evaporating dish is classified as total solids, and may consist of organic, inorganic, dissolved, suspended or volatile matter.

Interferences
Highly mineralized water with a significant concentration of calcium, magnesium, chloride and sulphate may be hygroscopic and require prolonged drying, proper desiccation and rapid weighing. Exclude large floating particles from the sample if it is determined that their inclusion is not desired in the final result.

Safety precautions
- Always use safety goggles, gloves and laboratory coat.
- Wear suitable protective gloves when removing evaporating dish from the oven.
- Clean all glassware and bottles well when finished analysis.
- Clean hands with antiseptic soap and disinfectant.

Apparatus
- Evaporating dishes: Dishes of 50–100 ml
- Muffle furnace for operation at 550°C or drying oven for operation at 103–105°C

- Water bath
- Desiccator (provided with a desiccant containing a colour indicator of moisture concentration)
- Analytical balance (capable of weighing to 0.1 mg)
- Magnetic stirrer and bar
- Wide-bore pipettes
- Forceps
- Beaker
- Procedure
- Preparation of evaporating dish

If volatile solids are to be measured, ignite clean evaporating dish at 550°C for 1 hour in a muffle furnace. If only total solids are to be measured, heat clean dish to 103–105°C for 1 hour. Store and cool the dish in the desiccator until needed. Weigh immediately before use.

Sample analysis

- Choose a sample volume that will yield a residue between 2.5 and 200 mg.
- Stir sample with a magnetic stirrer to homogeneous samples, pipet a measured volume of well-mixed sample from the approximate midpoint of the container but not in the vortex.
- If sample is contained solids in the range up to 20,000 mg/l, transfer carefully 20–50 ml of well-mixed sample into evaporating dish. If sample is contained solids more than 20,000 mg/l, transfer carefully 10–20 g of well-mixed sample into evaporating dish and weight.
- Evaporate to dryness on a water bath.
- Dry evaporated sample for at least 1 hour in an oven at 103–105°C, cool dish in the desiccator to balance temperature and weigh.
- Weighing until a constant weight is obtained, or until weight change is less than 4 per cent of previous weight or 0.5 mg, whichever is less.

Calculation

$$\text{mg total solids/L} \frac{(A-B) \times 1000}{\text{Sample volume (ml)}}$$

$$\% \text{ total solids} \frac{(A-B) \times 100}{C-B}$$

where A = Weight of dried residue + dish (mg)
B = Weight of dish (mg)
C = Weight of wet sample + dish (mg)

5.3 STANDARD OPERATING PROCEDURE FOR TOTAL SUSPENDED SOLIDS (TSS)

Scope and field of application

Suspended solids are useful determinants in the analysis of polluted, reused and wastewaters. They are used to evaluate the strength of domestic/industrial wastewaters and determine the efficiency of treatment units, such as settling tanks, biological filters, and the activated sludge.

Principle

A measured volume of well-mixed sample is vacuum filtered through a dried pre-weighed glass fibre filter. The filters and residue is dried to a constant weight at 103–105°C. The increase in weight of the filter represents the total suspended solids.

Interferences

- Exclude isolated large floating particles.
- Samples high in dissolved solids must be washed adequately.
- Loss in mass of the rinsed glass fibre filters must be taken into the final calculation.
- The larger the sample, the smaller the factor applied in the calculation, but avoid prolonged filtrations.

Safety precautions

- Always use safety goggles, gloves and laboratory coat.
- Wear suitable protective gloves when removing evaporating dish from the oven.
- Exercise care when using glassware, vacuum pumps and ovens.
- Cleaning all glassware and bottles well when finished analysis.
- Clean the hands with antiseptic soap and disinfectant.

Apparatus

- Evaporating dishes: Dishes of 50–100 ml
- Muffle furnace for operation at 550°C or drying oven for operation at 103–105°C
- Filtration apparatus
- Vacuum pump
- Whatman glass fibre filter
- Desiccator (provided with a desiccant containing a colour indicator of moisture concentration)
- Analytical balance (capable of weighing to 0.1 mg)
- Magnetic stirrer and bar
- Wide-bore pipettes
- Forceps
- Beaker

Procedure

- Prepare glass fibre filter paper and evaporating dish
- Use Whatman glass fibre filter paper and mark the filter paper.
- Place filter paper on the evaporating dish as a support.
- Filter paper is heated to 103–105°C for 1 hour. Store and cool filter paper in desiccator until needed. Weigh immediately before use.

Sample analysis

- Choose a sample volume that will yield a residue between 2.5 and 200 mg.
- Stir sample with a magnetic stirrer to homogeneous samples, pipette a measured volume of well-mixed sample from the approximate midpoint of the container but not in the vortex.
- Insert filter paper (GF/C paper) with wrinkled side up into filtration apparatus that connected vacuum pump and spray few distilled water into filter paper and suction.
- Transfer carefully well-mixed sample 20-50 ml into filter paper on filtration apparatus and continue suction.
- When complete filtration takes more than 10 minutes, increase filter diameter or

decrease sample volume.

- Wash filter with three successive 10 ml volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 minutes after filtration is complete. (Samples with high dissolved solids may require additional washings.)
- Carefully remove filter paper from filtration apparatus and transfer to an evaporating dish as a support.
- Dry sample for at least 1 hour in an oven at 103–105°C, cool dish in desiccator to balance temperature, and weigh.
- Weighing until a constant weight is obtained, or until weight change is less than 4 per cent of previous weight or 0.5 mg, whichever is less.

Calculation

mg total suspended solids/l(A–B) x 1000/Sample volume (ml)

where A = Weight of filter + dried residue (mg)
B = Weight of filter (mg)

5.4 STANDARD OPERATING PROCEDURE FOR TOTAL DISSOLVED SOLIDS (TDS)

Scope and field of application

Dissolved solids are useful determinants in the analysis of polluted, reused and wastewaters. They are used to evaluate wastewater that contains a high fraction of colloidal solids. The size of colloidal particles in wastewater is typically in the range of 0.01 to 1.0 µm.

Principle

A well-mixed sample is filtered through a standard glass fibre filter, and the filtrate is evaporated to dryness in a weighed dish and dried to a constant weight at 180°C. The increase in dish weight represents the total dissolved solids. This procedure may be used for drying at other temperatures.

Interferences

Highly mineralized waters with a considerable calcium, magnesium, chloride, and/or sulphate content may be hygroscopic and require prolonged drying, proper desiccation and rapid weighing.

Samples high in bicarbonate require careful and possibly prolonged drying at 180°C to insure complete conversion of bicarbonate to carbonate. Because excessive residue in the dish may form a water-trapping crust, limit sample to no more than 200 mg residue.

Safety precautions

- Always use safety goggles, gloves and laboratory coat.
- Wear gloves suitable to prevent high temperatures when removing evaporating dish from the oven.
- Exercise care when using glassware, vacuum pumps and ovens.
- Good cleaning all glassware and bottles when finished analysis.
- Clean the hands with antiseptic soap and disinfect.

Apparatus

- Evaporating dishes: Dishes of 50–100 ml
- Drying oven for operation at 103–105°C
- Filtration apparatus
- Vacuum pump
- Water bath
- Whatman glass fibre filter
- Desiccator (provided with a desiccant containing a colour indicator of moisture concentration)
- Analytical balance (capable of weighing to 0.1 mg)
- Magnetic stirrer
- Magnetic bar
- Wide-bore pipettes
- Forceps
- Beaker

Procedure

- Prepare the glass fibre filter paper and evaporating dish
- Prepare Whatman glass fibre filter paper.
- Heat clean dish to 103–105°C for 1 hour. Store and cool the dish in desiccator until needed. Weigh immediately before use.

Sample analysis

- Choose a sample volume that will yield a residue between 2.5 and 200 mg.
- Stir sample with a magnetic stirrer to homogeneous samples, pipette a measured volume of well-mixed sample from the approximate midpoint of the container but not in the vortex.
- Insert filter paper (GF/C paper) with the wrinkled side up into filtration apparatus that connects vacuum pump and spray some distilled water into the filter paper with suction.
- Transfer carefully 20–50 ml of the the well-mixed sample into the filter paper on the filtration apparatus and continue suction.
- Wash filter with three successive 10-ml volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 minutes after filtration is complete.
- Transfer total filtrate sample (with washings) to a weighed evaporating dish and evaporate to dryness on a water bath.
- Dry evaporated sample for at least 1 hour in an oven at 180°C; cool dish in desiccator to balance temperature and weigh.
- Weigh until a constant weight is obtained, or until weight change is less than 4 per cent of previous weight or 0.5 mg, whichever is less.

Calculation

mg total dissolved solids/l(A– B) x 1000/Sample volume (ml)

where A = Weight of dried residue + dish (mg)
B = Weight of dish (mg)

5.5 STANDARD OPERATING PROCEDURE FOR TOTAL FIXED SOLIDS AND TOTAL VOLATILE SOLIDS (TFS AND TVS)

Principle

The residue from the above methods is ignited to constant weight at 550°C. The remaining solids represent the fixed total, dissolved or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough estimate of the amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes.

