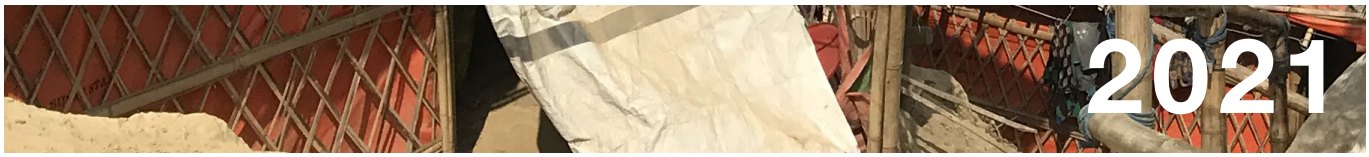
METHODOLOGIES & APPLICATION
FROM DOCUMENTED EXPERIENCE

UPM

Sampling for Faecal Sludge and Other Liquid Wastes in Emergency Settings



Sampling for Faecal Sludge and Other Liquid Wastes in Emergency Settings

is part of the series

Methodologies & Application from Documented Experience
MADE by UPM

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The findings and conclusions contained within are those of the authors and do not necessarily reflect
positions or policies of the Bill & Melinda Gates Foundation or other above mentioned institutions.

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Abbreviations & Acronyms

µS	Micro Siemens
BAU	Bangladesh Agricultural University
BF	Bathing Facility
BOD	Biological Oxygen Demand
BRAC	Bangladesh Rural Advancement Committee
°C	Celsius degree
c.	Circa
cm	Centimeter
$CaCO_3$	Calcium Carbonate
COD	Chemical Oxygen Demand
CSES	Center of Sustainable Environmental Sanitation
DC	Drainage Channel
DO	Dissolved Oxygen
DPHE	Department of Health and Engineering
EAWAG	Swiss Federal Institute of Aquatic Science and Technology Electrical
EC	Conductivity
FC	Faecal Coliforms
FOG	Fats Oil and Grease
FS	Faecal Sludge
FSM	Faecal Sludge Management
ID	Identification
i.e.	id est: In other words
ISO	International Standard Organization
l	liter
ml	milliliter
mg	milligram
N	Nitrogen
NO_2	Nitrite
NO_3	Nitrate
NGO	Non-Governmental Organization
NGOF	Non-Governmental Organization Forum
NH_4-N	Ammonium
PL	Pit Latrine
PPE	Personal Protective Equipment
SO_4	Sulphate
SS	Settleable Solids
STB	Septic Tank Bottom
STT	Septic Tank Top
TA	Total Alkalinity
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solids
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphorus
UNHCR	United Nations High Commissioner for Refugees
UPM	UPM Umwelt-Projekt-Management GmbH
USTB	University of Science and Technology Beijing
VS	Volatile Solids
VSS	Volatile Suspended Solids
WW	Wastewater

Preface

This publication is the result of the technical assistance provided by UPM Umwelt-Projekt-Management GmbH (UPM) and its partners, the Centre for Sustainable and Ecological Sanitation (CSES) of the University of Science & Technology Beijing (USTB), and the Bureau of Socioeconomic Research and Training (BSERT) of the Bangladesh University of Agriculture (BAU) to the United Nations High Commissioner for Refugees (UNHCR), the Department of Public Health Engineering (DPHE) and the local WASH sector in Cox's Bazar, Bangladesh, in cooperation with the Bill & Melinda Gates Foundation.

The goal of this technical assistance assignment was to provide support to the emergency WASH sector and local administration, regarding sanitation and faecal sludge management, with focus on value—recovery in emergency settings, in order to sustainably improve the living conditions of displaced populations and their hosting communities.

The present manual "Sampling for Faecal Sludge & Other Liquid Wastes in Emergency Settings" is a summary of the methodologies and applications tested during a liquid waste characterization study implemented by the UPM team in Cox's Bazar between October 2018 and April 2019.

The content of this manual has been used, in particular, during two on-site trainings targeting WASH & humanitarian response professionals: 1) Training Workshop on Compliance and performance monitoring of Faecal Sludge Treatment Plants (FSTP), in October 2019 in Cox's Bazar and 2) Faecal Sludge Sampling Training Workshop In Zahle, Lebanon in June 2019, organized in cooperation with UNICEF Lebanon.

A UPM team member records on-site measurements of liquid waste during a visit to Rohingya Camp at Cox's Bazar, Bangladesh, during April 2019.

Introduction

In crisis situation and in particular during the first phase of humanitarian emergecy, first responders and humanitarian organizations do not often have the time and know-how to review the primary sanitation situation in order to optimize its set-up.

The characterization of liquid waste represents then an essential initial step for the development of any efficient sanitation solution.

Detailed information of the waste streams can support the selection of the most appropriate technical solution, in order to fulfil final discharge requirements of local and international standards.

Standards for sampling and analysis of FS are not available as there are existing for other environmental fields, such as Standard Methods for the Examination of Water and Wastewater or Methods of Soil Analysis (Bassan, 2016).

In this publication and for the characterization study itself, the applied methods were adapted from Methods for the Characterization of Faecal Sludge in Vietnam (EAWAG, 2016), as well as DIN and ISO standards. These contents also include experiences of USTB-CSES and UPM from similar activities in China, Burkina Faso, Senegal and most recently in Bangladesh and Lebanon.

Overview

Contents of this manual are presented along five consecutive stages applied while sampling and analyzing liquid waste:

1	2	3	4	5
STRATEGY	**PREPARATION**	**COLLECTION**	**PRESERVATION**	**ANALYSIS**
• Objective • Source • Sampling Size • Geographical Scope • Analytic Parameters • Timing • Methodology • Quality Assurance / Quality Control	• Laboratory • Staff • Materials & Equipment • Transport	• PPE & Safety • Group • Sampling Methodologies • Preparation • Onsite Measurements • Quality Assurance / Quality Control • Transport	• Methods	• Preparation • Measurements • Quality Assurance / Quality Control • Data Log

Strategy Definition

To be able to achieve a successful sampling exercise, a strategy should be defined first, considering the following aspects:

STRATEGY

Objective

Usually defined by the assignment and the questions that need to be answered and which will determine the entire strategy.

- Characterization of FS for treatment design
- Monitoring WW treatment plant
- Understanding variations of FS
- …

Source

The location from where the sample will be taken. It is defined by the sanitation system and the type of the liquid waste to be assessed.

- Toilet (Pit latrines, compost toilets)
- Septic or Holding Tanks
- Effluent from discharge point of WWTP or FSTP
- …

Sampling Size

It is usually defined by the available budget and the required level of accuracy.

- Depending on required accuracy
- Available budget
- Organic Concentration
- …

Geographical Scope

Refers to the geographical area where the assessment should take place.

- City or District
- Refugee Camp
- Institution
- …

Analytical Parameters

A list of parameters for the analysis, which will depend on the assessment objective.

- Sample Volume
- Preparation
- Storage
- Lab Capabilities
- …

Timing

Definition of when and during what period (summer/winter) the sampling should take place.

- Summer / Winter
- Dry / Wet Session
- Duration of Sampling
- …

Methodology

Definition on the type of sample and how the sample should be collected.

- Will depend on Objective
- Will depend on Source

Quality Assurance / Quality Control

A set of principles to be implemented to ensure and control the quality of sampling and its measurements.

STRATEGY

Experience in Bangladesh

During the period from October 2018 to March 2019, UPM was commissioned by Bill & Melinda Gates Foundation to provide Technical Assistance to Emergency Response and WSH Grantees, working in the context of the Rohingya Crisis in the refugee camps located in Cox's Bazar, Bangladesh.

The objective for this assignment was to assess the range of characteristics of wastewater, greywater and faecal sludge from different sources in order to range the variations in key parameters by source (toilet pits, drainage channels, bathing facilities and septic tanks) across the camps. To fulfil this specific objective, the following strategy was followed:

Objective

Characterization of different liquid waste streams, including wastewater, greywater and faecal sludge.

Source

13 x Pit Latrines.
4 x Septic Tanks (4 x Top Layer, 4 x Bottom Layer).
6 x Bathing Facilities.
6 x Drain Channels.
Repeating the measurement of all parameters for each 10th sample. Calibration of the equipment

Geographical Scope

Refugee Camp (Nº3 and Nº4 Extension).

Analytical Parameters

pH, $CaCO_3$, DO, EC, Mineral Oil, SO_4^{2+}, Temperature, BOD_5, BMP, COD, FC, Helminths, FOG, NH_4-N, SS, TA, TKN, TOC, TP, TSS, TS, TSS, VSS.

Timing

October 2018 – March 2019.

Methodology

Grab Sample & Composed Sample,
Appropriate preservation, storage and transport.

Quality Assurance / Quality Control

Collection and processing of each 10^{th} sample blindly.
Repeating the measurement of all parameters for each 10^{th} sample.
Calibration of the equipment.
Appropriate preservation, storage and transport.

STRATEGY

Experience in Lebanon

In 13th of June 2019, UPM was commissioned by Bill & Melinda Gates Foundation to provide Technical Assistance to UNICEF Lebanon and local WASH partners who are working in informal settlements of Syrian refugees in Bekaa Valley, near Zahle, Lebanon.

The objective of the assignment was to train local NGOs partners with theory and practice on sampling for feacal sludge and wastewater, including the definition of sampling strategy which was defined as follows:

Objective
Characterization of Septage for design of WWTP and simplified sewer.

Source
30+ Holding Tanks.

Geographical Scope
Informal Settlements.

Analytical Parameters
pH, EC, TP, BOD_5, COD, TKN, T-Coliform.

Timing
June – July 2019.

Methodology
Grab Samples.

Quality Assurance / Quality Control
Collection and processing of each 10th sample blindly.
Repeating the measurement of all parameters for each 10th sample.
Calibration of the equipment.
Appropriate preservation, storage and transport.

