Effects of Varying Substrate to Inoculum Ratio for Anaerobic Co-Digestion of Fecal Sludge with Organic Waste

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Approval Sheet

It is certified that contents and form of thesis entitled **"Effects of Varying Substrate to Inoculum Ratio for Anaerobic Co-Digestion of Fecal Sludge with Organic Waste"** submitted by Ali Raza, Waqas Hassan and Muhammad Ahmed Ghani have been found satisfactory for the requirement of the degree.

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Declaration

We hereby declare that Ali Raza, Waqas Hassan and Muhammad Ahmed Ghani of Institute of Environmental Sciences and Engineering, NUST for the purpose of Final Year Project have written this thesis entitled "Effects of Varying Substrate to Inoculum Ratio for Anaerobic Co-Digestion of Fecal Sludge with Organic Waste". All the research and experiments conducted during this project are bona fide to best of our knowledge and the project is fully carried under the supervision of Engr. Nida Maqbool (Assistant professor at IESE). We further declare that this work has not been submitted for any other degree/diploma. The other publications, articles and websites that we referred during our research are mentioned at the respective places.

Abstract

Sanitation is a recognized basic human right by World Health Organization nevertheless; 1.7 billion people worldwide still lack access to latrines or other forms of basic sanitation. Additionally, it is a severe issue when fecal sludge is dumped in the open and released untreated into the environment, open fields, and aquatic bodies. Hence, there is a need to devise a solution that can assist individuals in accessing sanitation services effectively, while also exploring the potential of biogas production as a sustainable energy source in the process. To deal with this problem we set our focus to study anaerobic digestion of fecal sludge to provide a possible sustainable treatment method to improve sanitation conditions. The anaerobic digestion of fecal sludge was studied through Bio Methane Potential (BMP) test applied at different ratios i.e. 0.25, 0.5, 1, 2 and 4. Along with that BMP was operated for both Mono-digestion and Co-digestion of fecal sludge. In this study, biogas production and reduction of parameters of interest were analyzed for a period of 25 days. The fecal sludge samples were collected from pits connected to household and septic tanks connected to a restaurant to have a comparison of fecal sludge from domestic area and commercial area. The highest gas yield was observed for Substrate to Inoculum Ratio (SIR) 0.25 for both mono-digestion of fecal sludge from pits and co-digestion of fecal sludge from pits with food waste with a value of 45.20 ± 1.13 ml/g VS of substrate and 121.09 ± 3.10 ml/g VS of substrate respectively. Mono-digestion of fecal sludge from septic tank has the highest biogas production in SIR 0.5 with a value of 104.7 ± 1.27 ml/g VS of substrate. This shows that the fecal sludge from the septic tanks (commercial vicinity) has more potential in terms of Biogas production. The co-digestion of fecal sludge from pits with food waste produced 1.5-3 times more gas as compared to the mono-digestion of fecal sludge from pits. The maximum removal of Total Solids (TS) and Volatile Solids (VS) was achieved at SIR 0.25 for mono-digestion and co-digestion of fecal sludge from pits but for mono-digestion of fecal sludge from septic tank, it was achieved at SIR 0.5. The maximum removal of Chemical Oxygen Demand (COD) was achieved at SIR 0.25 for mono and co-digestion of fecal sludge from pits and at SIR 0.5 for mono-digestion of fecal sludge from septic tanks. As other researchers have noted comparable tendencies, it is rather typical to detect differences in the generation of biogas and removal percentages of COD, sCOD, TS, VS, etc. in different substrates.

Acknowledgement

First of all, we would like to thank Allah Almighty from whom blessings we have been able to complete our project successfully. We would also like to extend our deepest gratitude to our supervisor Engr. Nida Maqbool for her constant support, guidance and encouragement. We have been honored to have a supervisor who made sure to guide us throughout the process and her constant counselling enabled us to achieve success in our project. We are also extremely grateful to our family and friends who always support us, and pray for our success.

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Abbreviation

SIR	Substrate to Inoculum Ratio
TS	Total Solids
VS	Volatile Solids
TANC	Total Ammonia Nitrogen Concentration
TKN	Total Kjeldahl Nitrogen
COD	Chemical Oxygen Demand
SCOD	Soluble Chemical Oxygen Demand
VFA	Volatile Fatty Acids
M.S	Mono-digestion of Septic tank
C.P	Co-digestion of Pits
M.P	Mono-digestion of pits
AD	Anaerobic Digestion

Chapter 1

1 Introduction

1.1 Sanitation

Sanitation encompasses the provision of facilities and services aimed at safely disposing of human urine and feces. Inadequate sanitation stands as a significant global contributor to diseases, highlighting the critical importance of improving sanitation for better health outcomes both within households and communities. The term 'sanitation' also includes efforts to maintain hygienic conditions, like collection of garbage and wastewater disposal services (WHO, 2021).