Interferences

Negative errors in the volatile solids may be produced by loss of volatile matter during drying. Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subject to considerable error.

Safety precautions

- Always use safety goggles, gloves and laboratory coat.
- Wear gloves and use long clamp suitable to prevent high temperatures when removing evaporating dish from the oven and furnace.
- Exercise care when using glassware, vacuum pumps and ovens.
- Good cleaning all glassware and bottles when finished analysis.
- Clean the hands with antiseptic soap and disinfect.

Apparatus

- Evaporating dishes: Dishes of 50–100 ml
- Muffle furnace for operation at 550°C
- Oven for operation at 103–105°C
- Water bath
- Desiccator (provided with a desiccant containing a color indicator of moisture concentration)
- Analytical balance (capable of weighing to 0.1 mg)
- Vacuum pump
- Magnetic stirrer
- Magnetic bar
- Wide-bore pipettes
- Forceps
- Beaker

Procedure

Preparation of evaporating dish

Volatile solids are to be measured by igniting the samples in a clean evaporating dish at 550°C for 1 h in a muffle furnace. Store and cool the dish in desiccator until needed. Weigh immediately before use.

Sample analysis

- Choose a sample volume that will yield a residue between 2.5 and 200 mg.
- Stir sample with a magnetic stirrer to homogeneous samples, pipette a measured volume of well-mixed sample from the approximate midpoint of the container but not in the vortex.

- If sample is contained solids below 20,000 mg/l, transfer carefully 20–50 ml of well-mixed sample into evaporating dish. If sample is contained solids in the range up to 20,000 mg/l, transfer carefully 10–20 g of well-mixed sample into evaporating dish and weight.
- Evaporate to dryness on a water bath.
- Dry evaporated sample for at least 1 hour in an oven at 103–105°C, cool dish in desiccator to balance temperature, and weigh.
- Ignite dry sample at 550°C for 15–20 minutes.
- Weigh dish as soon as it has cooled to balance temperature. Weigh until a constant weight is obtained or until weight change is less than 4 per cent of previous weight or 0.5 mg, whichever is less.

Calculation

$$\text{mg total volatile solids/l} = \frac{(A-B) \times 1000}{\text{Sample volume (ml)}}$$

$$\text{\% total volatile solids} = \frac{(D-B) \times 100}{D-C}$$

$$\text{mg total fix solids/l} = \frac{(B-C) \times 1000}{\text{Sample volume (ml)}}$$

$$\text{\% total fix solids} = \frac{(B-C) \times 100}{D-C}$$

where A = Weight of dried residue + dish before ignition (mg)
B = Weight of dried residue + dish after ignition (mg)
C = Weight of dish (mg)
D = Weight of dried residue + dish (mg)

5.6 STANDARD OPERATING PROCEDURE FOR VOLATILE SUSPENDED SOLIDS (VSS)

Principle

The residue from the above methods is ignited to a constant weight at 550°C. The remaining solids represent the fixed total, dissolved or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in controlling wastewater treatment plant operation because it offers a rough estimate of the amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes.

Interferences

Negative errors in the volatile solids may be produced by loss of volatile matter during drying.

Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subject to considerable error.

Safety precautions

- Always use safety goggles, gloves and laboratory coat.

- Wear gloves and use long clamp suitable to prevent high temperatures when removing evaporating dish from the oven and furnace.
- Exercise care when using glassware, vacuum pumps and ovens.
- Good cleaning all glassware and bottles when finished analysis.
- Clean the hands with antiseptic soap and disinfect.

Apparatus

- Evaporating dishes: Dishes of 50–100 ml
- Muffle furnace for operation at 550°C
- Oven for operation at 103–105°C
- Filtration apparatus
- Whatman glass fibre filter
- Desiccator (provided with a desiccant containing a colour indicator of moisture concentration)
- Analytical balance (capable of weighing to 0.1 mg)
- Vacuum pump
- Magnetic stirrer and bar
- Wide-bore pipettes
- Forceps
- Beaker

Procedure

- Prepare glass fibre filter paper and evaporating dish.
- Use Whatman glass fibre filter paper and mark the filter paper.
- Place filter paper on the evaporating dish as a support.
- Heat filter paper to 103–105°C for 1 hour. Store and cool filter paper in desiccator until needed. Weigh immediately before use.

Sample analysis

- Choose a sample volume that will yield a residue between 2.5 and 200 mg.
- Stir sample with a magnetic stirrer to homogeneous samples, pipette a measured volume of well-mixed sample from the approximate midpoint of the container but not in the vortex.
- Insert filter paper (GF/C paper) with wrinkled side up into filtration apparatus that connected vacuum pump and spray some distilled water into the filter paper and suction.
- Transfer carefully 20–50 ml of the well-mixed sample into the filter paper on the filtration apparatus and continue suction.
- When complete filtration takes more than 10 minutes, increase the filter diameter or decrease sample volume.
- Wash filter with three successive 10-ml volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 minutes after filtration is complete. Note: Samples with high dissolved solids may require additional washings.
- Carefully remove filter paper from filtration apparatus and transfer to an evaporating dish as a support.
- Dry sample for at least 1 h in an oven at 103–105°C, cool dish in desiccator to balance temperature, and weigh.
- Ignite dry sample at 550°C for 15–20 minutes.
- Weigh until a constant weight is obtained, or until weight change is less than 4 per cent of previous weight or 0.5 mg, whichever is less.

Calculation

$$\text{mg of volatile suspended solids/l} = \frac{(A-B) \times 1000}{\text{Sample volume (ml)}}$$

where A = Weight of dried residue + dish before ignition, mg
B = Weight of dried residue + dish after ignition, mg

5.7 STANDARD OPERATING PROCEDURE FOR SLUDGE VOLUME INDEX

Principle

Sludge volume index (SVI) is the volume of millilitres occupied by 1 gram of a suspension after 30 minutes settling. SVI typically is used to monitor settling characteristics of activated sludge and other biological suspensions. Although SVI is not supported theoretically, experience has shown it to be useful in routine process control.

Safety precautions

- Always use safety goggles, gloves and laboratory coat.
- Cleaning all glassware and bottles thoroughly when analysis is complete.
- Clean hands with antiseptic soap and disinfectant.

Apparatus

- Imhoff cone or cylinder

Procedure

- Determine the suspended solids concentration of a well-mixed sample of suspension.
- Fill an Imhoff cone or cylinder to the 1-litre mark with a well-mixed sample.
- Settle for 30 minutes.
- Record the volume of sludge at 30 minutes as the settled volume.

Calculation

$$\text{Sludge volume index ml/g} = \frac{\text{Settled sludge volume (ml/l)x1000}}{\text{Suspended solids (mg/l)}}$$

Interpretation of SVI results:

- SVI: < 100 ml/g: Old sludge, possible pin floc, increasing effluent turbidity
- SVI: 100–250 ml/g: Normal operation, good settling, low effluent, turbidity
- SVI: > 250 ml/g: Bulking sludge, poor settling, high effluent turbidity

5.8 STANDARD OPERATING PROCEDURE FOR CHEMICAL OXYGEN DEMAND (COD)

Colorimetric method

Scope and application

- The COD (chemical oxygen demand) expresses the amount of oxygen originating from potassium dichromate that reacts with the oxidizable substances contained in 1 l of water under the working conditions of the specified procedure.

- It is an important and rapidly measured parameter to measure the amount of organic compounds in stream and industrial waste studies, and in operational control of wastewater treatment plants. It is also applicable for measurements on human excreta.

Summary

The water sample is oxidized with a hot sulphuric solution of potassium dichromate, with silver sulphate as catalyst. Chloride is masked with mercury sulphate. The concentration of unconsumed yellow $Cr_2O_7^{2-}$ ions or, respectively, of green Cr^{3+} ions is then determined photo metrically. The method is analogous to EPA 410.4, US Standard Methods 5220 D, and ISO 6060.

Interferences

Difficulties caused by the presence of chlorides in the sample are overcome by the addition of mercuric sulphate to samples before digesting. The chloride ion is then eliminated from the reaction by forming a soluble mercuric chloride complex.

A catalyst must be used to include some organic compounds (e.g. acetic acid), while other biological compounds (e.g. cellulose), which are not important, are included in the determination. Pyridine is not oxidized even in the presence of the catalyst.

Sampling

- Collect samples preferably in glass bottles.
- Test unstable samples without delay.
- Preserve samples by acidifying with concentrated sulphuric acid to pH 2.
- Determine COD on well-shaken samples. Settled samples may also be analysed if requested.
- Use a 3ml pipette to measure out samples.

Safety precautions

- Handle reagents with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed.
- After the analysis clean bottles and beakers with water and then dry.
- Dispose of the used gloves after completion of analysis.
- Clean hands using antiseptic soap.
- Avoid spillage and contact with skin. In the latter case, use copious quantities of cold water to wash skin and call for medical attention.

Sample preparation

- Analyse immediately after sampling.
- Homogenize the samples.
- Check the chloride content with the Merckoquant® Chloride Test. Samples containing more than 2000 or, respectively, 5000 mg/l Cl^- must be diluted with distilled water prior to determining COD.