STRATEGY

Defining Sampling Strategies

Here are three different examples on how to sample faecal sludge from one same source –a septic tank–, to illustrate how the objectives determine the methodologies for each case:

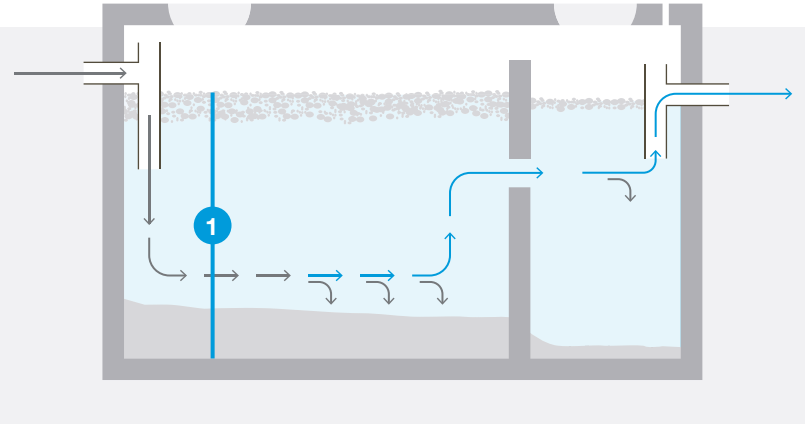
Sampling 1

OBJECTIVES

Characterization of FS for design of FSTP.

METHODOLOGY

Cross-section grab sample (Core Sampler Device).



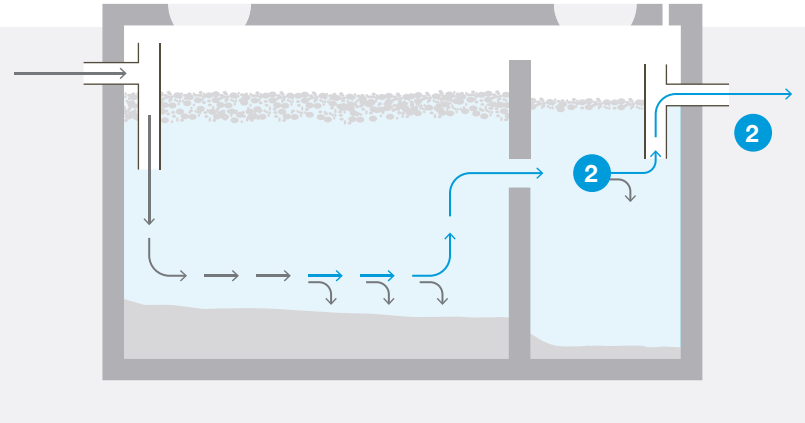
Sampling 2

OBJECTIVES

(a) Monitoring; (b) Design of WWTP; (c) Sewer Design.

METHODOLOGY

For (a): composed day sample middle layer (Mounted Jar Sampler). Also for (a): effluent pipe (Bucket), if accessible. For (b) & (c): grab sample middle layer (Mounted Jar Sampler).



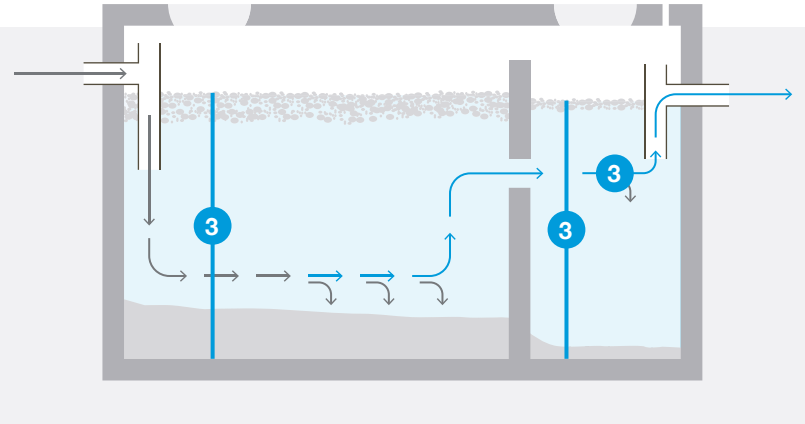
Sampling 3

OBJECTIVES

Understanding different steps of the treatment.

METHODOLOGY

Grab sample (Core & Mounted Jar Sampler).



STRATEGY

Analytical Parameters

To be able to identify the characteristics of different liquid waste streams according the objectives, a list of parameters to be evaluated and analyzed needs to be defined first.

In order to facilitate the preparation of this list, one should refer to the different standards for final output and technical options specificities.

A summary of the relevant parameters (and their specific significances) use to evaluate the quality of wastewater and faecal sludge are presented in Table 1.

TABLE 1: Analytical parameters and their significance.

Parameter	Significance
Ambient Temperature	• One of the essential parameters for biological treatment and operational control of any treatment unit.
Sample Temperature	• Predicting rates of biological activity, treatment processes, and pathogen die-off.
BOD_5	• BOD —Biological Oxygen Demand— is used to determine the strength of different liquid waste streams. • Measuring the amount of dissolved oxygen needed by aerobic biological organisms to break down organic material present in a given water sample at 20°C over a period of 5 days, presenting the biologically oxidizable organic matter content in liquid waste. • Major parameter in stream pollution control and one of the regulatory standards for effluent discharge. • Design parameter for treatment plants. • BOD_5:COD – How readily biodegradable is the wastewater?
BMP	• BMP —Biomethane Potential— describes the potential of biogas or methane gas production per unit mass of total solid or volatile solid matter at ambient or mesophilic temperatures.
COD	• COD —Chemical Oxygen Demand— is used to determine the strength of different liquid waste streams – i.e. the relative inputs of energy, chemicals, time etc. required to break the waste down to levels that the environment can absorb without ill-effects. • Measuring the amount of oxygen required for chemical oxidation of organic material by a strong chemical oxidant. COD is always equal to or higher than BOD since it is the total oxygen required for complete oxidation. • Design parameter for treatment plants. • One of the regulatory standards for effluent discharge. • BOD_5:COD — How readily biodegradable is the wastewater?
Carbonate Hardness	• High carbonates and bicarbonates concentrations ensure good capacity to maintain neutral pH (favorable for microorganisms) and reducing potential of acidification, by increasing buffer capacity. See also TA. • Determination of water hardness.

DO	• DO —Dissolved Oxygen— is used to evaluate pollution strength of WW. • Used as corrosion control indicator. • Indicator of aeration efficiency for aerobic treatment, i.e. activated sludge systems.
EC	• EC —Electrical Conductivity— indicates salinity that have long-term impacts on the soil. • EC value is used to calculate the measure of total dissolved solids (TDS) concentration. • There is high correlation between electrical conductivity (EC) and total dissolved solids (TDS). • The more salts dissolved in soil solution, the higher is the value of electrical conductivity in soil. • A general relationship to determine TSS from electrical conductivity i.e., TSS (mg/l) = EC x 640. ECe represents electrical conductivity of saturation extract.
Faecal Coliforms	• Used as indicator for pathogenic contamination. • Not necessarily good indicator for pathogen reduction, because many viral or protozoan pathogens are more resistant to disinfection processes, but is relatively simple to measure. • Part of regulatory standards for effluent discharge.
Helminths	• Used as indicator for faecal contamination. • *Ascaris lumbricoides* is the most commonly used indicator. The eggs are the most resistant to treatment process and can be identified relatively easily. • Can be used as metric indicator expressed in number of eggs, however more sensitive metric approach is to measure the number of viable eggs • Presents of Helminths suggest that any human contact with FS should be avoided.
FOG	• FOG —Fats, Oils and Greases— is a parameter in stream pollution control and one of the regulatory standards for effluent discharge, high concentration can create films on surface water. • Used for operation control: High concentration can reduce microbial degradation. • Can clog soil when infiltrating.
NH_4-N	• At high level is toxic for methanogenic bacterial populations. • Toxic for fish, oxygen depleting. • TKN – NH_4-N = organic.
pH	• Acidity is one of the most essential parameters for understanding the chemistry processes, such as acid-basic nature of the sludge or liquid, alkalinity, neutralization, biological stabilization, precipitation, coagulation, disinfection, and corrosion control. • Used for operational control of biological processes: usual range is pH 6-9. • One of the regulatory standards for effluent discharge.
Salinity	• Soil salinity is a major problem for agriculture under irrigation. Water and soil salinity are a common problem in various coastal districts in Bangladesh. It results in significant losses of ecosystem functions and services. • At 20°C one liter of water can dissolve about 357 grams of salt, a concentration of 26.3%.
SS	• SS —Settleable Solids— Determination of the need for a primary settling tank and its design. • Determination of the efficiency of sedimentation units.