Unfortunately, unsafe sanitation is one of the most pressing health and environmental challenges, particularly affecting the most impoverished populationsInfectious disorders including cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio are greatly increased by a lack of access to basic sanitation (WHO, 2019). Addressing sanitation disparities is crucial to reducing disease burdens and promoting better living conditions for vulnerable communities worldwide.

1.2 Global Sanitation Situation

Improving global access to safe sanitation stands as the most cost-effective and impactful approach to enhancing public health and saving lives. Poor sanitation contributed to around 775,000 premature deaths in 2017, or 1.4% of all deaths worldwide. Poor sanitation was the cause of 5% of fatalities in low-income countries (Ritchie & Roser, 2021). Shockingly, open defecation is still practiced by nearly 494 million people, with individuals defecating in open spaces like gutters, bushes, or open water bodies (WHO, 2022).

Economic costs associated with inadequate sanitation services totaled US$222.9 billion worldwide in 2015. This sum represents a sizeable portion of the Gross Domestic Product (GDP) of all the nations with inadequate sanitation. The effect of poor sanitation is most prominent in the Asia-Pacific region, accounting for nearly 77% of the total economic impact (Giribabu et al., 2019). These statistics underscore the urgent need to address sanitation disparities worldwide, as it not only impacts public health and lives but also carries substantial economic consequences for communities and nations.

1.3 Sanitation Situation in Pakistan

The sanitation situation in Pakistan mirrors the global scenario, with approximately 79 million people lacking access to basic sanitation facilities (Water-Aid, 2021). Shockingly, around 25 million people in Pakistan still practice open defecation (UNICEF, 2021), which has significant implications for public health. Poor water and sanitation conditions contribute to an alarming statistic, with approximately 53,000 Pakistani children under the age of five dying annually from diarrhea (UNICEF, 2021).

Wastewater management and treatment systems are inadequate, exacerbating the sanitation challenges in the country. The World Bank has been actively involved in supporting sanitation projects in urban areas of Pakistan, with the aim of enhancing access to proper sanitation facilities and promoting safe wastewater disposal (World Bank, 2020). Rural areas are particularly affected, as around 52% of households lack any sanitation system, in stark contrast to only 8% in the urban areas (Khan, Fatima et al., 2021). This disparity further underscores the need for comprehensive and targeted efforts to address the sanitation needs of the population, especially in rural communities.

1.4 Sewer and Non Sewer Sanitation in Pakistan

Decentralized fecal management, which includes septic tank and pit latrines, presents a viable solution for sanitation in areas without sewers. This method is popular in South Asia, Central Asia, and South Africa, where it is the predominant method of service delivery (WHO, UNICEF, 2021). As a result of incorrect fecal sludge management, sanitation without sewers is typically inadequately managed, which causes environmental problems (Peal et al., 2020). Nevertheless, when properly managed, decentralized sanitation systems can be very effective in promoting public health and environmental well-being.

Figure 1.1 (Maqbool, 2022) depicts the state of safe sanitation in bigger cities of Pakistan. The green portion of the bar represents the safely managed fecal sludge, while the red portion indicates the unsafely managed fecal sludge.

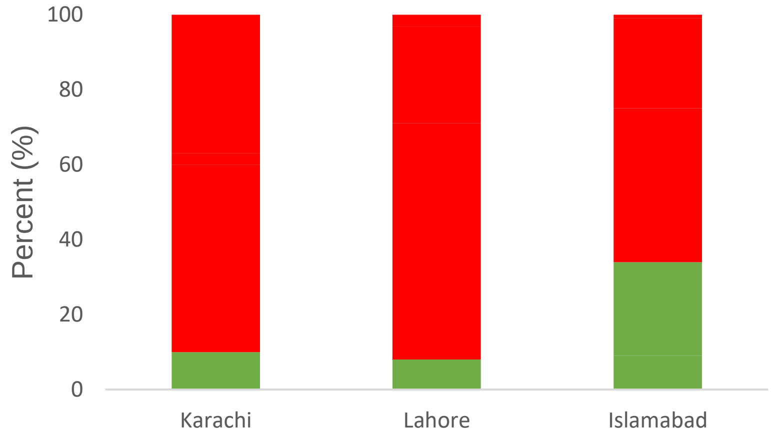

Figure 1.1 Situation of safe sanitation in the major cities of Pakistan

1.5 Non Sewer Sanitation in Pakistan

The three basic kinds of toilets are Non-Flush, Flush, and No Toilet. Flush toilets are connected to a variety of systems, including sewage lines, septic tanks, pits, or open drains, and are thought of as an upgraded variant of the standard toilet. In Pakistan, 83% of households have access to flush toilets, compared to 27% who are sewer-connected, 21% who use septic tanks, 17% who use pit latrines, and 18% who use open drains (PBS, 2021).