Procedure

- Pipette solution A and solution B into an empty cell (free of scratches and organic impurities) according to the desired measuring range and mix (Table x). Take care not to exceed the stated volumes.

- Suspend any bottom sediment present in the cell by swirling.
- Tightly attach the screw cap to the cell.
- In all subsequent steps the cell must be held only by the screw cap.
- Vigorously mix the contents of the reaction cell.
- Heat the reaction cell for 120 minutes at 148°C in the preheated thermo reactor. Remove the hot reaction cell from the thermo reactor and allow to cool in the cell rack. Do not cool with cold water.
- Wait 10 minutes, shake the cell and return to the rack for complete cooling to room temperature (cooling time at least 30 minutes).
- Measure the sample in the photometer

Table 2: Reagent details

Measuring range (mg/l COD)	4.0–40.0	10–150	100–1500	500–10000
Solution A Cat. No. Volume	1.14538.0065 0.30 ml	1.14538.0065 0.30 ml	1.14538.0065 0.30 ml	1.14679.0495 2.20 ml
Solution B Cat. No. Volume	1.14681.0495 2.85ml	1.14682.0495 2.85 ml	1.14539.0495 2.30 ml	1.14680.0495 1.80 ml

5.9 STANDARD OPERATING PROCEDURE FOR AMMONIACAL NITROGEN

Scope and application

- Ammonia is naturally is present in surface and wastewaters.
- All these forms of nitrogen as well as nitrogen gas are biochemically interconvertible and are components of the nitrogen cycle.
- The method covers the range from about 10 to 25 mg/l for titrimetric procedure.

Summary

- The sample is buffered at pH 9.5 with borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds.
- It is distilled into a solution of boric acid when titration is to be used.
- The ammonia in the distillate is determined titrimetrically with standard sulphuric acid and a mixed indicator together with a pH meter.

Interferences

Glycine, urea, glutamic acid, cyanates and acetamide hydrolyze very slowly in solution on standing but of these only urea and cyanates hydrolyze on distillation at pH 9.5.

Sampling

- Most reliable results are obtained on fresh samples.
- Destroy residual chlorine immediately after sample collection to prevent its reaction with ammonia.
- If an immediate analysis is not possible, preserve samples by acidifying to pH between 1.5 and 2.0 with 0.8 ml conc. H_2SO_4/L and store at 4°C.
- If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination.

Safety precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Use eye and hand protection when preparing acid or handling color reagent.

- Prepare and keep color reagent in fume hood.

Apparatus
- VELP distillation unit
- pH meter

Reagents
- Ammonia-free water
  Eliminate traces of ammonia in distilled water by adding 0.1ml sulphuric acid to 1l distilled water and redistill. Alternatively treat distilled water with enough bromine or chlorine water to produce a free halogen residue of 2–5 mg/L and redistill after standing for 1 hr.
- 0.1N NaOH
  Dissolve 4 g NaOH in 1l distilled water.
- 1N NaOH
  Dissolve 40 g NaOH in 1 l ammonia-free distilled water.
- Borate buffer solution
  Add 88 mL of 0.1N NaOH solution to 500 ml of 0.025M di-sodium tetra borate-hydrous ($Na_2B_4O_7$. $10H_2O$) solution—(9.5 g $Na_2B_4O_7.10H_2O$ hydrous per litre) or (5.0 g $Na_2B_4O_7$ anhydrous per liter) and dilute to 1l.
- Mixed indicator solution
  Dissolve 200 mg methyl red indicator in 100 ml 95 per cent ethyl or isopropyl alcohol or ethanol. Dissolve 100 mg methylene blue in 50 ml 95 per cent ethyl or isopropyl alcohol or ethanol. Combine solutions. Prepare monthly.
- Indicating boric acid solution
- Dissolve 20 g H_3BO_3 in ammonia-free distilled water, add 10 ml mixed indicator solution and dilute to 1l. Prepare monthly.
- Standard sulphuric acid titrant (0.02N)
- Dissolve 0.5 ml concentrated sulphuric acid in distilled water and dilute to 1litre. Weigh out about 1.325 g anhydrous sodium carbonate, previously dried at 270°C. Dissolve in distilled water and make up to 250 ml in a volumetric flask—this is 0.1N.

Note: Do not keep longer than 1 week. Titrate the sulphuric acid solution against 25 ml of sodium carbonate solution using bromocresol green-methyl red mixed indicator. Calculate the normality of the sulphuric acid.

Normality of H_2SO_4 solution = 25 x 0.1/Vol. of *H_2SO_4 used*

Sample preparation
- Weigh out 1.8–2 g of well-mixed faecal sludge sample.
- Place the weighed out sample into a blender with 250 ml of distilled water and blend it for 30–45 sec.
- Transfer the blended mixture into a volumetric flask and make up to 1L with distilled water.
- Transfer the 1l solution to a plastic bottle and store at 4°C

Procedure
- Preparation of equipment: Add 500 ml ammonia-free water and 20 ml borate buffer to a distillation flask and adjust pH to 9.5 with 6N NaOH solution.
- Add a few glass beads and use this mixture to steam out the distillation apparatus until

distillate shows no traces of ammonia.
- Add appropriate amount (see Table 3: Sample volume selection table) of sample to distillation flask.
- Add 20 ml borate buffer to distillation flask.
- Distill for 5 min and collect 100 ml distillate into the 50 ml indicating boric acid solution.
- Titrate ammonia in distillate with standard 0.02N sulphuric acid; titrate until indicator turns pale lavender.
- Carry a blank through all steps of the procedure and apply the necessary correction to the results.

Table 3: Sample volume selection table

Ammonia nitrogen (mg/l)	Sample volume ml
5–10	250
10–20	100
20–50	50.0
50–100	25.0

Calculation

NH_3 *(mg/L) = (A–B) × 280/sample (ml)*

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 (0.02N, 1ml = 0.28mg NH3-N) 1l – 280 mg NH3 – N

NH_3 *in wet sample (mg/g) = (A–B) x 280 x V/Sample (ml) x M*

Concentration = Mass/molar mass

NH_3 *in wet sample (g/g) =* NH_3 *in wet sample* (mg/g)/1000

NH_3 *in dry sample (g/g) =* NH_3 *in wet sample (g/g)/Total solids (g/g)*

where:
M = mass of sludge used in sample preparation (g)
V = Volume of dilution (L)

5.10 STANDARD OPERATING PROCEDURE FOR TOTAL KJELDAHL NITROGEN (TKN)

Scope and field of application
- The kjeldahl methods determine nitrogen in the tri-negative state. They fail to account for nitrogen in the form of azide, azine, azo, hydrazine, nitrate, nitrite, nitrile, nitro, nitroso, oximine and semi-carbazone.
- Kjeldahl nitrogen is the sum of organic nitrogen and ammonia nitrogen. Organic nitrogen includes proteins, peptides, nucleic acids and urea.
- The macro-kjeldahl method is applicable to samples containing high concentrations of organic nitrogen but requires a relatively large sample volume for low concentrations.
- In the semi-micro-kjeldahl, which is applicable to samples containing high

concentrations of organic nitrogen, the sample volume should be chosen to contain organic plus ammonia nitrogen in the range of 0.2–2 mg.

Summary

- In the presence of sulphuric acid (H_2SO_4), potassium sulphate (K_2SO_4), and copper sulphate (CuSO4) catalyst, amino nitrogen of many organic materials is converted to ammonium.
- Free ammonia also is converted to ammonium.
- After addition of base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulphuric acid.
- The ammonia may be determined colorimetrically, by ammonia-selective electrode, or by titration with a standard mineral acid.
- The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentration.

Interferences

- The most reliable results are obtained on fresh samples.
- If an immediate analysis is not possible, preserve samples for Kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated sulphuric acid and storing at 4°C.
- Do not use $HgCl_2$ because it will interfere with ammonia removal.

Sampling

- Nitrate: During kjeldahl digestion, nitrate in excess of 10 mg/l can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N_2O, resulting in negative interference.
- Inorganic salts and solids: The acid and salt content of the kjeldahl digestion reagent is intended to produce a digestion temperature of about 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 increase in temperature, add more sulphuric acid to maintain an acid-salt balance.

Safety precautions

- Handle concentrated sulphuric acid with care.
- Always use safety goggles, gloves and laboratory coat while working in the laboratory.
- After the analysis clean bottles and beakers with clear water and keep for drying.
- Dispose of used gloves after completing analysis.
- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.
- Avoid spillage and contact with skin. In the latter case, use copious quantities of cold water to wash skin and call for medical attention.

Apparatus

- Digestion apparatus: Kjeldahl flasks with a total capacity of 800 ml yield the best results. Digest over a heating device adjusted so that 250 ml water at an initial temperature of 25°C can be heated to a rolling boil in about 5 minutes. The temperature range should be 375–385°C for effective digestion.
- Distillation apparatus
- 250 ml of TKN digestion tubes

- Titrate apparatus
- Glass bead
- Erlenmeyer flask
- Cylinder
- Volumetric flask

Reagents

Prepare all reagents and dilution in ammonia-free water. All of the reagents listed for the determination of nitrogen (ammonia), are required, plus the following:

- Digestion reagent:

Dissolve 134 g K_2SO_4 and 7.3 g $CuSO_4$ in ~800 ml water. Carefully add 134 ml conc. H_2SO_4. When it has cooled to room temperature, dilute the solution to 1 l with water. Mix well. Keep at a temperature close to 2°C to prevent crystallization.