SO_4	• High concentration of sulphate causes odor and corrosion of sewer in anaerobic conditions by converting to hydrogen Sulphide.
TA	• TA —Total Alkalinity— is not harmful to human beings, but water supplies with less than 100 mg/l are desirable for domestic use. • Measures the buffering capacity of water.
TDS	• TDS —Total Dissolved Solids— is a measure of the dissolved combined content of all inorganic and organic substances present in a liquid in molecular, ionized, or micro-granular (colloidal sol) suspended forms. • TDS is one of the parameters for determination of WW strength. • High concentration of TDS effluent discharged in rivers can harm aquatic life. • TS = TSS + TDS.
TKN	• TKN —Total Kjeldahl Nitrogen— is the total concentration of organic nitrogen and ammonia. • Is an essential nutrient for plant growth, and uncontrolled discharge can lead to algal blooms, eutrophication, toxicity to fish and to other water organisms. • It is important indicator for biological treatability of WW.
TN	• TN —Total Nitrogen— is equal to TKN + NO_2 + NO_3
TOC	• TOC —Total Organic Carbon— is the amount of carbon found in organic compounds in the sample. • Is one of relevant parameters for balanced carbon–nitrogen ratio (C:N), which is important in aerobic and anaerobic digestion of FS.
TP	• Phosphorus is important nutrient for plant growth, uncontrolled discharge into the water bodies at high concentrations can lead to eutrophication. • Phosphorus (P) can be classified as total-P, inorganic-P (orthophosphate, H_2PO_4-, HPO_{42}, PO_{43}-) and organic-P. • Inorganic-P, called phosphate are significant for environmental control. COD:TP ratio indicates if biological process can be conducted properly.
TS	• TS —Total Solids— is a measure of the suspended and dissolved solids in water, which is equal to TSS + TDS. • It is also referred to as Dry Matter (DM) content. • TS is used to design FS treatment technologies and monitor operational • processes.
TSS	• TSS —Total Suspended Solids— is the dry-weight of suspended particles, that are not dissolved, in a sample of water that can be trapped by a filter that is analyzed using a filtration apparatus. • Soil clogging is generally accelerated under increasing hydraulic loading rates or under increasing concentrations of organic matter and suspended solids. • TSS - Suspended solids are of 3 forms: floaters, in suspension and sinkers. • TSS is important parameter for evaluation of the strength of WW and for determination of the efficiency of treatment unit. • TSS is one of the regulatory standards for effluent discharge.
TVC	• TVC TVC —Total Viable Coliforms—: drinking water samples are collected to ensure that the cold water supply is free from specific pathogenic indicator bacteria. • A low TVC level indicates an overall low level of bacteria in the system, whereas a much higher TVC level indicates that the system may be suffering from biofilm contamination.
VS	• VS —Volatile Solids— are considered to be the organic portion of TS. • Is also used as an indicator for potential biodegradability. • Removal rate of VS can also determine efficiency of treatment unit.

VSS	• VSS —Volatile Suspended Solids— is a organic fraction of the TSS. • It is used as an indicator of what is or can be degraded during aerobic or anaerobic processes.
Ratio BOD_5:COD	• Typical values for the ratio of BOD_5:COD for untreated municipal wastewater are in the range from 0.3 to 0.8. If the BOD_5:COD ratio for untreated wastewater is 0.5 or greater, the waste is considered to be easily treatable by biological means.
Ratio BOD_5:TOC	• The corresponding BOD_5:TOC ratio for untreated wastewater varies from 1.2 to 2.0.
Ratio C:N / TOC:TN	• Even if the C:N ratio for FS with 3:1 is high on nitrogen for anaerobic digestion (optimal C:N for digestion 16–25:1), it can be digested. If higher biogas production is required, one can include other organic wastes with high carbon content. • Balanced C:N ratio is essential for composting (25:1–30:1) as well as for biogas production (16:1–25:1).
Ratio COD:TKN	• High COD:TKN ratio indicates that organic concentrations are suitable for nitrogen removal by biological denitrification. • The COD:TKN ratio principally determines which biological wastewater treatment process configuration is the most appropriate.
Ratio COD:TP	• COD:TP indicates whether sufficient organic matter is available for biological phosphorus removal.
Ratio VS:TS	• The ratio of VS to TS is used as an indicator of the relative amount of organic matter and the biochemical stability of FS.
Ratio VSS:TSS	• The ratio of VSS to TSS is used as an indicator of the relative amount of organic matter and is a part of VS.

Specific questions may also help to establish the necessary parameters to fulfil the objectives for the assessment. These questions will differ depending on different contexts.

As an example, here are the questions used for the experience of UPM in Cox's Bazar:

→ What is the strength of the different wastewater streams (concentrations of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), and fats, oils, and greases (FOG), pH, organic fraction (VS)?

→ Identify the soil and filter media clogging potential of percolated (treated) wastewater (soil clogging potential is generally accelerated under increasing hydraulic loading rates or under increasing concentrations of organic matter and suspended solids).

→ How rreadily biodegradable is the wastewater (BOD_5:COD) and the sludge (VSS:TSS)?

→ How settleable is the wastewater, strength before/after pre-settling (SS:TS)?

→ What is the capacity for denitrification (COD, BOD_5:TKN) – i.e. what is the risk of eutrophication?

→ Is the pH, C:N and BOD_5:COD ratio suitable for in-situ anaerobic digestion, especially in the various units that are designed to use anaerobic digestion?

→ How effective are different treatment processes at pathogen reduction, and which variables appear to have a significant impact on the effectiveness of each process?

Laboratory

During the sampling process, some parameters can already be tested on-site while others can be measured only in a field laboratory or required further analysis by an external professional laboratory, as illustrated in Table 2.

TABLE 2: Parameters and recommended place for analysis.

Parameter	Remarks
Carbon Hardness, DO, EC, Mineral Oil, pH, Temperature, TDS, Salinity.	On-Site
BMP, SS, TDS, TS, TSS, VS, VSS.	Field Laboratory
BOD_5, COD, FOG, NH_4-N, TA, TKN, TOC, TP, E. Coli, Helminths.	External Laboratory

Field Laboratory

The field laboratory can be set up in the utility room of an apartment, as close as possible to the sampling location.

The location should fulfil following criteria:

- → Sufficient space.
- → Backup generator, in case of frequent blackouts in the area.
- → Tiled kitchen with ventilation.
- → Functioning water supply and sanitary facilities.
- → Acceptance of the owner.

In addition, the laboratory needs to be equipped with a fridge and a deep freezer to ensure appropriate sample storage. A washing machine to maintain the hygiene of personal protective clothing is also necessary. Procurement of equipment might require purchase overseas and import, if the equipment is not available in country. Note that some specific pieces of equipment are not allowed to be shipped by plane and may require payment of high import tax.

The sample analysis needs to be conducted always as close to the date of collection as possible, according the *first in – first out* principle.

External Laboratory

The identification and initial contact with existing professional laboratories can support the first evaluation round for the selection of these laboratories.

After the first round, the selected laboratories should be double checked with two random samples by certified laboratory (i.e.: COD, BOD_5, NH_4-N and TP). Quality control of the measurements should be assessed by submitting one duplicate sample to the selected laboratory and processed blindly. This approach is not only helping to ensure the quality of the measurements, but also to determine if the selected laboratory is suitable and if it can be considered for future measurements.

For the experience of Cox's Bazar, the criteria for laboratory selection was as described in Table 3.

In case some issues with laboratory occur during the testing work, such as: not respecting agreed deadline, questionable methodology and/or results, it is then suggested to organize a surprise visit to check actual work and processes.

TABLE 3: Criteria for External Laboratory selection in Cox's Bazar.

Lab	Near Source	Para-meters	Acceptance of FS	Price	Timing	Remarks	Selected
A	No	6/10	Yes	Low	15—30d	Selected to measure TOC.	Yes
B	No	2/10	Yes	Low	15d	Selected for Biological Assessment.	Yes
C	Yes	1/10	Only WW	Average	?	Field Laboratory.	No
D	No	9/10	Price discrimi-nation for FS	High for FS	15d	Overcharging samples for FS.	No
E	No	8/10	Yes	Average	15d	Announced to refuse the smelly FS samples.	No
F	Yes	7/10	Yes	Average	15d	Problematic to reach.	No
G	No	8/10	Yes	Low	?	No measurements of COD; BOD was possible at the moment.	No
H	No	5/10	Yes	Average	15d	Certified Lab, used to to verify selected labs with 2 random samples.	Yes
I	Yes	7/10	Yes	Average	15d	Logistic support and convenient sample collection in Chittagong, plus flexibility.	Yes

Staff

Team Composition

The staff for sampling should be composed by:

- → Two people for sampling, mixing, measuring and filling up containers.
- → One person with clean hands for data log, pictures and observation.

In Cox's Bazar, it was agreed to involve two teams, one for WW and one for FS sample collection. Each of the two teams participating in the on-site sampling consisted of five people, with the roles and responsibilities as follows:

- → One person in charge of on-site parameter measurements and directing the team.
- → One person responsible for taking notes and recording parameter readings as well as taking pictures for documentation purposes (i.e. NGO field officer).
- → Two heavily-protected persons involved in direct sampling.
- → One lightly-protected person involved in provision of water, bleach, ethanol and tissues for rinsing or cleaning equipment.

Staff Training

Prior to the sampling, all staff involved in the sample collection (i.e. NGOs staff and community representatives) should receive a basic training. This training should ideally be provided in the local language, or translated into it, to make sure all participants fully understand the content and also to allow them to ask questions if needed.

The purpose of this training is to introduce safety measurements and sampling methodologies, in order to ensure not only appropriate use of personal protective/sampling equipment, but also quality of the sampling.

Recommended topics for this training:

- → Introduction explaining the objectives of the sampling.
- → Distribution of PPE and guidance on how to wear it properly.
- → Safety protocol for before, during and after sampling session.
- → Group formation
- → Introduction of sampling devices.
- → Introduction of sampling methodology.
- → Introduction of sampling containers.
- → Exercise with clean water.
- → Cleaning after collection.