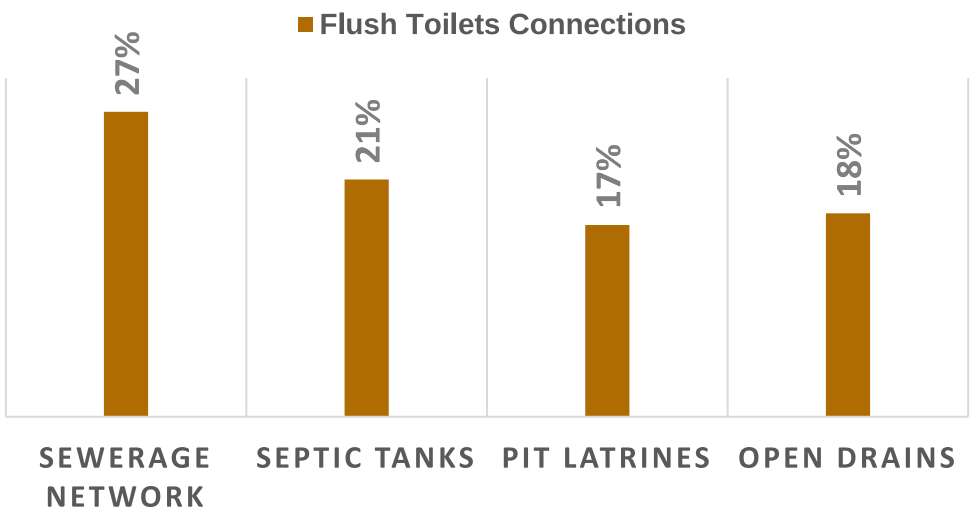

Figure 1.2 Type of flush connections in Pakistan

1.6 Fecal Sludge and Onsite Sanitation System

Fecal sludge is the waste generated from on-site sanitation systems, where it is not conveyed through a sewer network. It can vary in its state, ranging from a slurry to a semi-solid consistency, and is produced as a result of collecting, storing, or treating blackwater, excreta, and sometimes grey water. Systems that produce fecal sludge include on-site sanitation methods such as pit latrines, open ablution blocks, aqua privies, septic tanks, and dry toilets. Fecal sludge management entails tasks such as waste storage, collection, transportation, treatment, and reuse or safe disposal.

Fecal sludge exhibits significant variation in its consistency, quantity, and concentration (Strande & Brdjanovic, 2014). Municipal wastewater management often involves the use of Onsite Sanitation System (OSS) technologies like pit latrines and septic tanks. These facilities handle human waste, and in the case of pit latrines, natural decomposition takes place when the filtrate seeps into the groundwater and soil. In septic tanks, biomass is decomposed partially and the filtrate is further soaked with a drainage system. From a public health perspective, these containment systems help reduce the risk of pathogens over time, as they separate excrement from the household (Besamykina et al., 2021).

1.7 Fecal sludge Management and its importance

Fecal sludge management (FSM) refers to the collection and treatment of fecal sludge from non-sewered sanitation systems, such as pit latrines and septic tanks. These on-site sanitation systems are situated within or in close proximity to residential properties. In contrast, wastewater management mainly focuses on sewered sanitation systems (Eawag/Sandec, 2008).

Figure 1.2 indicates that 38% of flush toilets in Pakistan are connected to septic tanks and pit latrines, highlighting the need for an effective fecal sludge management system to address this issue. FSM encompasses several components (Hawkins & Muxímpua, 2015), aiming to ensure proper handling and treatment of fecal sludge for improved sanitation and environmental health. which are as follow;

1.7.1 Capture and Storage

The collection and storage of fecal sludge is the initial stage in a fecal sludge management system. The sludge is collected and stored in a pit, septic tank, or another onsite sanitation system.

1.7.2 Collection/Emptying

Fecal sludge from containment systems must be timely removed when they are full of sludge. Hand tools or other mechanical tools like vacuum trucks or other machinery can be used to empty this sludge manually or mechanically.

1.7.3 Transportation

Fecal sludge is then transported from the collection points to the treatment or disposal facilities. To reduce health risks, it's crucial to use safe vehicles and adhere to good hygiene practices while traveling.

1.7.4 Treatment

Faeces are treated using procedures to minimise volume, eliminate germs, and lessen environmental and health concerns. Anaerobic digestion, composting, drying beds, artificial wetlands, and other technologies are examples of treatment methods that can be used, depending on the resources that are available and the amount of treatment that is needed..