- Sulphuric acid — 0.1 N

Dissolve 2.8 ml of concentrated sulphuric acid into 1 l of distilled water

- Sodium hydroxide — 35 per cent

Dissolve 350g of NaOH into 1 l of distilled water

- Sodium hydroxide-sodium thiosulfate reagent:

Dissolve 500 g NaOH and 25 g $Na_2S_2O_3$ $5H_2O$ in water and dilute to 1 l.

- Borate buffer solution:

Add 88 ml 0.1N NaOH solution to 500 ml approximately 0.025M sodium tetraborate ($Na_2B_4O_7$) solutions (9.5 g $Na_2B_4O_7$ $10H_2O$/L) and dilute to 1 l.

- Sodium hydroxide: NaOH, 6N.
- Mixed indicator

Mix methyl red (20mg) and bromocresol green indicator (100mg) top up to 100 ml ethanol. Make up every month.

Procedure

Sample preparation

- Weigh out between 1.8 g and 2 g of well mixed faecal sludge sample.
- Place the weighed out sample into a blender with 250 ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1 l with distilled water.
- Transfer the 1 l solution to a plastic bottle and store in the cold room.

Instrument set up

- Add 1000 ml of 32 per cent NaOH into reagent bottle 2, screw in bottle (clockwise) and push the bubbling tube to the bottom.
- Temperature range is set, follow program I- 420°C for 60 minutes,
- Place suction cap onto tubes and open tap until a steady flow of water is reached (2l/min).
- Set pump to Mode A, air flow No: 4 until temperature of heating block reaches 200°C.
- Then set pump to Mode B: air flow No: 4 until end of digestion.

- Reactivate Mode B—100 per cent of the maximum air flow—if SO_3 gas emission is too much.

Digestion

- Place 50 ml of mixed diluted sample into 300ml kjeldahl flask for raw or primary sewage, wastewater or 140 ml for ponds, rivers or final effluents.
- Add a glass rod to the tube, and 5 boiling stones.
- Slowly add 10 ml of concentrated sulphuric acid, 1 kjeltab (or 2 spatulas powder) and swirl to dissolve. Wait approx. 15 minutes or overnight if sample as a high organic/fat content and then place onto digestion unit.
- Boil briskly at 420°C until dense fumes of SO_3 are evolved and a pale green colour is obtained.
- The required temperature, i.e. 420°C , is usually reached after an hour.
- Keep pump running for 30 minutes after samples are fully digested and heating block is switched off.
- If sample is too little before it is fully digested, add 10 ml concentrated sulphuric acid and remember to increase the volume of sodium hydroxide used during the distillation.
- Digestion takes about 3 hours. The colour changes from blue to dark green to black to colourless/pale green.
- Switch off the heating block, pump and the water supply.
- Replace water in the water bath and replace the NaOH in reagent bottle 2.

Distillation

- Prepare absorption solution by placing 25 ml of 4% boric acid in a 250 ml conical flask and insert under the condenser outlet with the tip below the surface of boric acid.
- Lower collected distillate free from contact with the condenser tip and continue distillation for 1 or 2 minutes to cleanse the condenser.

Enter distillation programme as follows:
Volume of water — 50 ml
Volume of NaOH — 50 ml (if 10 ml sulphuric acid used in digestion)
— 200 ml (if 30 ml sulphuric acid used in digestion)
Phenolphthalein indicator — 10 drops

Sample in tube turns purple with addition of NaOH — above pH 11 before distillation
Distillation time — 3 minutes

Titration

- Titrate the distillate against 0.1 N sulphuric acid with mixed methyl red (0.02 g) bromocresol green indicator (0.1 g) top up to 100 ml ethanol.
- Colour change: from blue to pale pink

Calculation

Nitrogen (mg/l) = (*Titration–Blank*)(0.1)(14)(1000)/*Sample volume* (ml)

where:
0.1 — Normality of sulphuric acid used in titration
14 — Atomic weight of nitrogen
1000 — Conversion of g to mg

5.11 STANDARD OPERATING PROCEDURE FOR ORTHO-PHOSPHATE AND TOTAL PHOSPHATE

Scope and application

The measurement of total phosphorus and phosphate is essential for performance studies on the struvite reactor. The phosphate concentration in influent and effluent gives an indication of the performance of the reactor operation whereas the total P values (influent and effluent) demonstrate the effectiveness of the filtration material used. The recovery can be calculated based on these measurements.

(Phosphate) Measuring range 0.02–11.46 mg/l P_2O_5
(Total phosphate) Measuring range 0.11–11.46 mg/l P_2O_5

Principle

In sulphuric solution orthophosphate ions react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue (PMB) that is determined photometrically.

Interferences

- Sample for phosphate analysis must be pretreated by filtration (0.45μm) to remove most of the turbidity (interferes with photometric measurement).
- In the case of total P, the sample must not be filtrated! The filtration step would remove already precipitated struvite during urine storage and thus render the analysis false.
- In any case urine should be diluted at least 1:100 to avoid matrix effects.
- Other interferences are mentioned in operational manual of test kits.

Sampling

Collect samples preferably in glass bottles.

Safety precautions

- Handle concentrated acids with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- After the analysis clean bottles and beakers with clear water keep them for drying.
- Dispose of the used gloves after completing analysis.
- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.
- Avoid spillage and contact with skin. In the latter case, use copious washings with cold water and call for medical attention.

Apparatus

- Heating block for total P measurement.
- Spectrophotometer.
- Glassware: Use acid-washed glassware for determining low concentrations of orthophosphates.

Note: Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCL and rinse well with distilled water. Preferably reserve the glassware only for phosphate determination and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

Reagents

Phosphate test
- PO_4^{-1} – Sulphuric Acid (≥25% - <50%)
- PO_4^{-2} – Non-hazardous

Total phosphate test
- P-1K – Sodium nitrate (≥50% - ≤100%)
- P-2K – Sulphuric acid (≥10% - <15%)
- P-3K – Non-hazardous

Calibration

To check the photometric measurement system (test reagents, measurement device and handling) and the mode of working, Spectroquant® CombiCheck 10 can be used. Besides a standard solution with 0.80 mg/l PO4-P, CombiCheck 10 also contains an addition solution for determining sample-dependent interferences (matrix effects).

Sample preparation

Faecal samples are diluted by blending 1.8–2 g sample into 1 l of distilled water, as described in detail in the following:
- Weigh out 1.8–2 g faecal sample using an analytical balance and add to a blender with 100 ml distilled water and blend.
- Add blended sample to a 1 l volumetric flask and dilute to 1lusing distilled water.
- Swirl flask until sample is completely dissolved.

Filtration
- Filter paper dimensions: diameter = 47 mm, pore size = 0.45 microns
- Filter the diluted solution using a Buchner funnel.
- Collect the filtrate for analysis.

Procedure

Note: Procedures according to Merck operational Manual for test kits (Phosphate 1.14848.0001 and total P 1.14543.0001)

Ortho-phosphate measurement
- Pipette 5.0 ml pretreated (diluted and filtered) sample into a test tube.
- Add 5 drops of reagent PO4-1 and mix.
- Add 1 level blue micro spoon of reagent PO4-2 and shake vigorously until the reagents completely dissolved.
- Leave to stand for 5 minutes (reaction time), then fill the sample into the cell and measure in the photometer.

Total phosphorous measurement
- Digestion for the determination of total phosphorus (wear eye protection!)
- Pipette 5.0 ml pretreated sample into a reaction cell.
- Add 1 dose Reagent P-1K, close the cell tightly, and mix.
- Heat the cell at 120 °C in the preheated thermo reactor for 30 min.
- Allow the closed cell to cool to room temperature in a test-tube rack.

Do not cool with cold water!
- Shake the tightly closed cell vigorously after cooling.

- Add 1 dose reagent P-2K, close the cell tightly, and mix.
- Add 1 dose reagent P-3K, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), then measure the sample in the photometer.

Calculations

Wet sample concentration (*g/g*) = A × V / 1000 × M
Dry sample concentration (*g/g*) = *We sample concentration* (*g/g*)/*total solids* (*g/g*)

where
A – Spectroquant reading concentration
V – Volume of dilution (L)
M – Mass of Sludge used in sample preparation (g)

5.12 STANDARD OPERATING PROCEDURE FOR BIOLOGICAL OXYGEN DEMAND (BOD)

Scope and application
Biochemical oxygen demand (BOD) is an empirical standardized laboratory test that measures oxygen requirement for aerobic oxidation of decomposable organic matter and certain inorganic materials in water, polluted waters and wastewater under controlled conditions of temperature and incubation period. The quantity of oxygen required for above oxidation processes is a measure of the test. The test is applied for freshwater sources (rivers, lakes), wastewater (domestic, industrial), polluted receiving waterbodies, marine water (estuaries, coastal water) and also for finding out the level of pollution, assimilative capacity of waterbody and also performance of waste treatment plants.