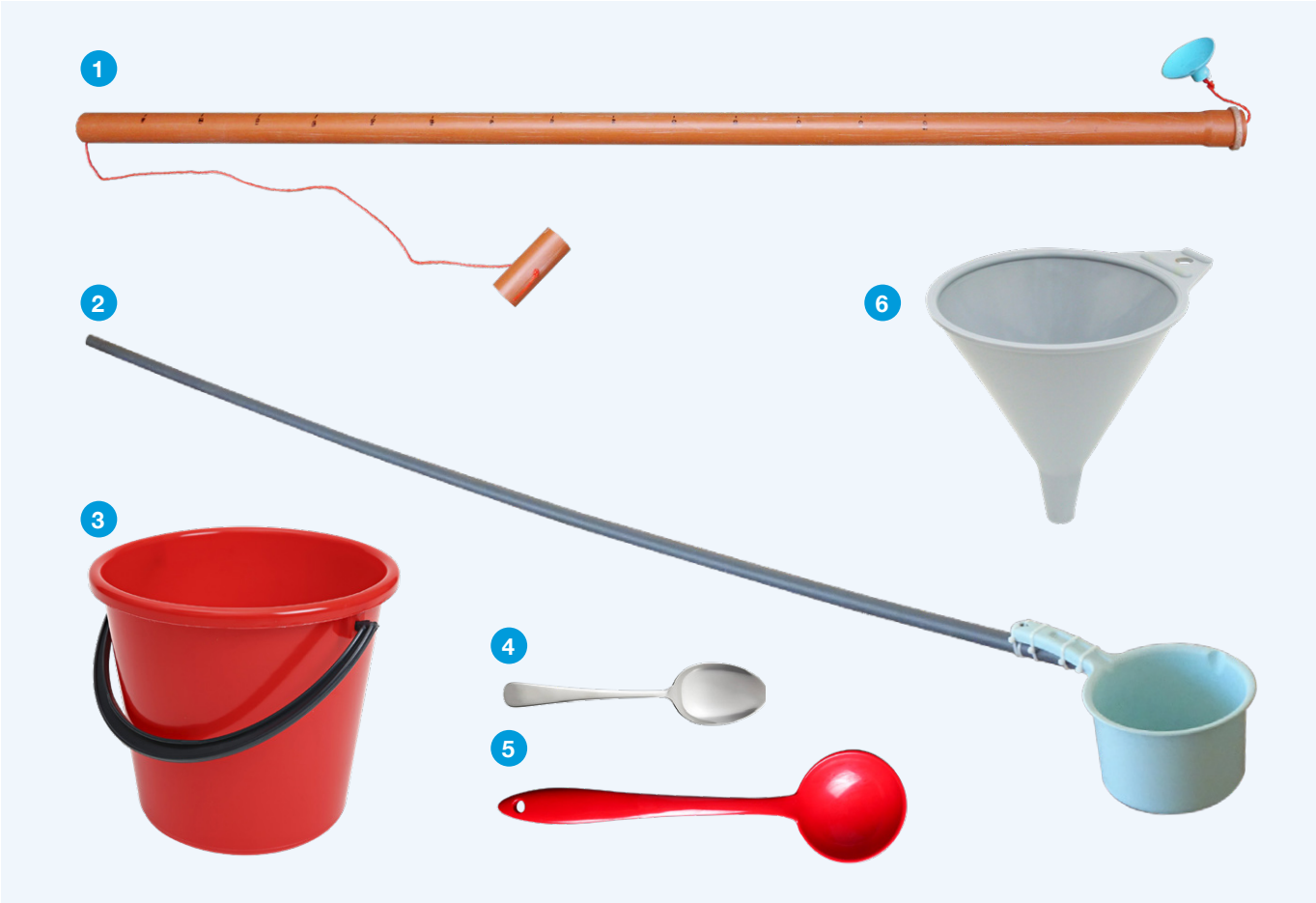
Materials & Equipment

Personal Protective Equipment (PPE)

How to wear this equipment is explained in detail in section 5.1: PPE & Safety.

1. Cap
2. Protective glasses
3. Mask, with activated carbon filter if possible
4. Overall
5. Laboratory gloves
6. Heavy duty gloves
7. Rubber boots
8. Plastic bags for personal belongings
9. First Aid Kit

Sampling Equipment



Core sampler and Mounted jar sampler are self-made devices, built with locally available materials. How to build these tools is explained in detail in section 4.4 DIY Sampling Devices.

1. Core sampler
2. Mounted jar sampler
3. Bucket of 10L with 1L graduation
4. Spoon
5. Large ladle
6. Funnel

Measuring Equipment

1. Beaker (250ml)
2. Falcon Tubes (50ml)
3. DO-meter
4. Multi-Meter (pH, TDS, EC, salinity)

Cleaning Materials

1. Water canister ca 5L filled with water
2. Ethanol, bleach, soap
3. Tissues
4. Rubbish bag (no rubbish should be left behind)
5. 2 x boxes or containers for transport of all materials

Labelling & Documentation

1. Permanent marker
2. Writing board / data sheets
3. Pen
4. Camera
5. Mobile phone or GPS devices

Transport (Logistics)

1. Vehicle (Best is a double cabin pick up, with open and separated loading area)
2. Multiple containers with variables sizes (depending on required volume)
3. Cool boxes and ice packs

DIY Sampling Devices

To collect faecal sludge samples from different types of source, two devices can be easily made using materials and tools obtainable at local hardware and plumbing stores: Core Sampler & Mounted Jar.

The former allows to collect a layered sample representative of an entire whole, while the latter allows to collect samples accessing the source from a certain distance.

Other specific features of these two devices and their use are explained in detail in section 5.4 Sampling Methodologies.

Core Sampler

Materials

1. 190cm of PVC pipe, diameter 2”, transparent if available
2. 1 Tennis ball or polystyrene ball
3. 1–2 cable ties
4. 1 Plunger, made of hard rubber
5. A piece of sand paper
6. 400cm of nylon string, 3–8mm
7. 1 Tube of silicone, including pistol
8. 1 Metal saw, including blade
9. Cutter knife and/or scissors
10. Measuring tape
11. Permanent marker

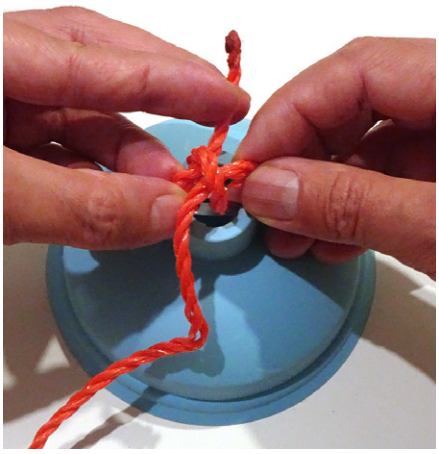
Instructions

1. Remove the handler from the plunger, leaving only the bottom rubber piece; this is to minimize surface to be cleaned later. Perforate twice the upper area of the rubber plunger to insert a cable tie.

2. Close the tie cable firmly around the rubber plunger and cut the unnecessary material.

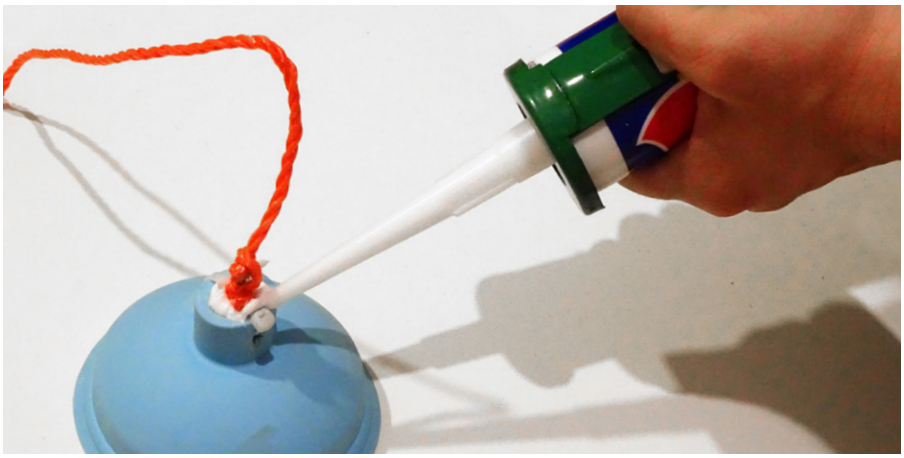
3. Prepare the nylon string by carefully melting with fire the tip of it, in order to seal it.

4. Tie the nylon string to the cable tie attached in the rubber piece. Best is to do a *butterfly nod* which allows the string to remain in the center of the nod.



5. Simple nods can be made to reinforce the first nod and avoid loose string.

6. Use silicone to fill the empty space between the plunger, the cable tie and the string, to avoid gaps where waste could accumulate. Let the silicone dry well, according specifications of the product.

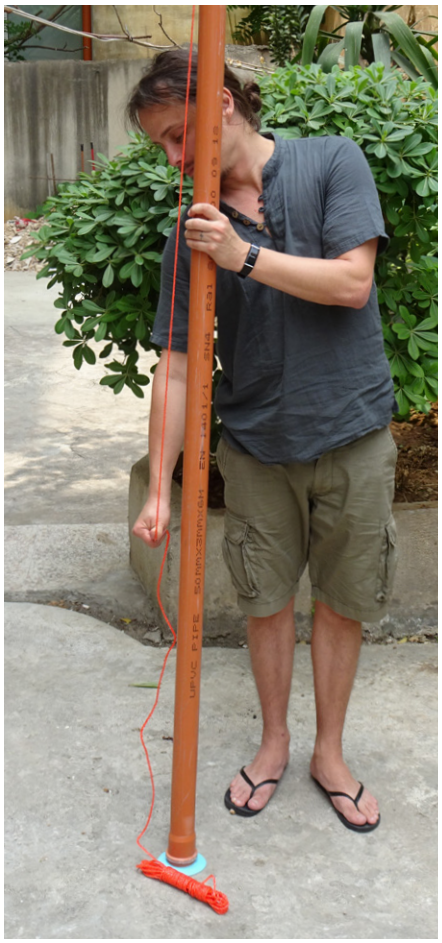


7. Prepare the PVC pipe, soften the borders of both extremities with the help of a cutter knife and sand paper.

8. Insert the string inside the pipe and pull it. Check the diameter of the rubber plunger compared with the border of the pipe in the other end.

9. Minimize the diameter of the plunger by cutting the rubber to leave around 2cm outside the pipe: this is particularly important if the access to the sludge to be sampled will occur through small ducts; confirm this aspect and prepare the device accordingly.

10. Estimate a comfortable length for the string to be pulled, keeping the plunger tight while holding the pipe. Cut the string and seal the extremity by melting with fire as previously done.