1.7.5 Reuse or disposal

The treated sludge can be safely disposed of in an environmentally friendly way after treatment, such as through land application or landfilling. Alternately, it may be treated further and used for advantageous functions like energy production or fertilizing crops, fish farming. Treated fecal sludge can be used for landscaping, land reclamation, or construction purposes, particularly in regions with limited access to other soil amendments.

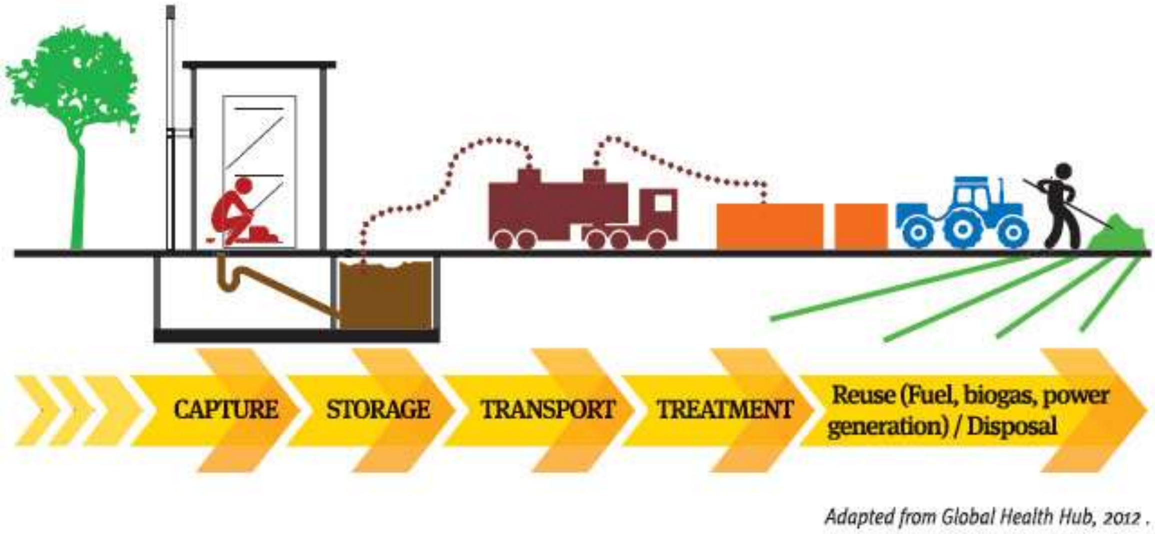

Figure 1.3 Process for Fecal Sludge Management

1.8 Problem Statement

Discharge of fecal sludge without any treatment into open fields and water bodies is a common practice in Pakistan and is huge risk to environment and public health. The problems this is causing are;

- Direct discharge into water bodies results in water pollution and water borne diseases.
- Open dumping near populated areas results in spread of different diseases such as diarrhea, cholera and typhoid.
- A potential resource of affordable energy is being lost in the form of fecal sludge.

1.9 Current Treatment Methods

Fecal sludge can be treated using a variety of techniques ranging from simple, low-cost methods to more complex, costly ones. This includes composting, drying beds, constructed wetlands and anaerobic digestion.

Chapter 2

2 Literature Review

2.1 What is fecal sludge?

Faecal sludge, often referred to as septage, is a mixture of semi-solid and liquid waste that builds up in onsite sanitation systems like septic tanks and pit latrines. It is created when mixed excreta and black water are collected, stored, or treated. Grey water may also be included, which results in raw or partially digested slurry. The main components of fecal sludge are sludge, effluent, and scum. It is characterized by an unpleasant odor, unattractive appearance, and contains various impurities like grease, grit, hair, debris, and pathogenic microorganisms.

Currently, the management of onsite sanitation systems is largely based on inadequate local practices, with a lack of comprehensive septage management practices (Strande & Brdjanovic, 2014). As a result, there is a need for improved development and management of onsite sanitation systems to address the challenges posed by fecal sludge and ensure effective waste management and public health protection.

2.2 Method utilized for Treatment of Fecal Sludge

2.2.1 Composting

Composting is a cost-effective and straightforward method for treating fecal sludge and organic waste, particularly in settings with limited resources. This approach promotes sustainability and circular economy principles by converting waste into a valuable product and organic fertilizer (Sun et al., 2017; Manga et al., 2017). The final compost product's quality, stability, and safety are crucial for composting to be successful. Compost quality may be impacted by a variety of composting conditions, with turning frequency playing a key role (Nakasaki et al., 2009).