Principle

Titrimetric method
This test measures the oxygen utilized for the biochemical degradation of organic material (carbonaceous demand) and oxidation of inorganic material such as sulphides and ferrous ions during a specified incubation period. It also measures the oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. Temperature effects are held constant by performing a test at fixed temperature. The methodology of BOD test is to compute a difference between initial and final DO of the samples incubation. A minimum -500ml of sample is required for the test. DO is estimate by iodometric titration.

Since the test is mainly a bio-assay procedure, it is necessary to provide standard conditions of temperature, nutrient supply, pH (6.5-7.5), adequate population of microorganisms and absence of microbial-growth-inhibiting substances. The low solubility of oxygen in water necessitates strong wastes to be diluted to ensure that the demand does not increase the available oxygen. A mixed group of microorganisms should be present in the sample; otherwise, the sample has to be seeded. Generally, temperature is controlled at 20°C and the test is conducted for five days, as 70–80 per cent of the carbonaceous wastes are oxidized during this period. The test can be performed at any other temperature provided the correlation between BOD5 20°C is established under same experimental condition is equivalent to BOD3, 27°C) for Indian conditions. While reporting the results, the incubation

period in days and temperature in °C is essential to be mentioned.

Interference

Since DO estimation is the basis of BOD test, sources of interference in BOD test are the same as in the DO test. In addition, lack of nutrients in dilution water, lack of an acclimated seed organisms and presence of heavy metals or other toxic materials such as residual chlorine are other sources of interferences in this test. Neutralize the sample to pH to 7.5 using a pH meter. Remove high residual chlorine by adding predetermined sodium sulphate solution.

Equipment and apparatus

- BOD bottles 300 ml capacity (clean with a detergent, rinse thoroughly and drain before use) with a water seal.
- Incubator or water-bath to be controlled at 20°C or at any desired temperature 10°C. Exclude all light to prevent photosynthetic production of DO.

Reagents and standards

All reagents listed in DO estimation are used for BOD. In addition following reagents are required:

- Phosphate buffer: Dissolve 8.5 g KH_2PO_4, 21.75g K_2HPO_4, 33.5g $Na_2HPO_4.7H_2O$ and 1.7g NH_4C; in distilled water and dilute to 1000 ml. The pH should be 7.2 without further adjustment. Discard reagent if there is any sign of biological growth.
- Magnesium sulphate: Dissolve 22.5g $MgSO_4.7H_2O$ in about 700 ml of distilled water and dilute to 1 litre.
- Calcium chloride: Dissolve 27.5g anhydrous $CaCl_2$ in about 7000 ml of distilled water and dilute to 1 litre.
- Ferric chloride: Dissolve 0.25g $FeCl_3.6H_2O$ in about 700 ml of distilled water and dilute to 1 litre
- Sodium sulphate solution 0.025N: Dissolve 1.575g Na_2SO_3 in distilled water and dilute to 1000 ml. Solution should be prepared daily.
- Acid and alkali solutions 1N: Prepare 1N H_2SO_4 and 1N NaOH or neutralization of caustic or acidic samples.
- Nitrification inhibitor: 2-chloro-6-(trochloromethyl) pyridine (nitrification inhibitor 2570-24 [2.2% TCMP])

Procedure

Preparation of dilution water

- The source of dilution water may be distilled water, tap or receiving-stream water free of biodegradable organics and bio-inhibitory substances such as chlorine or heavy metals.
- Aerate the required volume of dilution water in a suitable bottle by bubbling clean-filtered compressed air for sufficient time (one to two hours) to attain DO saturation at room temperature or at 20°C/27°C.
- Before use stabilize the water at 20°C/27°C.
- Add 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solutions, in this order, for each litre of dilution water. Mix well. The quality of dilution water may be checked by incubating a BOD bottle full of dilution water for five days at 20°C for 3 days at 27°C. DO uptake of dilution water should not be more than 0.2 mg/l and preferable not more than 0.1 mg/l.

- For wastes that are not expected to have sufficient microbial population, seed is essential. Preferred seed is effluent from a biological treatment system. Where this is not available, supernatant from domestic wastewater (domestic sewage) settled at room temperature for at least one hour but not longer than 36 hours is considered sufficient in the proportion 1–2 ml/l of dilution water. Adopted microbial population can be obtained from the receiving water microbial population can be obtained from the receiving water body preferably 3–8 km below the point of discharge. In the absence of such situation, develop an adapted seed in the laboratory.
- Determine BOD of the seeding material. This is seed control. From the value of seed control determine seed DO uptake. The DO uptake of seeded dilution water should be between 0.6 mg/L and 1 mg/L.

Sample preparation

- Neutralize the sample to pH 7, if it is highly acidic or alkaline.
- The sample should be free from residual chlorine. If it contains residual chlorine remove it by using $Na_2S_2O_3$ solution as described below.
- Take 50 ml of the sample and acidify with addition of acetic acid. Add about 1g KI. Titrate with 0.025N $Na_2S_2O_3$, using starch indicator. Calculate the volume of $Na_2S_2O_3$ required per litre of the sample and accordingly add to the sample to be tested for BOD.
- Certain industrial wastes contain toxic metals, e.g. planting wastes. Such samples often require special study and treatment.
- Bring samples to 20 ffl 1°C before making dilutions.
- If nitrification inhibition is desired, add 3 mg 2-chloro-6-(trichloromethyl) pyridine (TCMP) to each 300 ml bottle before capping or add sufficient amount to the dilution water to make a final concentration of 30 mg/L. Note the use of nitrogen inhibition in reporting results.
- Samples having high DO contents, DO ≥ 9mg/L should be treated to reduce the DO content to saturation at 20°C. Agitate or aerate with clean, filtered compressed air.

Dilution of sample

Dilutions that result in a residual DO of at least 1mg/l and DO uptake of at least 2 mg/l produce reliable results. Make several dilutions of the pre-treated sample so as to obtain about 50 per cent depletion of DO or DO uptake of 2 mg/l. Prepare dilutions as follows:

- Siphon out half the required volume of seeded dilution water in a graduated cylinder or volumetric flask without entraining air. Add the desired quantity of mixed sample and dilute to the appropriate volume by siphoning dilution water. Mix well with plunger type mixing rod to avoid entraining air.
- Sample processing.
- Siphon the diluted or undiluted sample in three labelled bottles and stopper immediately.
- Keep one bottle for determination of the initial DO and incubate two bottles at 27°C for three days. See that the bottles have a water seal.
- Prepare a blank in triplicate by siphoning plain dilution water (without seed) to measure the O_2 consumption in dilution water.
- Also prepare a seed blank in triplicate to measures BOD of seed for correction of actual BOD.
- Determine DO in a BOD test can in the blank on initial day and end of incubation period by winkler method as described for DO measurement.
- DO estimation in a BOD test can also be done by membrane electrodes. A DO probe

with a stirrer is used to determine initial and final DO after incubation in BOD samples. The semi-permeable membrane provided in the DO probe acts as a diffusion barrier against impurities between sensing element and sample.

DO Measurement

Reagents

- Manganese sulphate: Dissolve 480 g $MnSO_4.4H_2O$ or 400g $MnSO_4.2H_2O$ in distilled to 1000 ml. Filter if necessary. This solution should not give colour with starch when added to an acidified solution of Kl.
- Alkali iodide-azide reagent: For saturated or less than saturated samples: Dissolve 500g NaOH (or 700g KOH) and 150g KI (or 135g NaI) in distilled water and dilute to 1000ml. Add 10g sodium azide, NaN_3 dissolved in 40 ml distilled water. This solution should not give colour with starch solution when diluted and acidified.
- For supersaturated samples: Dissolve 10 g NaN_3 in 500 ml distilled water. Add 480 g NaOH and 750g Nal and stir to dissolve the contents.

Cautions: Do not acidify this solution because toxic hydrozoic acid fumes may be produced.

- Sulphuric acid: H_2SO_4, conc., 1 ml is equivalent to about 3ml alkali-iodide-azide reagent.
- Starch indicator: Prepare a paste or solution of 2.0 g of soluble starch powder as well as 0.2 g salicylic acid as preservative in distilled water. Pour this solution in 100 ml boiling distilled water. Continue boiling for a few minutes, cool and then use.
- Stock sodium thiosulphate, 0.1 N: Dissolve 24.82 g $Na_2S_2O_3.5H_2O$ in distilled water. Preserve by adding 0.4g solid NaOH or 1.5 ml of 6N NaOH and dilute to 1000ml.
- Standard sodium thiosulphate, 0.025N: Dilute 250ml stock $Na_2S_2O_3$ solution to 1000 ml with freshly boiled and cooled distilled water. Add preservative before making up the volume.

Note: This should be standardized with standard dichromate solution for each set of titrations.