11. Prepare a holder to pull the string easily: it can be a piece of the same pipe, a tennis ball or a polystyrene ball. Perforate the object, insert the string through it and tie the string firmly.

12. Prepare a bucket with clean water to test the tightness of the plunger, grabbing liquids with the device and releasing them carefully, avoiding spitting.

13. Finally, draw in the device a scale for reference, marking each 10cm, starting from the base of the plunger. This will allow to estimate the depth of the source container when sampling.

Mounted Jar

Materials

1. 150cm uPVC or PVC pipe, diameter 1/2”
2. 1 plastic water ladder, with horizontal handler
3. 5–10 cable ties
4. Cutter knife and/or scissors
5. Measuring tape
6. Permanent marker

Instructions

1. Place the ladle at one extremity of the pipe, ensuring a firm position. Tie the handle of the ladle to the pipe by using cable ties. If the ladle comes already with a perforation, use it to increase tightness. Check that the ladle is firmly attached to the pipe. If not, add cable ties. With scissors, or cutter knife, to cut all unnecessary material.

2. Use the permanent marker and the measuring tape to write a scale on the device, marking each 10cm counting from the outer border of the ladle; this will help to measure distances when sampling.

PPE & Safety

Sampling of faecal sludge, waste and wastewater has potential to put human health at risk due to the presence of pathogens and other harmful components. It is essential to ensure proper handling of sampling and sample material.

According to ISO 5667-15, any human exposure to pathogenic organism or pollutant should be prevented by using respirators, safety glasses and appropriate protective gloves. It is necessary to provide safety training to the staff involved in sampling activities, to rise the awareness about potential health risks and encourage a correct use of their personal protective equipment —PPE—.

BEFORE SAMPLING

- → One should start to wear the PPE as close as possible to the source of sampling, in a clean and private environment.
- → Apply mosquito repellant if necessary.
- → Put on first protective eyewear, mask and cap.
- → Then wear the laboratory gloves.
- → Wear protective sampling clothes (overall and boots), which are only worn at the sampling site. Store clean clothes and shoes in a closed bag in a clean place.
- → Finally, put on the heavy duty rubber gloves.

DURING SAMPLING

- → It is strictly prohibited for the sampler to touch any of the sanitary paraphernalia (i.e. bleach, alcohol, tissues and hand sanitizers) or any equipment other than the sampling equipment.
- → When wearing gloves, do not touch the mask, glasses or face. Do not bring the hands near the head area.
- → Equipment that had come into contact with the waste material is required to be rinsed with water and bleached after each sampling.
- → The sampling area should also be kept clear of people.
- → No smoking, drinking or eating is allowed during the sampling, specially while wearing PPE.
- → The cool box should be labeled with the Biological Hazard symbol.

AFTER SAMPLING

- → Once all parameters are analyzed, clean all materials and devices with water and bleach directly on the sampling site, taking care not to spread FS in the environment.
- → Once all materials and devices are properly cleaned and all containers are inside a cool box, take off the long rubber gloves.
- → The sampling area must be cleaned before leaving the site. No trash should be left behind. If spills occur, the area should be washed with water and bleach.
- → Spray the shoes with bleach before taking them off. Put the sampling shoes inside a box/ bag.
- → Take off all sampling clothes and place them into a separated bag.
- → Take off laboratory gloves, and put them into the trash bag.
- → Wash hands using soap or hand sanitizer.

COLLECTION

Quality Assurance

Quality Assurance is a set of principles to follow during collection and analysis to ensure the quality of the samples:

→ For each of the samples, the parameter reading instruments should be calibrated before and during their use.

→ Appropriate transportation: Transport of samples should be always done inside an ice box with ice (cool and dark).

→ The samples should be delivered to the laboratory in the same day of sampling, or, if not possible, timely preserved.

COLLECTION

Quality Control

Quality Control it is recommended method to verify reliability of analysis results.

The method followed in Cox's Bazar for quality control was:

→ Duplicated collection every 10 samples.

→ Duplicate analysis for all parameters every 10th sample.

Relative error should not exceed 10% and if so, further investigation and troubleshooting would be required.

COLLECTION

Sampling Methodologies

Sampling methodology will depend on the objectives and the source. In case sampling of greywater and faecal sludge is required, it is recommended to always start with the greywater.

There are two sample types:

→ **Grab Sample**

→ **Composed Sample**.

A Grab Sample is the collection of one sample at a random point and time.

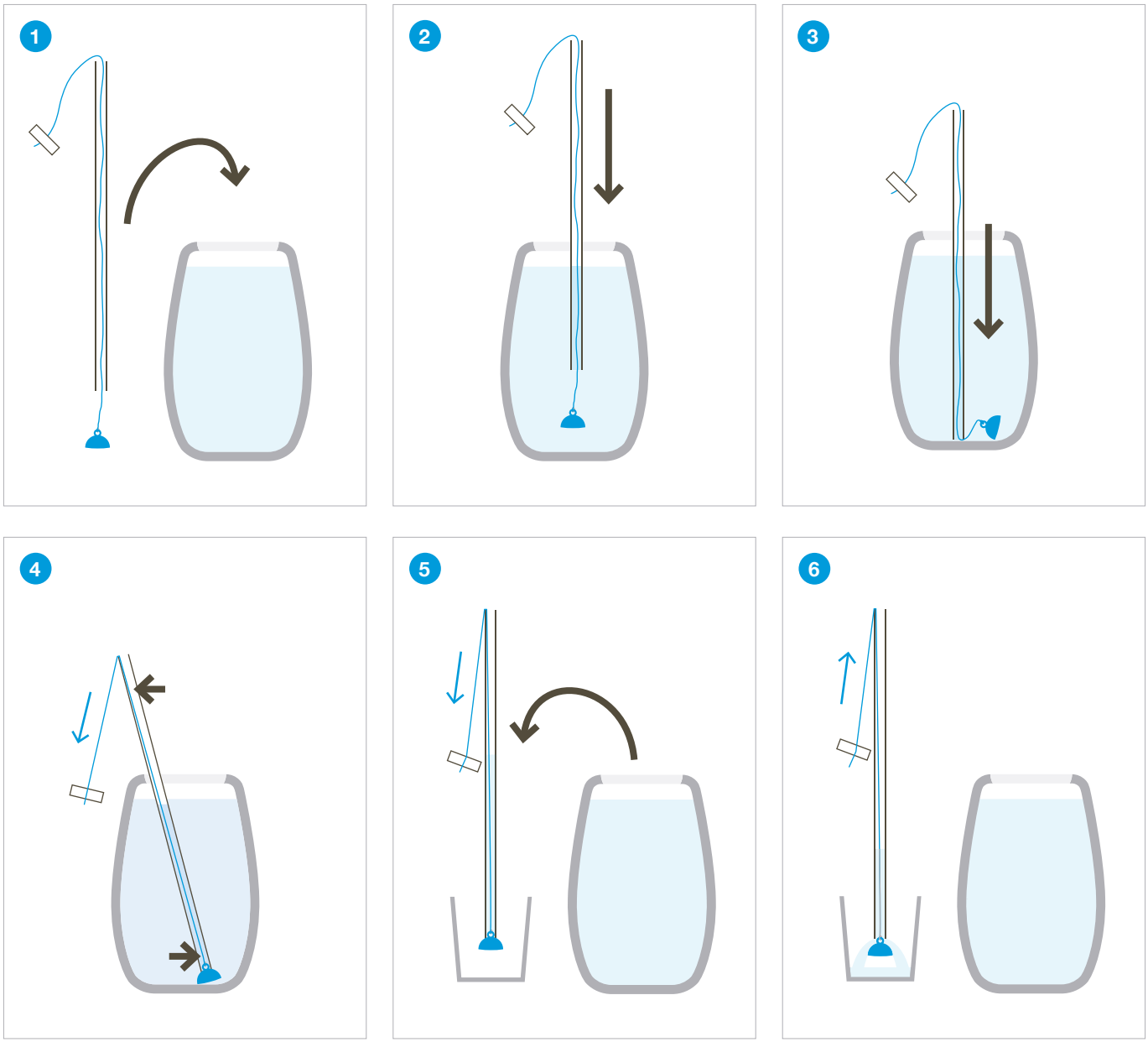
A Composed Sample can be generated either from continuous sampling (e.g. placing a barrel under the toilet pit for 24h) or several grab samples, during a defined period of time (e.g. for one day, each 2h).

In the experience of Cox's Bazar, both types of sampling methodologies were used during the study. Grab sampling was applied for drainage channels and septic tanks, Composed sampling was used for pit latrines and bathing facilities. The collection containers used for each of the waste source samples included 1.5L bottles and falcon tubes.

Applicable methodologies and protocols to be applied according different source for the sampling can be found here.

Composed Sample with Core Sampler

1. Firstly, the device needs to be open, so the slurry can enter.
2. Insert the device into the container, making sure the plunger is away from the border of the pipe.
3. Stop the insertion once the device touches the bottom of the collection container.
4. Incline slightly and close the device, by pulling up the string and hold it tight.
5. Carefully put the device back to the initial straight angle and pull out the core sampler.
6. Release the sample into 10L bucket by letting go on the string. Repeat from different points, until the required amount is collected (i.e. 8L).



Faecal Sludge from Toilet Pit

Prior to Sampling

→ One day before (12h to 24h) the sampling, a 250L barrel is mounted under the toilets.

During Sampling day

→ After the specified period of time (12–24h), the content of the barrel is stirred and the sample collected using the core sampler device.

Faecal Sludge from Septic Tank

Grab Sample (Top Layer) with Mounted Jar Sampler

→ Submerge the device into the septic tank at depth of about 10cm.

→ Move horizontally across the length of the tank.

→ Pull the device out and empty the content carefully into a small bucket.

→ Repeat from different points, until the required amount is collected (i.e. 8L).