The frequency of turning significantly influences the composting process by affecting aeration conditions and oxygen supply, thus influencing the rate of biodegradation (Waqas et al., 2018). In low-resource settings where many composting facilities are operated manually, optimizing the turning frequency is particularly crucial. A common aeration technique is to modify the turning frequency according to the process stage (Ricardo et al., 2017).

In order to investigate the effects of turning frequency and aeration rate on the process and pathogen inactivation, several studies on composting various organic wastes have been carried out. Contradictory findings have been found (Brito et al., 2008; Tirado et al., 2010). Despite these discrepancies, composting remains a promising and environmentally friendly approach for managing organic waste, as long as the process is carefully managed and adapted to local conditions. Table 2.1 provides a list of a few composting plants around the world.

Table 2.1: Composting Treatment Plants in the world

S. No	Plant Name	City, Country	Capacity	Status	Reference
1	Bio-En Power	Ontario, Canada	110,000 tons-per-year	Operational	http://www.bio-enpower.com/
2	Earnside Energy	Glenfarg – Perth, Scotland	30,000 Tonnes per year	Operational	https://bio-capital.co.uk/portfolios/earnside-energy/
3	Redstow Renewables	Swaffham – Norfolk, England	74,000 Tonnes per year	Operational	https://bio-capital.co.uk/portfolios/redstone-renewables/

2.2.2 Constructed Wetlands

Constructed Wetlands (CW) are constructed systems that efficiently treat many types of wastewater by combining biological and physical processes. Fecal sludge (FS) treatment using them is affordable and simple, and treatment effectiveness is guaranteed (Panuvatvanich et al., 2009). Numerous studies (Hu et al., 2020; Kim et al., 2018; Magri et al., 2016) have used CW for FS treatment and the resultant leachate for irrigation. Sludge treatment reed beds (STRB) are CW, particularly those that are reed-planted, that may be used as drying beds for sludge gathered from sources such as septic tanks, ASP, and ABR (Kim et al., 2018). These treatment systems greatly reduce the amount of water in the sludge and use the nutrients for plant development. Moreover, CW employs various mechanisms, including sedimentation, volatilization, sorption, biodegradation, and interception, to treat the leachate. The presence of plants in CW positively impacts microorganisms, resulting in enhanced degradation of organic compounds present in wastewater. (Jain et al, 2022). Table 2.2 shows the list few Composting plants in the world.

Table 2.2: Constructed Wetlands Treatment Plants in the world

S. No	Plant Name	City, Country	Capacity	Status	Reference
1	Arcata Marsh & Wildlife Sanctuary	California, USA	2.3 million gallons per day	Operational	https://www.cityofarcata.org/340/Arcata-Marsh-Wildlife-Sanctuary
2	Western Treatment Plant	Melbourne, Australia	40 billion litres of recycled water	Operational	https://www.melbournewater.com.au/water-and-environment/water-management/sewerage/western-treatment-plant
3	Ak-Chin Water Reclamation Facility	Arizona, USA	0.6 million gallons per day	Operational	https://carollo.com/solutions/ak-chin-indian-community-water-and-wastewater-capital-improvements-project/

2.2.3 Unplanted Drying Beds

Unplanted sludge drying beds have an under-drain at the bottom to catch leachate. They are built with shallow filters filled with sand and gravel. Spreading the sludge over the bed's surface allows liquid to travel through the sand and gravel to the bottom when water evaporates from the sludge's top into the atmosphere during the dewatering process (Figure 7.1). As long as the sludge loading rate is properly chosen and the sludge deposition spots are well-planned, the drying bed's operation and design are very straightforward.

According to fecal sludge's (FS) chemical composition, between 50 and 80 percent of its volume is discharged as liquid leachate. Before being released, this liquid has to be collected and treated (Tilley et al., 2014). The sludge can be removed from the bed manually or automatically after the necessary level of dryness has been reached. Depending on the desired end-use, extra processing can be needed to improve stabilization and further eliminate pathogens.

Factors such as cost-effectiveness and ease of operation should be considered during the installation of a drying bed and should be weighed against its potential for odour generation and large footprint. (Strande & Brdjanovic, 2014). Table 2.3 shows the list few Composting plants in the world.