Procedure

- Collect sample in a BOD bottle.
- Add 1 ml $MnSO_4$ followed by 1ml of alkali-iodide-azide reagent to a sample collected in 250 to 300 ml bottle up to the brim. The tip of the pipette should be below the liquid level while adding these reagents. Stopper immediately. Rinse the pipettes before putting them to reagent bottles.
- Mix well by inverting the bottle two to three times and allow the precipitate to settle leaving 150 ml clear supernatant. The precipitate is white if the sample is devoid of oxygen, and becomes increasingly brown with rising oxygen content.
- At this stage, add 1 ml conc. H_2SO_4. Replace the stopper and mix well till precipitate goes into solution.
- Take 201 ml of this solution in a conical flask and titrate against standard $Na_2S_2O_3$ solution using starch (2 ml) as an indicator. When 1 ml $MnSO_4$ followed by 1 ml alkali-iodide-azide reagent is added to the samples as in above, 2 ml of original sample is lost. Therefore 201 ml is taken for titration which will correspond to 200mL of original sample.

Note: 200 x 300/ (300-1) = 201 ml

Calculation

1ml of 0.025N $Na_2S_2O_3$ = 0.2 mg of O_2

DO in mg/l = (0.2 x 1000) x (0.025N) ml of thiosulphate/200

When dilution water is not added

BOD as O_2 mg/L = (D1 –D2) x 1000/ % dilution

When dilution water is added

BOD O_2 mg/l = (D1 – D2) – (B1 –B2) x 1000/ % dilution

When material is added to sample or to seed control

BOD O_2 mg/L = (D1 – D2) – (B'1 x B'2) x F x 1000/ % dilution

Where
D1 = DO of sample immediately after preparation, mg/l
D2 = DO of sample after incubation period, mg/l
B1 = DO of blank (seeded dilution water) before incubation, mg/l
B2 = DO of blank (seeded dilution water) after incubation, mg/l
F = Ration of seed in diluted sample to seed in seed control (vol. of seed in diluted sample/ Vol. of seed in seed control)
B'1 = DO of seed control before incubation, mg/l
B'2 = DO of seed control after incubation, mg/l

5.13 STANDARD OPERATING PROCEDURE FOR TESTING TOTAL COLIFORM, FAECAL COLIFORM (MOST PROBABLE NUMBER [MPN] METHOD)

Scope and field of application
The most probable number (MPN) of bacteria present can be estimated from the number of tubes inoculated and the number of positive tubes obtained in the confirmatory test, using specially devised statistical tables. It is applicable to the analysis of drinking water quality, salt or brackish waters as well as muds, sediments and sludge.

Principle
In the multiple-tube fermentation method, a series of tubes containing a suitable selective broth culture medium is inoculated with test portions of a water sample. After a specified incubation time at a given temperature, each tube showing gas formation is regarded as 'presumptive positive' since the gas indicates the possible presence of coliforms. However, gas may also be produced by other organisms, and so a subsequent confirmatory test is essential. The two tests are known respectively as the presumptive test and the confirmatory test. For the confirmatory test, a more selective culture medium is inoculated with material taken from the positive tubes. After an appropriate incubation time, the tubes are examined for gas formation as before.

Sampling
- Collect samples in clean sterile, nonreactive borosilicate glass or plastic bottles or pre-

sterilized plastic bags appropriate for microbiological use.

- Keep sampling bottle closed until it is to be filled.
- Do not place cap down on any surface.
- Avoid external contamination during sample collection and do not contaminate inner surface of stopper or cap and bottle neck.
- Fill container without rinsing, replace stopper/cap immediately.
- When the sample is collected, leave ample air space in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination. Reject sample bottles that are overfilled and request resampling or, alternatively, add overfilled samples to a larger sterile sample bottle in the laboratory to assure adequate mixing.
- Sampling frequency and the number of samples to be collected will depend on ultimate data usage needs.
- Start microbiological analysis of water samples as soon as possible after collection to avoid unpredictable changes in the microbial population.
- For the most accurate results, ice sample during transport to the laboratory if they cannot be processed within one hour after collection.
- Maintain samples in the dark and keep cool with ice at <8°C but do not frozen during a maximum transport time of six hours.
- Do not hold for more than 24 hours.

Safety precautions

- Sterile work area to reduce contamination from airborne particles and aerosols
- Good personal hygiene
- Wash your hand before and after working with cell
- Sterile lab ware, reagents and media
- Sterile handling
- Always wipe your hand and work are with 70 per cent ethanol

Apparatus

- Water bath
- Autoclave
- Cabinet (Laminar Airflow)
- Incubator
- Test-tubes and racks
- Durham tubes
- Pipettes and tips
- Balance
- Vortex

Culture media

- Brilliant green lactose bile broth (BGLBB)
  Suspend 40 gm of the powder (Himedia) in 1 l of distilled water. Mix well and dissolve by heating with frequent agitation. Dispense 10 ml into test tubes with Durham gas collecting tubes for gas detection. Sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2–8°C. The colour is green.
- MacConkey broth

Add 40 g powder (Himedia) to water (1000 ml), mix thoroughly, and heat to boiling to dissolve. Sterilize by autoclaving for 15 minutes at 121°C.

- Normal saline: 9 g of sodium chloride in 1000 ml of distilled water. Sterilize by autoclaving for 15 minutes at 121°C

Sample preparation

Homogenization

- Sample homogenization procedures are based on whether the sample is a liquid or a solid liquid samples: Homogenize 300 ml of sample in a sterile blender on high speed for one to two minutes. Adjust the pH to 7.0–7.5 by adding 1.0 N hydrochloric acid or 1.0 N sodium hydroxide, if necessary.
- Solid samples: 30 gm of sample weighed directly into the sterile blender jar. Add 270 ml of sterile dilution water to rinse any remaining sample into the blender. Cover and blend on high speed for one minute. This is the 'homogenized' sample. A volume of 1 ml of the 'homogenized' sample contains 0.1 g of the original sample. Adjust the pH to 7–7.5 by adding 1 N hydrochloric acid or 1 N sodium hydroxide, if necessary.

Procedure

Multiple-Tube Fermentation Technique for faecal coliform and Standard Total Coliform Fermentation Technique, five tubes per dilution, are used (1 ml, 0.1 ml and 0.01 ml sample).

Presumptive

- Collect a water sample into the sterile bottle. Return the sample to the lab. (Previous studies have found such high concentrations of coliform that we need to dilute the sample first.)
- Prepare sterile test tubes of 9 ml sterile normal saline, label the test tubes 10–1 to 10–5 indicating the dilution factor.
- Aseptically add 1 ml of sample to the 9 ml of sterile dilution water in the first test tubes (10–1) by using 1000 μl auto-micropipette and mix by vortex.
- Take 1 ml (use new tip) of this dilution and add to the next test tube (10^{-2}) and mix by vortex.
- Repeat this procedure for remaining test tube.
- Arrange fermentation tubes in rows of five each in a test tube rack.
- Add 1 ml of serially diluted sample to 5 fermentation tubes containing macConkey broth (10 ml/tube)
- Incubate the inoculated tubes at 35 +/ 0.5°C for 24 hours.
- Check each tube for the presence of acid and gas production in the inner shell vials. If gas production is not readily apparent, shake the tubes gently and check for rising gas bubbles.
- Record positive presumptive results (gas produced) on the MPN test data sheet.
- All positive presumptive tubes should be carried into the faecal coliform confirming test procedure. Transfers should be made as soon as the gas production is noted in a fermentation tube.

Faecal coliform test

- From all the presumptive coliform positive tubes, inoculate in to tube containing Brilliant Green Lactose Bile Broth (BGLBB)/EC medium.
- Incubate at 44°C (+/-0.5°C) for 24 hours.
- Record positive results (gas production) and compare on the MPN test data sheet.

Calculation

MPN/100 ml

$$MPN/100ml = \frac{\text{No. of Positive tube} \times 100}{\sqrt{\left\{\begin{matrix}\text{ml of sample in} \\ \text{negative tubes}\end{matrix}\right\} \times \left\{\begin{matrix}\text{ml of sample in} \\ \text{all tubes}\end{matrix}\right\}}}$$

After selecting the valid series, the MPN/100 ml is determined by matching the selected series with the same series on the MPN reference chart. If the selected series does not match the sample dilution series at the top of the MPN reference chart, the results must be calculated using the following formula:

MPN/100 ml = MPN from chart x (ml sample for first column of chart/ml sample in first dilution of the selected series)

5.14 STANDARD OPERATING PROCEDURE FOR HELMINTH EGGS ENUMERATION

Scope and field of application

The prevalence of helminth infections in people living with basic water and sanitation in third-world countries such as most of those in Africa is generally high. Due to the extreme hardiness of the eggs of the roundworm, *Ascaris lumbricoides*, they are used in the sanitation field as a 'marker for the safe reuse of human waste'. Other commonly found helminths are *Trichuris trichiura*, *Taenia sp.* and in areas with very sandy soils, *hookworm sp*. It is generally accepted that if any of the various waste treatments used are successful in inactivating *Ascaris* eggs, then all harmful bacteria and viruses should also be killed.

Principle

Helminth eggs are thought to adhere to soil particles, possibly as a result of charge interactions with or adsorption of eggs to the particles. Many waste samples, even if they are not from Urine-Diversion Toilets, are often contaminated with silica particles, hence the use of ammonium bicarbonate as a wash solution. 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 mediums.