Grab Sample (Bottom Layer) with Mounted Jar Sampler

→ Repeat all steps for both preparation and sampling as defined above. The only difference will be the submerging depth of the sampler. This time, the sampler should be immerged until it touches the bottom surface of the tank.

Faecal Sludge from Holding Tank

Grab Sample (Middle Layer) with Core Sampler

→ First, make sure the sampler is closed, by holding the string tight.

→ Insert the sampler until it touches the bottom of the container.

→ Remove the core sampler, to estimate the middle point.

→ Insert the core sampler until the middle layer, then open it, and close it again.

→ Carefully pull the core sampler out and empty sample into a 10L bucket, by releasing the string.

→ Repeat from different points, until the required amount is collected (i.e. 8L).

Graywater from Bathing Facility

Composed Sample with Core Sampler

→ Firstly, the device needs to be open, so the slurry can enter.

→ Insert device until it touches the bottom of the collection container.

→ Tilt slightly and close the device by pulling up the string and holding it tight.

→ Carefully pull out the core sampler and empty sample into a 10L bucket by releasing the string.

→ Repeat from different points, until the required amount is collected (i.e. 8L).

Wastewater from Drain Channel

Grab Sample with Mounted Jar Sampler

→ Applicable wherever a sufficient flow can be identified.

→ The device is placed inside the channel against the flow of the wastewater.

→ Wait until the device is full.

→ Pull the device out of the channel and empty the contents carefully into a small bucket.

→ Repeat steps until the required amount is collected (i.e. 8 l).

Sample Preparation

Once the sample is collected in a bucket, the sample should be properly mixed, bottled and labeled.

1. Mix the content of the bucket with a ladle and the help of a spoon if necessary.

2. After the sample is mixed, use a ladle and funnel to distribute the sample:

   i. into labelled containers.

   ii. into glass beaker (100ml) for on-site measurements.

3. Close container, wash it and bleach it.

4. Store inside ice box.

Bottle & Samples Labelling

In the context of Bangladesh, empty PVC water bottles were used as container for liquid samples and food storage container were prepared for solid parts, but never used. From that experience, it was found that water bottles from the local brand Fresh were most suitable due to the thickness of the bottle and the presence of a lid which could be closed watertight after opening, preventing dripping of the sample.

Each of the container was labelled with an assigned code, to keep record of the date on which it was collected and make a clear distinction between the collected samples.

The nomenclature for labelling was established as indicated hereunder:

[**Source Type**] + [**Camp Nº**] + [**Zone**] + [**Block**] + [**Source ID**] (if available) + [**Date**] + [**Time**] + [**Name**].

The codes for source types were:

BF : Bathing Facility
DC : Drainage Channel
PL : Pit Latrine
STT : Septic Tank (Top Layer)
STB : Septic Tank (Bottom Layer)

Samples Distribution

Distribution of the samples in plastic bottles is recommended as described in Table 4, where the sample volume may vary if they are being sent to external laboratories, depending on the specific requirements per parameter.

TABLE 4: Container type and sample volume.

Parameter	Volume (l)	Remarks
BOD_5, COD, FOG, TA, TKN, TOC, TP.	1.5	Filled up to the top.
Faecal Coliforms, Helminths	0.25	Leave some air (2.5 cm) on the top of the bottle.
NH_4-N	0.25	Leave some air (2.5 cm) on the top of the bottle to add H_2SO_4.
SS, TS, TVS, TSS/VSS	1.5	Filled up to the top. For SS test, 1L is required.
Storage	0.5	To be storage in the deep freezer in case further or repeated analysis is required.

COLLECTION

On-Site Measurements

Some parameters can be measured on-site for each of the samples.

The applicable methodology and protocol to be applied for this task can be found in Table 5.

TABLE 5: Protocol for on-site measurements.

Parameter	Unit	Device	Protocol
Carbonate Hardness	mg/l $CaCO_3$	QUANTOFIX® Carbonate Hardness [1]	• Carbonate hardness is usually expressed either in degrees KH (from the German "Karbonathärte"), or in parts per million calcium carbonate (ppm $CaCO_3$ or milligrams $CaCO_3$ per liter, mg/l). Normally, if more than 150 mg/l as $CaCO_3$, it is considered as hard water. • Distribute c. 100ml of already homogenized sample into a glass beaker, mixing using a ladle. • Perform the measurements as per instruction.
DO	mg/l	Extech DO – Meter Model DO210	• Distribute c. 100ml of already homogenized sample into a glass beaker, using a ladle. • Calibrate and preparer the DO meter as per instruction. • Submerge about 10cm of the probe into the glass beaker with the sample. • Start rotating gently the probe, until the value is stabilized • Read the value and write it down. • Clean the probe with water, disinfect with ethanol and dry with a tissue. • Duplicate the measurement for every 10th sample to ensure the reproducibility.
EC	µS/cm	Extech pH / Conductivity / TDS / Salinity / Temperature EC500	• Distribute c. 100ml of already homogenized sample into a glass beaker, using a ladle. (Same sample can be used for DO measurements). • Calibrate the device as per instruction, with tenderized solutions of three ranges. Standardizing solutions of 84µS/cm, 1413µS/cm or 12.88mS/cm. • Switch on the device, set up and submerge the probe as per instruction. • Wait until the value is stabilized. • Read the value and write it down. • Clean the probe with water, disinfect with ethanol and dry with tissue paper. • Duplicate the measurement for every 10th sample, to ensure the reproducibility.
Mineral Oil	Yes/No	Macherey-Nagel, 90760	• Distribute c. 100ml of already homogenized sample into a glass beaker, using a ladle; • Use the test stripes as per instruction. • This test paper allows rapid and reliable detection of oil contaminations of water and soil. The sensitivity depends largely on the nature of the respective hydrocarbon. To determine oil in water, move the paper back and forth a few times in the sample. In case of volatile hydrocarbons, the color reaction of the test paper has to be evaluated immediately.
pH		Extech pH / Conductivity / TDS / Salinity / Temperature EC500	• Distribute c. 100ml of already homogenized sample into a glass beaker, using a ladle. • Calibrate the device with three-point calibration as per instruction, with prepared (pH 4, 7, and 10) buffer solution. • Submerge the probe as per instruction. • Wait until the value is stabilized. • Read the value and write it down. • Clean the probe with water, disinfect with ethanol and dry • wit a tissue. • Duplicate the measurement for every 10th sample, to ensure the reproducibility.

[1] This method is also used for the quick and easy control of water in swimming pools and aquariums. For strong turbid wastewater, it does not work. Well water and drinking water can be measured to estimate the risk of scaling. Samples with high SS and dark colors are difficult to measure.

Salinity	ppm	Extech pH / Conductivity / TDS / Salinity / Temperature EC500	• Since salinity values are calculated from conductivity, sample preparation and calibration are done as described for EC. • Switching of the measuring mode is done according to the instructions. • Duplicate the measurement for every 10th sample, to ensure the reproducibility.
SO_4	ppm	VISOCOLOR® ECO	• Sulphate ion is one of the major anions occurring in natural waters, and the source of sulphate in wastewater stream. • Sulphate reacts with barium ions forming a precipitate of barium sulphate. Under defined conditions, this turbidity can be used for concentration measurements. Turbidities of the sample interfere and have to be filtered. Good reproducibility can be obtained for drinking, surface and ground water. For polluted wastewaters low concentrations are measured. • The measurements are performed according to the instructions. • Duplicate the measurement for every 10th sample, to ensure the reproducibility.
Temperature	°C	Extech pH / Conductivity / TDS / Salinity / Temperature EC500	• Temperature values always display when the device is turn on and can be obtained while conducting other measurements. • Ambient temperature should also be monitored.

COLLECTION

Transportation

For sample collection and transport, a plastic container can be used which is also in line with ISO 5667-15:2009 standards for preservation, handling and storage of samples of sewage and waterworks sludge.

For the experience in Cox's Bazar, each team was carrying two small intermediate cooling boxes in which the collected samples were immediately placed inside. They were then transferred into larger cooling boxes which were placed in a transport vehicle close to the sampling site. This was repeated after each sampling procedure from the specific waste sources.

Once the sample collection is completed, the samples should be transported back to the field laboratory and stored inside a refrigerator or freezer, until further transport or the measurements.

Samples assigned to external laboratory should be delivered in the same day of sampling. In case these samples are required to take public transportation (such as bus, plane, etc.), then they should be transported inside an icebox filled with icepacks, to keep samples in cool and dark environment.

The icebox should be marked on the outside with the Biological Hazard symbol.

In Cox's Bazar, the samples were delivered to third party laboratories by bus and plane.

PRESERVATION

Methods

If the collected samples cannot be processed immediately, the samples should then be preserved.

An overview of different preservation techniques and maximum recommended storage time is presented in Table 6.