Table 2.3: Unplanted Drying Beds Treatment Plants in the world

S. No	Plant Name	City, Country	Capacity	Status	Reference
1	Cape Flats Wastewater Treatment Works	Cape Town, South Africa	150 M liters/day	Operational	https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/6904656
2	Los Angeles-Glendale Water Reclamation Plant	Los Angeles, USA	20 million gallons of per day	Operational	https://www.lacitysan.org/san/faces/home/portal/s-lsh-wwd/s-lsh-wwd-cw/s-lsh-wwd-cw-p/s-lsh-wwd-cw-p-lagwrp
3	Newtown Creek Wastewater Treatment Plant	New York, USA	170 million US gallons per day	Operational	https://en.wikipedia.org/wiki/Newtown_Creek_Wastewater_Treatment_Plant

2.2.4 Anaerobic digestion

Absence of oxygen is the defining characteristic of anaerobic circumstances. Different faecal sludge management (FSM) systems, such as septic tanks, settling tanks, and anaerobic and facultative waste stabilisation ponds, where oxygen is reduced, include anaerobic digestion as a component.. Anaerobic fermentation is also used for sludge treatment. In addition to producing biogas, a useful energy source that is predominantly made up of methane (55-75%) and carbon dioxide (30-45%) (Arthur et al., 2011), anaerobic digesters are essential for stabilising fecal sludge. Anaerobic processes produce less sludge or microbial biomass than aerobic metabolisms because they are less energy-efficient (Strande & Brdjanovic, 2014). There are several advantages to using anaerobic digestion to handle organic waste and fecal sludge, making it an important strategy for managing fecal sludge. Table 2.4 shows the list few Composting plants in the world.

Table 2.4: Anaerobic Digestion Treatment Plants in the world

S. No	Plant Name	City, Country	Capacity	Status	Reference
1	Edmonton Waste Management Centre	Edmonton, Canada	40,000 tonnes per year	Operational	https://www.edmonton.ca/programs_services/garbage_waste/edmonton-composting-facility
2	Upper Blackstone clean water	Massachusetts, USA	45 million gallons per day	Operational	https://www.ubcleanwater.org/
3	Basingstoke Anaerobic Digestion Plant	Basingstoke, England	50 MWh of electricity every day	Operational	https://www.stantec.com/uk/projects/b/basingstoke-advanced-anaerobic-digestion-facility
4	La Farfana wastewater treatment plant	Maipu , Chile	8.8 m^3/s	Operational	https://www.suezwaterhandbook.com/case-studies/wastewater-treatment/La-Farfana-wastewater-treatment-plant-Chile

2.2.1.1 Benefits

There are certain benefits of using this treatment method (USEPA, 2023).

Diversified Farm Revenue

Anaerobic digestion of organic waste streams offers several benefits that can create additional revenue streams and contribute to sustainability.

Firstly, anaerobic digestion facilities can receive "tipping fees" for managing non-farm organic waste, providing a direct source of income while also generating biogas for energy production.

Second, the digested manure's liquid and solid by-products include beneficial organic nutrients that may be used as fertilizer. The digested solids can also be used to substitute peat moss or as bedding for animals.

The creation of biogas, a sustainable energy source, as a result of the decomposition of manure is another noteworthy benefit. This biogas may be utilized locally to generate power, heat homes, and provide fuel, or it can be exported to the grid to support more environmentally friendly energy practices.

Rural Economic Growth

The effectiveness of anaerobic digestion systems depends on the availability of skilled professionals with expertise in various areas, including site work, concrete, plumbing, electrical work, permitting, and engineering. These experts are essential during the planning and construction stages of the system. skilled professionals in these fields ensures the smooth implementation of the facilities. Once operational, these systems require skilled labor to efficiently maintain and operate them. The production of valuable products like nutrients, manure solids, and renewable energy can give rise to businesses that capitalize on these resources, fostering growth in the market. Cooperative business models can be adopted to distribute risks and rewards among farms and private investors, promoting collaboration and sustainable practices. Additionally, anaerobic digestion can open doors for agro-tourism, providing an opportunity to people to visit and learn about environmental improvements, and gain insight into the origin of their food.

Conservation of Agricultural Land

Anaerobic digestion offers significant benefits to soil health and local water resources. By changing the nutrients in manure into a more readily available form for plants to use, anaerobic digestion enhances soil health and fertility. The process helps reduce nutrient run-off, preventing the potential pollution of nearby water bodies, and also effectively destroys pathogens, contributing to a safer environment for both human and ecological health. Overall, anaerobic digestion plays a critical role in promoting sustainable agriculture practices and safeguarding water resources in the local area.

Energy Independence

Anaerobic digestion systems offer numerous advantages for on-farm energy needs and beyond. Biogas produced from the digestion process serves as a versatile source of energy that can be utilized to meet various on-farm requirements. It can produce electricity to light barns and houses, offer energy for cooling milk, provide heat for warming barns and on-site greenhouses and act as fuel for on-site vehicles. By relying on biogas, farmers can operate independently of traditional utilities, reducing their dependency on external energy sources and operating "off the grid."