- Ammonium bicarbonate is used as both a wash solution and also to dissociate the eggs from the soil particles.
- Filtration, using 100µm and/or 20µm sieves is used to separate larger and smaller particles from the eggs both after washing and after flotation.
- Flotation, using a solution of zinc sulphate at a specific gravity of 1.3 is used to float eggs with a relative density of <1.3 out of the matter retained with them on the 20µm sieve.

Safety precautions

- Sterile work area to reduce contamination from airborne particles and aerosols
- Good personal hygiene
- Wash your hand before and after working

Table 4: MPN test data sheet (Source: APHA 1998)

Combination of positives	MPN index/100 mL	Confidence limits		Combination of positives	MPN index/100 mL	Confidence limits	
		Low	High			Low	High
0-0-0	<1.8	–	6.8	4-0-3	25	9.8	70
0-0-1	1.8	0.090	6.8	4-1-0	17	6.0	40
0-1-0	1.8	0.0920	6.9	4-1-1	21	6.8	42
0-1-1	3.6	0.70	10	4-1-2	26	9.8	70
0-2-0	3.7	0.70	10	4-1-3	31	10	70
0-2-1	5.5	1.8	15	4-2-0	22	6.8	50
0-3-0	5.6	1.8	15	4-2-1	26	9.8	70
1-0-0	2.0	0.10	10	4-2-2	32	10	70
1-0-1	4.0	0.70	10	4-2-3	38	14	100
1-0-2	6.0	1.8	15	4-3-0	27	9.9	70
1-1-0	4.0	0.71	12	4-3-1	33	10	70
1-1-1	6.1	1.8	15	4-3-2	39	14	100
1-1-2	8.1	3.4	22	4-4-0	34	14	100
1-2-0	6.1	1.8	15	4-4-1	40	14	100
1-2-1	8.2	3.4	22	4-4-2	47	15	120
1-3-0	8.3	3.4	22	4-5-0	41	14	100
1-3-1	10	3.5	22	4-5-1	48	15	120
1-4-0	10	3.5	22	5-0-0	23	6.8	70
2-0-0	4.5	0.79	15	5-0-1	31	10	70
2-0-1	6.8	1.8	15	5-0-2	43	14	100
2-0-2	9.1	3.4	22	5-0-3	58	22	150
2-1-0	6.8	1.8	17	5-1-0	33	10	100
2-1-1	9.2	3.4	22	5-1-1	46	14	120
2-1-2	12	4.1	26	5-1-2	63	22	150
2-2-0	9.3	3.4	22	5-1-3	84	34	220
2-2-1	12	4.1	26	5-2-0	49	15	150
2-2-2	14	5.9	36	5-2-1	70	22	170
2-3-0	12	4.1	26	5-2-2	94	34	230
2-3-1	14	5.9	36	5-2-3	120	36	250
2-4-0	15	5.9	36	5-2-4	150	58	400
3-0-0	7.8	2.1	22	5-3-0	79	22	220
3-0-1	11	3.5	23	5-3-1	110	34	250
3-0-2	13	5.6	35	5-3-2	140	52	400
3-1-0	11	3.5	26	5-3-3	170	70	400
3-1-1	14	5.6	36	5-3-4	210	70	400
3-1-2	17	6.0	36	5-4-0	130	36	400
3-2-0	14	5.7	36	5-4-1	170	58	400
3-2-1	17	6.8	40	5-4-2	220	70	440
3-2-2	20	6.8	40	5-4-3	280	100	710
3-3-0	17	6.8	40	5-4-4	350	100	710
3-3-1	21	6.8	40	5-4-5	430	150	1100
3-3-2	24	9.8	70	5-5-0	240	70	710
3-4-0	21	6.8	40	5-5-1	350	100	1100
3-4-1	24	9.8	70	5-5-2	540	150	1700
3-5-0	25	9.8	70	5-5-3	920	220	2600
4-0-0	13	4.1	35	5-5-4	1600	400	4600
4-0-1	17	5.9	36	5-5-5	>1600	700	–
4-0-2	21	6.8	40				

Results to two significant figures.

- Always wipe your hand and work area with 70 per cent ethanol

Apparatus

- Compound microscope with 10x and 40x objectives
- Bench-top centrifuge with a swing-out rotor that can spin a minimum or 8 x 15 ml plastic conical test tubes (Falcon tubes)
- Sink with hose attached to tap for washing using high water pressure
- Top balance (scale for weights up to 200 gm and accurate to 2 decimal places)
- Magnetic stirrer and bar magnets
- Vortex mixer
- Hydrometer that can measure SG between 1.2 and 1.3 or 1.3 and 1.4
- 100µm mesh stainless steel flat sieve, diameter 200 mm
- 20µm mesh stainless steel flat sieve, 200 mm
- 20µm mesh stainless steel flat sieve, 100 mm
- Plastic test tube racks to hold the 15 ml Falcon tubes
- Plastic 200 ml beakers
- Plastic 'hockey-stick' shaped stirring rods
- Plastic 3 ml pipettes
- Non-sterile gloves
- Applicator sticks and wooden tongue depressors
- Microscope slides (76 x 26 x 1.2 mm)
- Cover glasses 22 x 40 mm

Chemicals and Reagents

- Zinc sulphate ($ZnSO_4$)
  $ZnSO_4$ (heptahydrate) is made up by dissolving 500g of the chemical in 880 ml de-ionized or distilled water.

Note: A hydrometer must be used to adjust the specific gravity (SG) to 1.3, use more chemical if the SG is too low or add more water if it is >1.3.

- Ammonium bicarbonate ((NH_4HCO_3)
  Dissolve 119 gm of ammonium bicarbonate in 1lt de-ionized water (use a magnetic stirrer and bar magnet)—store in a glass jar.

Procedure

- Weigh 10 or 20 gm into a 200 ml plastic beaker, on a top-pan balance.
- Add 50–80 ml NH_4HCO_3 a magnetic stirring bar, mix on magnetic stirrer for 20 minutes.
- Pour mixture over 100µm sieve which fits on top of a 20µm sieve (wet sieves with tap H_2O first).
- Rinse beaker with tap H_2O and pour over sieves.
- Wash magnet and remove, wash 100µm sieve well (using 'hockey stick' or gloved hand) over 20µm filter, checking bottom sieve for fluid build-up.
- Separate sieves and then rinse the 20µm sieve well and wash the material to one side of the sieve.
- Rinse all material off 20µm filter into original rinsed-out beaker.
- Pour beaker contents into 4 x 15 ml conical test tubes.
- Centrifuge at 3000 rpm in centrifuge with swing-out rotor for five minutes.
- Pour off supernatant: deposits left in four test tubes.
- Place test tubes in rack with applicator stick in each (as a stirring rod) and pipette in

$ZnSO_4$, 3 ml at a time, vortexing in between addition of the chemical, until tubes are filled to 14 ml mark.
- Centrifuge at 2000 rpm for five minutes.
- Pour supernatant flotation fluid over smaller diameter 20µm sieve. Wash out test tubes and keep one aside for reuse.
- Wash material on 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.
- Centrifuge at 3000 rpm for 5 minutes to obtain the final deposit.
- Pour off supernatant water and pipette up the deposit, place it on a counting chamber (hemocyometer), place a 22 x 40 mm cover slip on top, examine and count every helminthes egg, classifying them as viable, potentially viable or dead.
- Calculate the number of eggs per ml sample

5.15 STANDARD OPERATING PROCEDURE FOR HEAVY METALS ANALYSIS

Flame atomic absorption spectrophotometry

Scope and application

In flame atomic absorption spectrometry, a sample is aspirated into a flame and atomized. A light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the flame. For some metal, atomic absorption exhibits superior sensitivity over flame emission. Because each metal has got its own characteristic absorption wavelength a source lamp composed of the elements is used, this marks the method relatively free from spectral or radiation interference. The amount of energy at the concentration of the element in the sample over a limited concentration range. Most atomic absorption instruments also are equipped for operation in an emission mode, which may provide better linearity for some elements.

Interferences

Chemical interference

Many metals can be determined by direct aspiration of sample into an air-acetylene flame. The most troublesome type of interference is termed 'chemical' and results from the lack of absorption by atoms bound in molecular combination in the flame. This can occur when the flame is not hot enough to dissociate the molecules or when the dissociated atom is oxidized immediately to a compound that will not dissociate further at the flame temperature.

Background correction

- Molecular absorption and light scattering caused by solid particles in the flame can cause erroneously high absorption values resulting in positive errors. When such phenomena occur, use background correction to obtain accurate values. Use any one of three types of background correction.
- Continuum-source background correction: Continuum-source background corrector utilizes either a hydrogen filled hollow cathode lamp with metal cathode or a deuterium arc lamp. When both line sources are placed in the same optical path and are time-shared, the broadband background from the elemental signal is subtracted electronically, and the resultant signal will be background-compensated.
- Zeeman background correction: This correction is based on the principle that a magnetic field splits the spectral in to two linearly polarized light beams parallel and

perpendicular to the magnetic field. One is called the pi component and other sigma component. These tow line beams have exactly the same wavelength and differ only in the plane of polarization. Zeeman background correction provides accurate background correction at much higher absorption levels than is possible with continuum sources background correction system.