TABLE 6: Methods for sample preservation

Parameter	Preservation	Container Type	Maximum Storage Time	Source
BOD_5	1–5°C, dark and airtight	P o G	48 hours	Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020, U.S. EPA, EMSL, 1979
	-18°C, dark and airtight	P	1 month	
BMP	-5°C, dark and airtight	P o G	48 hours	
	-18°C, dark and airtight	P	1 month	
COD	1–5°C, dark and airtight	P	48 hours	Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020, U.S. EPA, EMSL, 1979
	Add of H_2SO_4 until pH reaches 1–2	P o G	6 months	ISO 5667-3:2012
	-18°C, dark and airtight	P	6 months	
Faecal Coliforms	5 +/- 3°C, dark and airtight	Sterile P o G	24 hours	ISO 5667-15:2009
Helminths				
FOG	1–5°C, dark and airtight	G	1 month	ISO 5667-15:2009
	-18°C, dark and airtight	P	6 months	
	Add sodium sulphate 25g on 50g sample	G	6 months	

NH_4-N	1–5°C, dark and airtight	P o G	48 hours	Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020, U.S. EPA, EMSL, 1979.
	Onsite filtration, add of H_2SO_4 until pH reaches 1–2	P o G	21 days	ISO 5667-3:2012
	Onsite filtration, -18°C, dark and airtight	P	1 month	
SS	1–5°C, dark and airtight	P o G	7 days	Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020, U.S. EPA, EMSL, 1979.
TA	1–5°C, dark and airtight	P o G	14 days	ISO 5667-15:2009
TKN	1–5°C, dark and airtight	P o G	24 hours	ISO 5667-15:2009
	-18°C, dark and airtight	P o G	6 months	ISO 5667-3:2012
	Add of H_2SO_4 until pH reaches 1–2	P o G	1 month	
TOC	1–5°C, dark and airtight	P o G	1 month	ISO 5667-15:2009
	-18°C, dark and airtight	P	6 months	
TP	1–5°C, dark and airtight	P o G	48 hours	Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020, U.S. EPA, EMSL, 1979.
	-18°C, dark and airtight	P	6 months	ISO 5667-3:2012
TS, TVS	1–5°C, dark and airtight	P o G	7 days	ISO 5667-15:2009
TSS / VSS	1–5°C, dark and airtight	P o G	7 days	ISO 5667-15:2009

ANALYSIS

PPE & Laboratory Safety

In order to maintain work safety, a safety protocol need to be strictly followed while handling FS or chemicals due to the high harm potential they represent for human health.

For the experience in Cox's Bazar, the following protocol was adapted from EAWAG's *Methods for the Characterization of Faecal Sludge in Vietnam*:

- → Always read safety related indications on the chemical boxes and bottles prior to use;
- → Always wear laboratory jacket, closed shoes, long wears;
- → Always wear laboratory glasses when handling FS, WW or chemicals;
- → Always wear laboratory gloves when handling FS, WW or chemicals;
- → Always wear active carbon mask when handling FS, WW or chemicals;
- → Always clean the working space and your hands with soap and sanitizer at the end of analysis;
- → Ensure that the chemicals and products are stored in a closed and safe location;
- → Never eat, drink or smoke in the laboratory, never bring food or drinks into the laboratory;
- → Do not use fridge or freezer otherwise than for storage of the sample.

Data Log

During field and laboratory measurements, a data log is required. It includes information about date, time, location, sample ID, ambient temperature, responsible personnel for sampling as well as the respective measured parameters.

Following example shows an empty sheet for the data log used for on-site measurements in Cox's Bazar experience.

ON SITE MEASUREMENTS

Name Initials:

Sample #	ID	Collection Date	Time	Ambient Temperature (°C)	Sample Temperature (°C)	pH	EC (µS/cm)	BOD_5 (mg/l)	COD (mg/l)	Total Coliform (cfu/100ml)	TKN (mg/l)	TP (mg/l)	TSS (mg/l)

For the laboratory, separated data sheets for each of the parameters or group of parameters need to be prepared, with date, names and measured values, as in following example:

LABORATORY ANALYSIS

Name Initials:

Sample #	ID	Collection Date	BOD_5 (mg/l)	COD (mg/l)	Total Coliform (cfu/100ml)	TKN (mg/l)	TP (mg/l)	TSS (mg/l)

Analysis Protocols

Preservation Protocol Using H_2SO_4 (Liquid & Semi-Solids)

SUBSTRATE

Total Sample

TIME

30 minutes

MATERIALS

- 250ml of faecal sludge or wastewater.
- 4 x 50ml Falcon tubes.
- Centrifuge.
- 1.5µm porosity filter.
- Buchner funnel.
- Vacuum pump.

PRESERVATION OF NH_4-N SAMPLES

1. Prepare the sample for filtration by:
   - → Distribute the sample into 4 x 50ml Falcon tubes.
   - → Centrifuge for 5 minutes, to separate solids and liquids for easier filtration.
   - → Place 1.5µm filter in Büchner funnel with wrinkled surface up.
2. Filter the sample:
   - → Turn on the vacuum pump.
   - → Pull only the supernatant over the filter paper.
   - → Pump until the filter paper appears dry.
   - → Distribute c.100ml of supernatant into a plastic bottle.
   - → Store inside the deep freezer at -18ºC.

Analysis Protocol for TS/VS (Liquid & Semi-Solids)

SUBSTRATE

Total Sample

TIME

27–28 hours

MATERIALS

- 100ml of faecal sludge or wastewater.
- 2 evaporating dishes (crucibles).
- Stirrer, ladle and pipette.
- Pliers or tongs.
- 500ml beaker.
- Scale (0.1mg).
- Drying oven.
- Muffle furnace.

NOTE

To convert % in mg/l the density should be measured. Typically, FS density can be considered as (1L = 1kg).

TS & VS (LIQUID)

1. Prepare evaporating dish (crucible) by:
   → Putting clean evaporating dish (crucible) for **1 hour** in a muffle furnace at **550°C**.
   → Let it cool down to about 100–200°C. *Do not open the muffle furnace straight away*.
   → Put into desiccator using tongs or pliers. *Do not use hands to manipulate*.
2. Cool down evaporating dish (crucible) for 15 minutes in desiccator:
   → Remove and put on the scale. *Do not use hands to manipulate*.
3. Weight the evaporating dish (crucible) $\mathbf{P_1}$ = weight of dish.
4. Mix the sample with magnetic stirrer in a 500ml glass beaker.
5. Pipet from midpoint of the beaker **V** = **40ml** into evaporating dish (crucible).
6. Weight the evaporating dish (crucible) $\mathbf{P_2}$ = weight of wet sample + dish.
   → Put into the oven.
7. Dry in the oven for approx. 1 hour (until water has evaporated), at 97–98°C.
   → Dry evaporated sample in oven for **24 hours** at **105°C**.
8. Cool down evaporating dish (crucible) for 15 minutes in desiccator.
   → Remove and put on the scale. *Do not use hands to manipulate*.
9. Weight the evaporating dish (crucible) $\mathbf{P_3}$ = **weight of dry sample** + **dish**.
   → Remove and put on the muffle furnace. *Do not use hands to manipulate*.
10. Leave in the muffle furnace for **2 hours** at **550°C**.
    → Wait until the furnace cooled down to 100–200°C.
    → Remove and put in desiccator. *Do not use hands to manipulate*.
11. Cool down the filter in desiccator for 15 minutes.
    → Remove and put on the scale. *Do not use hands to manipulate*.
12. Weight the evaporating dish (crucible) $\mathbf{P_4}$ = **weight of ignited sample** + **dish**.

$$\%TS = \frac{P_3 - P_1}{P_2 - P_1} \times 100 \;\; ; \;\; \%VS = \frac{P_3 - P_4}{P_3 - P_1} \times 100 \;\; ; \;\; \%FS = \%TS - \%VS$$

TS & VS (SEMI-SOLID)

1. Prepare crucible by:
   → Putting clean crucible for **1 hour** in a muffle furnace at **550ºC**.
   → Let it cool down to about 100–200ºC. *Do not open straight away*.
   → Put into dissector using tongs or pliers. *Do not use hands to manipulate*.
2. Cool down crucible for 15 minutes in desiccator.
   → Remove and put on the scale. *Do not use hands to manipulate*.
3. Weight the crucible $\mathbf{P_1}$.
4. Homogenize the sample with ladle. Use ladle (the ladle should be always emptied to keep all particle for analyzing). Fill **V** = **40ml**, according to sludge density.
5. Weight the crucible $\mathbf{P_2}$.
   → Put it into oven.
6. Dry in the oven for **24 hours** at **105ºC**.
7. Cool down crucible for 15 minutes in desiccator.
   → Remove and put on the scale. *Do not use hands to manipulate*.
8. Weight the crucible $\mathbf{P_3}$.
   → Remove and put on the muffle furnace. *Do not use hands to manipulate*.
9. Leave in the muffle furnace for 2 hours at 550ºC.
   → Wait until the furnace cooled down to 100–200ºC.
   → Remove and put on the scale. *Do not use hands to manipulate*.
10. Cool down the filter in dissector for 15 minutes.
    → Remove and put on the scale. *Do not use hands to manipulate*.
11. Weight the evaporating dish (crucible) $\mathbf{P_4}$ = **weight of ignited sample** + **dish**.

$$TS\,[g/l] = \frac{P_3 - P_1}{V} \times 1000 \;\; ; \;\; VS\,[g/l] = \frac{P_3 - P_4}{V} \times 1000 \;\; ; \;\; VS\,[ml] \;\; ; \;\; P\,[g]$$

Analysis Protocol for TSS/VSS (Liquid & Semi-Solids)

SUBSTRATE

Total Sample

TIME

27–28 hours

MATERIALS

- 40ml of faecal sludge or wastewater.
- 0.45µm glass membrane filter paper.
- Filter apparatus with pump.
- Distilled water.
- 2 evaporating dishes (crucibles).
- Stirrer, ladle and pipette.
- Pliers or tongs.
- 500ml beaker.
- Scale (0.1mg).
- Drying oven.
- Muffle furnace.

NOTE

To convert % in mg/l the density should be measured. Typically, FS density can be considered as (1L = 1kg).