Reduction of greenhouse gas emissions

Anaerobic digestion has a crucial role in reducing greenhouse gas emissions. It captures methane gas that would have been released into the atmosphere and help mitigate its potent greenhouse effect. Moreover, the displacement of fossil fuel energy use with biogas contributes to the overall reduction of greenhouse gas emissions, making it an environmentally beneficial technology.

Sustainable Food Production

Anaerobic digestion offers numerous benefits for both animal and human health by reducing pathogens present in manure. Additionally, this process converts nutrients in manure to a more readily accessible form for plants, hence increasing crop productivity and yield on the farm. By recycling nutrients locally, anaerobic digestion contributes to a food production system that is sustainable economically and environmentally.

Food Waste management

Anaerobic digesters can deal with food waste from sources like restaurants and grocery stores, diverting such waste from landfills. This added benefit not only reduces food waste but also enhances the efficiency of the farm digesters.

Odor Reduction

The ability of anaerobic digestion to control and eliminate odors from domestic sludges was the driving force behind its development. Under anaerobic conditions, methane and carbon dioxide are typically the byproducts of microbial degradation of carbonaceous materials and have no odor.

2.2.1.2 Drawbacks of using Anaerobic Digestion

Along with all these advantages, there are also some drawbacks in using anaerobic digestion.

Lower Biodegradation Efficiency

The anaerobic digestion process may exhibit lower biodegradation efficiency, typically ranging from 30 to 50%. This means that a significant portion of the organic matter may not be effectively degraded during the digestion process. Additionally, anaerobic digestion may require long hydraulic retention times going up to 30 days, to achieve the desired level of decomposition (Tanaka et al., 1997; Appels et al., 2008; Tyagi and Lo, 2011).

Complex Sludge Matrix

Complex sludge has physical and chemical barriers that prevent anaerobic enzymatic breakdown. Due to these obstacles, the rate of hydrolysis is slowed down, less biogas is produced, and the dewaterability of the sludge is poor. Consequently, the resulting biosolids fall under Class B category, which means they are pathogen subservient, i.e., they might contain pathogens that are inactive but can become active when conditions are favorable. (Tang et al., 2010; Tyagi and Lo, 2011; Anjum et al., 2016)

Comparatively Low COD Removal

Around 85% to 90% of organic pollutants may be efficiently reduced by anaerobic digestion. However, to achieve satisfactory removal of Chemical Oxygen Demand (COD), a second step is often necessary, typically involving aerobic treatment. In many cases, businesses pay local governments based on the volume and COD content of their waste. As a result, some manufacturers may opt to forgo the additional expense of secondary treatment and instead choose to pay higher fees (Stuart, 2006).

Required Expertise

Due to its complexity, anaerobic digestion cannot be considered a simple or "black-box" process. A thorough awareness of all the nuances involved in the process is necessary for successful functioning. This level of expertise might not always be readily available in developing regions. In developed countries, implementing anaerobic digestion systems may require hiring skilled personnel, which adds to both the initial investment and ongoing operational costs (Stuart, 2006).

Capital Investment

The main challenge in implementing aerobic digesters often lies in the significant development costs associated with their construction. Whether it is a large-scale industrial project or a smaller on-farm digester, the capital expenditure required for building aerobic digesters can be a potential deterrent (Stuart, 2006). The relatively high costs involved can sometimes hinder the widespread adoption of these systems.

2.3 Anaerobic Digestion Process Description

In the absence of oxygen, anaerobic digestion is a biological process that breaks down organic matter into simpler molecules. Methane, one of the primary byproducts of this process, is a renewable energy source that is also ecologically beneficial. For anaerobic fermentation to be effective, the substrate must have a moisture content of more than 90% (Vikhareva et al., 2022).

Hydrolysis, acidogenesis, acetogenesis, and methanogenesis are the four steps of anaerobic digestion (Vikhareva et al., 2022). Each stage's speed and efficiency depend on the consortium of microorganisms present, which exist in symbiotic relationships and occupy different ecological niches. These microorganisms work together to drive the overall process of anaerobic digestion.

2.3.1 Hydrolysis

Complex organic substances including polysaccharides, proteins, lipids, and nucleic acids are broken down into smaller, soluble components during the first stage of anaerobic fermentation. Microorganisms, such as amylase, lipase, cellulase, cellobiase, xylanase, and protease, secrete hydrolytic enzymes during this breakdown process, which are essential in breaking down these complex materials into simpler ones. The process of using organic material anaerobically ends in hydrolysis. The majority of bacteria participating in anaerobic fermentation are strict anaerobes, including Bacteroides, Clostridia, and Bifidobacteria. Streptococci and Enterobacteriaceae are examples of facultative anaerobes. In the presence of oxygen, facultative anaerobes can switch between aerobic and anaerobic modes of metabolism, while strict anaerobes can only survive and function in oxygen-free environments.