- Smith-Hieftje background correction: This correction is based on the principle that absorbance measured for a specific element is reduced as the hollow cathode lamp is increased while absorption of non-specific absorption substance remains identical at all current levels. When this method is applied, the absorption at a high current mode is subtracted from the absorption at a low-current mode. Under these conditions, any absorbance due to non-specific background is subtracted out and corrected for.

Apparatus and equipment

- Atomic absorption spectrometer: It consists of a light source emitting the line spectrum of an element, a device for vaporizing the sample, a means of isolating an absorption line, and a photoelectric detector with its associated electronic amplifying and measuring equipment.
- Burner: The most common type of burner is a premix, which introduces the spray into a condensing chamber for removal of large droplets. The burner may be fitted with a conventional head containing a single slot: a tree-slot boling head which may be preferred for direct aspiration with an air-acetylene flame, or a special head for use with nitrous oxide and acetylene.
- Readout: Most instruments are equipped with either a digital or null meter readout mechanism. Most modem instruments are with microprocessor or stand-alone control computers capable of integrating absorption signals over at high concentrations.
- Lamps: Use either a hollow-cathode lamp or electrode less discharge lamp (EDL). Use one lamp for each element being measured. Multi-element hollow-cathode lamps generally provide lower sensitivity than single element lamps. EDLs take a longer time to warm up and stabilize.
- Pressure reducing valves: Maintain supplies of fuel and oxidant at pressures somewhat higher than the controlled operating pressure of the instrument by using suitable reducing valves. Use a separate reducing value for each gas.
- Vent: Place a vent about 15–30 cm above the burner to remove fumes and vapours from the flame. This precaution protects laboratory personnel from vapours, protects the instrument from corrosive vapours, and prevents flame stability from being affected by room drafts. A damper of variable speed blower is desirable for modulating airflow and preventing flame disturbance, select blower size to provide the airflow recommended by the instrument manufacturer. In laboratory locations with heavy particulate air pollution, use clean laboratory facilities.

Reagents and standards

- Air: Air is cleaned and dried through a suitable filter to remove oil, water and other foreign substances. The sources may be a compressor or commercially bottled gas.
- Acetylene: Standard commercial grade in which acetone is always present in acetylene cylinders. This prevents the entering and damaging the burner head by replacing a cylinder when its pressure has fallen to 689 kPa (100 psi) acetylene.
- Caution: Acetylene gas represent an explosive hazard in the laboratory. Follow instrument manufacturer's direction in plumbing and using this gas. Do not allow gas

contact with copper, brass with >65 per cent copper, silver or liquid mercury; do not use copper or brass tubing, regulators, or fittings.

- Nitrous oxide: The gas is commercially available in cylinders. Fit nitrous oxide cylinder with a special non-freezable regulator or wrap a heating coil around an ordinary regulator to prevent flashback of the burner caused by regulation in nitrous oxide flow through a frozen regulator.
- Caution: Use nitrous oxide with strict adherence to manufacturer's directions. Improper sequencing of gas flow at start-up and shutdown of instrument can produce explosions from flashback.
- Metal-free water: Use metal-free water for preparing all reagents and calibration standards and as dilution waste. Prepare metal-free water by deionizing tap water and/or by using one of the following processes, depending on the metal concentration in the sample: single distillation, reinstallation, or sub-boiling.
- Standard solution: Prepare standard solutions of known metal concentrations. Stock standard solution can be obtained from several commercial sources that should be used as secondary standard solution for calibration of instrument and the prepared standards.

Procedure

Preliminary digestion for metals

To reduce interference by organic matter and to convert metal associated with particulate to a form (usually the free metal) that can be determined by inductively coupled plasma spectroscopy, use one of the digestion techniques. Use the least rigorous digestion method required providing complete and consistent recovery compatible with the analytical method and the metal being analysed.

Closed system digestion (microwave-assisted digestion)

Nitric acid will digest most samples adequately. Nitrate is an acceptable matrix for both flame and electro-thermal atomic absorption. Some samples may require addition of perchloric, hydrochloric, or sulphuric acid for complete digestion. Confirm metal recovery for each digestion and analytical procedures used. As a general rule, HNO_3 alone is adequate for clean samples or easily oxidized materials; HNO_3-H_2SO_4 or HNO_3-HCl digestion is adequate for readily oxidizable organic matter; HNO_3-$HClO_4$ or HNO_3-$HClO_4$-HF digestion is necessary for difficult-to-oxidize organic matter or minerals. Dry ash formation is helpful if large amounts of organic matter are present.

Microwave digestion system

Scope and application

The microwave region of the electromagnetic spectrum lies between infrared radiation and radio frequencies and corresponds to wavelength of 1cm to 1m frequencies of 30GHz–300MHz respectively used for RADAR transmission and the remaining wavelength range is used for telecommunication. In order not to interfere with these uses, domestic and industrial microwave heaters are required to operate at either 12.2 cm (2.45 GHz) or 33.3cm (900M Hz) unless the apparatus is shielded in such a way that no radiation losses occur. Laboratory/domestic microwave ovens generally operate at 2.45 GHz.

Principle

Microwave system is designed with time-controlled programmable power supply, having a corrosion resistant, well-ventilated cavity and having all electronics protected against corrosion for safe operation. Use a unit having a rotating turntable with a minimum speed of 3 rpm to ensure homogeneous distribution of microwave radiation. Only laboratory-grade microwave equipment and closed digestion containers with pressure release that are specifically designed for hot and acid may be used.

Microwave sample preparation is now a standard analytical tool employing a variety of microwave equipment, including both low to high pressure closed vessels and atmospheric-pressure open-vessels. Both open vessels and closed-vessel microwave systems use direct absorption of microwave radiation through essentially microwave transparent vessel materials. Atmospheric pressure microwave systems can generate more stable temperature condition. In comparison, closed vessel microwave dissolution systems are limited by the temperature and pressure safety tolerances of the reaction vessel and the microwave absorption characteristics of the solution.

Microwave digestion rotor (MDR) technology provides analysts with unsurpassed performance capabilities and the highest standard of safety in closed vessel microwave digestion. The rotor consists of high-density polypropylene core. The compact core has the required strength to withstand the extreme pressure generated inside the vessel during digestion. Special plastic compression screws are located on the top of the rotor next to each niche. The vessel cover is also made of PFA (Teflon). Microwave digestion of organic samples frequently involves exothermic reactions, which instantaneously generate large amount of decomposition gases (CO_2 and NOx) as the sample is oxidized. The relief valve is capable of instantaneously venting excess decomposition gases and resealing the vessel for completion of the digestion procedure.

The acid used in microwave digestion may be classified in two main groups:

- Non-oxidizing acids such as hydrochloric acid, hydrofluoric acid, phosphoric acid, dilute sulphuric acid and perchloric acid.
- Oxidizing acids, such as nitric acid, hot concentrated perchloric acid, concentrated sulphuric acid and hydrogen peroxide.

Operating conditions

Install a hollow cathode lamp for the desired metal in the instrument and roughly set the wavelength. Set slit width according to manufacturer-suggested setting for the element being measured. Turn on instrument; apply to the hollow-cathode lamp the current suggested by the manufacturer, and let instrument warm-up until energy source stabilizes, generally about 10 to 20 min. Readjust current as necessary after warm-up. Optimize wavelength by adjusting wavelength dial until optimum energy gain is obtained. Align lamp in accordance with manufacturer's instruction. Install suitable burner head and adjust burner head position. Turn on air and adjust flow rate to that specified by manufacturer to give maximum sensitivity for the metal being measured. Turn on acetylene, adjust flow rate to value specified, and ignite flame. Let flame stabilize for a few minutes.

Aspirate a blank consisting of deionized water containing the same concentration of acid in standard and samples. Adjust the instrument to zero, aspirate a standard solution and adjust aspiration rate of the nebulizer to obtain maximum sensitivity. Adjust burner both vertically and horizontally to obtain maximum response. Aspirate blank again and

check zero reading of the instrument, aspirate a standard near the middle of the linear range. Record absorbance of this standard when freshly prepared and with a new hollow cathode lamp. Refer to the data on subsequent determination of the same element to check consistency of instrument setup and aging of hollow cathode lamp and standard. The instrument now is ready to operate. When analysis is complete, extinguish flame by turning off first acetylene and then air.

Standardization

Select at least three concentrations of each standard metal solution to bracket the expected metals concentration of a sample. Aspirate blank and adjust zero of the instrument. Then aspirate each standard in turn into flame record absorbance. Prepare a calibration curve by plotting on linear graph paper absorbance of standard versus their concentrations. For instruments equipped with direct concentration readout, this step is unnecessary. With some instrument it may be necessary to convert percent absorption by using a table generally provided by the manufacturer.

Calculations

Calculate concentration of each ion, in µg/l for trace elements, and in mg/L for more common metals, by referring to the appropriate calibration curve. Alternatively read concentration directly from the instrument readout if the instrument is so equipped. If the sample has high values multiply it by the appropriate dilution factor.

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