TSS & VSS (LIQUID)

1. Prepare the **0.45µm** filter by:
   - → Placing on funnel with wrinkled surface up.
   - → While vacuum is applied, wash the disc with three successive 20ml volumes of distilled water.
   - → Remove all traces by continuing to apply vacuum for 3 minutes.
   - → Remove membrane filter from the funnel with pliers or tongs. *Do not use hands to manipulate*.
   - → Put into drying oven for about 1.5 hours at 105°C
   - → Put into desiccator using tongs/pliers. *Do not use hands to manipulate*.
   - → Cool down in desiccator for 15 minutes.
2. Prepare evaporating dish (crucible) by:
   - → Putting clean evaporating dish (crucible) for 1 hour in a muffle furnace at 550°C.
   - → Let it cool down to about 100–200°C. *Do not open the muffle furnace straight away*.
   - → Put into desiccator using tongs or pliers. *Do not use hands to manipulate*.
   - → Cool down in desiccator for 15 minutes.
3. Weight evaporating dish + filter $\mathbf{P_1}$ = **weight of dry filter** + **dish**.
4. Stir the sample in 500ml glass beaker with magnetic stirrer. While stirring, pipette a measured V = 100ml volume onto the 0.45µm filter with the suction pump on.
5. Wash with 3 successive 10ml of volume of distilled water, allowing complete drainage between washing.
   - → Continue suction for about 3 minutes after filtration is complete.
6. Carefully remove the filter paper from filtration apparatus and transfer it to the evaporating dish.
7. Put the filter into the oven and dry for **24 hours** at **105°C**.
   - → After drying, remove and put down into desiccator. *Do not use hands to manipulate*.
8. Cool down the filter in dissector for **15 minutes**.
   - → Remove and put on the scale. *Do not use hands to manipulate*.

9. Weight evaporating dish + filter P_2 = **weight of dry filter** + **sample** + **dish**.
   → Remove and put in the muffle furnace. *Do not use hands to manipulate*.
10. Leave in the muffle furnace for **2 hours** at **550°C**.
   → Wait until the furnace cooled down to 100–200°C
   → Remove and put inside the desiccator. *Do not use hands to manipulate*.
11. Cool down the filter in dissector for **15min**.
   → Remove and put on the scale. *Do not use hands to manipulate*.
12. Weight the evaporating dish (crucible) P_3 = **weight of filter** + **ignited sample** + **dish**.

$$TSS\,[g/l] = \frac{P_2 - P_1}{V} \times 1000 \;\; ; \;\; VSS\,[g/l] = \frac{P_2 - P_3}{V} \times 1000 \;\; ; \;\; V\,[ml] \;\; ; \;\; P\,[g]$$

TSS & VSS (SEMI-SOLIDS)

1. Prepare the **0.45µm** filter by:
   → Placing on Büchner funnel with wrinkled surface up.
   → While vacuum is applied, wash the disc with three successive 20ml volumes of distilled water.
   → Remove all traces by continuing to apply vacuum for 3 minutes.
   → Remove membrane filter from the funnel with pliers or tongs. *Do not use hands to manipulate*.
   → Put into drying oven for about 1.5 hours at 105°C.
   → Put into desiccator using tongs/pliers. *Do not use hands to manipulate*.
   → Cool down in dissector for 15 minutes.
2. Prepare evaporating dish (crucible) by:
   → Putting clean evaporating dish (crucible) for **1 hour** in a muffle furnace at **550°C**.
   → Let it cool down to about 100–200°C. *Do not open the muffle furnace straight away*.
   → Put into dissector using tongs or pliers. *Do not use hands to manipulate*.
   → Cool down in dissector for 15 minutes.
3. Weight evaporating dish + filter P_1 = **weight of dry filter** + **dish**.
4. Weight empty Falcon tube of 50ml (make sure the tube is dry), P_2.
5. Homogenize the sample with ladle. The ladle should be always emptied to keep all particle for analyzing.
6. Weight the Falcon tube + raw sample P_3.
   → **V** = $P_3 - P_2$
7. Centrifuge for **20 minutes**.

8. Carefully distribute the supernatant evenly on **0.45µm** filter, while pump is on.
9. Recover the solid part in the tube with distilled water and dispense it on the filter while pump is on.
   → Continue suction for 3 minutes. If the suction is taking longer than 15 to 30 minutes, it means that the filter is clogged and needs to be replaced.
   → Carefully put the filter on the dish and into the drying oven. *Do not use hands to manipulate*.
10. Put the filter into the oven and dry for **24 hours** at **105ºC**.
   → After drying, remove and put down into desiccator. *Do not use hands to manipulate*.
11. Cool down the filter in desiccator for **15 minutes**.
   → Remove and put on the scale. *Do not use hands to manipulate*.
12. Weight evaporating dish + filter $\mathbf{P_4}$ = **weight of dry filter** + **dry sample** + **dish**.
   → Remove and put in the muffle furnace. *Do not use hands to manipulate*.
13. Ignite in the muffle furnace for **2 hours** at **550ºC**.
   → Wait until the furnace cooled down to 100–200ºC
   → Remove and put inside the desiccator. *Do not use hands to manipulate*.
14. Cool down the filter in desiccator for 15 minutes.
   → Remove and put on the scale. *Do not use hands to manipulate*.
15. Weight the evaporating dish (crucible) $\mathbf{P_5}$= **weight of filter** + **ignited sample** + **dish**.

$$TSS\,[g/l] = \frac{P_4 - P_1}{V} \times 1000 \;\; ; \;\; VSS\,[g/l] = \frac{P_4 - P_5}{V} \times 1000 \;\; ; \;\; V\,[ml] \;\; ; \;\; P\,[g]$$

Analysis Protocol for SS & Sludge Volume Index

SUBSTRATE

Total Sample

TIME

1.5 hours

MATERIALS

- 100ml sample.
- Imhoff cone.
- Timer.

SETTLEABLE SOLIDS (LIQUID)

1. Prepare Sample by:
   → Mix the sample with magnetic stirrer.
2. Fill an Imhoff cone to the 1l mark with well mixed sample.
3. Settle for **45 minutes**, then:
   → Gently agitate near the sides of the cone with a rod or by spinning and let settle for another **15 minutes**.
4. Record the volume of settleable solids in the cone as ml/l.

SETTLEABLE SLUDGE VOLUME (SSV)

1. Prepare 1l Sample = $\mathbf{V_1}$
   → Put 1l in 1000ml measuring jar.
   → Mix the sample with magnetic stirrer.
2. Determine volume occupied at measured time interval, e.g. 5, 10, 15, 20, 30, 45, and 60 minutes $\mathbf{V_2}$.
3. Report settled sludge volume ml/l for indicated time intervals, $\mathbf{V_3}$ = SSV.

$$V_3(t_i)\,[\,ml/l] = V_1 - V_2(t_i)$$

Analysis Protocol for Bio-Methane Potential (BMP)

SUBSTRATE

Total Sample

TIME

30–45 days

MATERIALS

- 200ml of faecal sludge or wastewater.
- 250ml Erlenmeyer flask.
- 250ml measuring cylinder.
- 50ml of inoculum.
- Perforated #6 plugs with glass tube.
- Gas analyzer.
- Flexible hose.

EXAMPLE

A 200g of feedstock materials with TS: 7.5% and VS (TS): 75%, has a VS of 11.25g. Seeding sludge (inoculum) with TS: 6.5% and VS (TS): 61% (Acceptable > 50%), 2% by weight of organic mass are 0.225g (VS), therefore 5.8g of seeding sludge would be necessary.

NOTE

- The seeding sludge should have VS (%) > 50%.
- Before its use, the seeding sludge should be stored for a week at the test (ambient) temperature.
- Batch should contain 2% by weight of organic mass from seeding sludge.
- Set one batch only with seeding sludge.

BMP

1. If not already done, determine the TS & VS of substrate and seeding sludge.
2. Calculated the required amount of seeding sludge.
3. Measure the weight of dry Erlenmeyer Flask, $\mathbf{P_1}$.
4. Put 200g of well mixed substrate $\mathbf{P_2}$, $\mathbf{P_2} - \mathbf{P_1} = 200g$.
5. Put the calculated amount of seeding sludge $\mathbf{P_3}$, $\mathbf{P_2} - \mathbf{P_1} - 200g = \mathbf{P_3}$.
6. Close with the perforated plug and mixed carefully.
7. Fixed two hoses inside measuring cylinder and put in container with water.
8. Connect one of the two hoses end to a glass tube on the top of the pipe.
9. Use another hose end to adjust the water level by suction with a manual hand pump, ensure the headspace of 20ml to prevent water entering gas analyzer.

   → When water level adjusted, close the hose end with the clam.
10. Determine gas volume by the amount of replaced water in ml on the daily basis.
11. Analyze the biogas with gas analyzer when reached 150ml mark: CH_4 , CO_2 , H_2S & O_2.
12. Continue approx. for 30–40 days until the gas production decreases.

Contributors

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Bangladesh Agricultural University was established as the only university of its kind in Bangladesh in 1961. It started functioning with the College of Animal Husbandry and Veterinary Science at Mymensingh as its nucleus. The university has six faculties and 43 departments covering all aspects of agricultural education and research. BAU was the second highest budgeted public university in Bangladesh for the year 2013–2014. It is ranked number one university of Bangladesh according to the webomertrics university ranking 2017. The Bureau of Socio-Economic Research and Training at BAU was established in 1977 at the BAU Faculty of Agricultural Economics & Rural Sociology to promote research, training and extension activities of the faculty staff. The Bureau conducts nationally and internationally funded research projects, while also provides research consultancy and advisement for Government and Non-Government Organisations. The Bureau publishes twice yearly *The Bangladesh Journal of Agricultural Economics*, in addition to reports and monographs based on the research projects completed by the faculty members.

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UPM Umwelt-Projekt-Management GmbH

Established in Munich (Germany) in 1991 with the mission to contribute to climate protection and sustainable energy production, UPM Umwelt-Projekt-Management GmbH (UPM), is strong corporate network specialized in climate change mitigation, adaptation and sustainable development, and is a leader player in international carbon trading markets. UPM established UPM Environment Engineering Project Management Consulting (Beijing) Co. Ltd, its subsidiary in China, in 2008, in order to support clients' servicing and project development in Asia.

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FIELDS OF EXPERTISE INCLUDE:

Sectors: Climate Change Mitigation/Adaptation; Sustainable Development Goals (SDGs); Renewable Energies (biogas, biomass, wind, solar); Ecological Sanitation, Wastewater Treatment, Fecal Sludge Treatment/Management (FSM), Waste-to-Value in emergency context; Citywide Inclusive Sanitation (CWIS).

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