2.3.2 Acidogenesis
Certain bacteria assist in the breakdown of more complex molecules during the second stage of anaerobic degradation, which results in the creation of organic acids and alcohols. These bacteria that produce acid are essential to the process. Volatile fatty acids are produced at this phase of anaerobic breakdown by the obligate acid-producing bacteria.

2.3.3 Acetogenesis
The third step of anaerobic decomposition of organic acids and alcohols results in significant volumes of acetic acid, carbon dioxide, and hydrogen being generated. At this stage, common bacteria like Acetobacterium woodii and Clostridium aceticum are active. The process of turning simple organic molecules into acetate, known as acetogenesis, can be inhibited by hydrogen gas buildup. Because of this, the partial pressure of hydrogen (H2) in the anaerobic mixture has a significant impact on the process as a whole. At this point, the efficiency and development of the anaerobic degradation process can be significantly impacted by the presence and concentration of hydrogen gas.

2.3.4 Methanogenesis
Methanogenesis, which produces both carbon dioxide (CO2) and methane (CH4), is the final stage in the bacterial breakdown of organic materials. There are two different kinds of bacteria engaged in this process. In the first, methane is produced by redox processes in which hydrogen serves as an electron donor and carbon dioxide as an acceptor. The second group utilizes acetate, a type of organic acid, to produce both carbon dioxide and methane. Both of these reactions occur simultaneously during methanogenesis. Figure 2.1 (Rea,J. 2014) shows all four steps of anaerobic digestion.

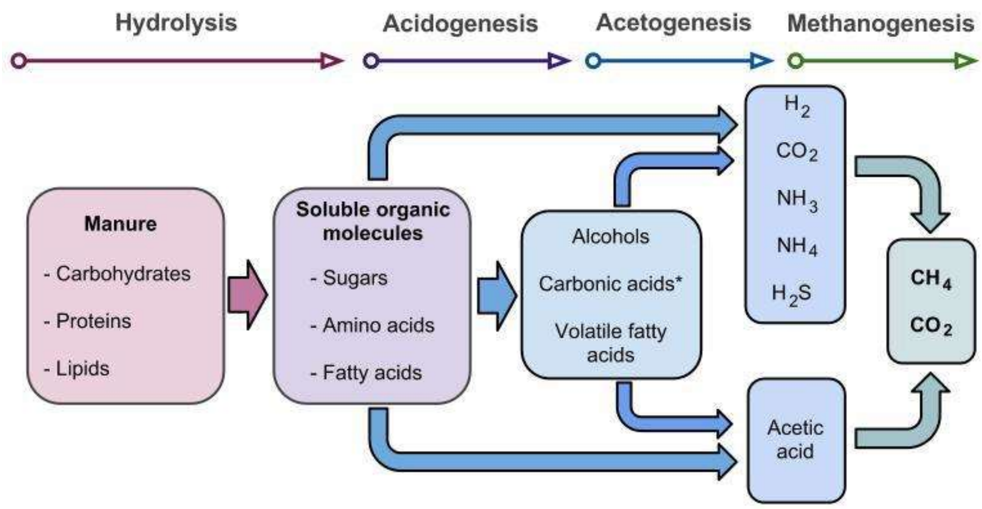

Figure 2.1: Steps of Anaerobic Digestion

2.4 Operational parameters
There are different working boundaries that impact the biogas creation. Significant boundaries that truly influence the biogas age are temperature, pH, pace of natural burden, tumult,

molecule size, maintenance time and so on. Abrupt changes in these boundaries can hurtfully the biogas age (ALI et al., 2018).

2.4.1 pH

In anaerobic digestion, pH is important since it tells whether the substrates are acidic or alkaline. The development and activity of microorganisms during the anaerobic digestion process depend on maintaining an ideal pH range. The optimal pH range for improved microbial efficiency in the digester is between 6.0 and 8.0, according to (ALI et al., 2018). It is essential to keep the pH within the ideal range to guarantee the stability and effectiveness of anaerobic digestion. Regular monitoring and control of pH levels are essential to create a favorable environment for microorganisms and to maximize the biogas production process. (Dubrovskis et al.,2010).

2.4.2 NH_4^+-N

During the digestion of protein-rich substrates, ammonia is produced, and its presence can slow down the digestion process and affect its effectiveness. High ammonia concentrations at elevated pH levels can be inhibitory to digestion, but certain manure systems can adapt to handle even higher ammonia levels, maintaining some level of functionality despite the ammonia presence. (Labatut, R. A., & Gooch, C. A.2014).

2.4.3 Volatile solids (VS)

Total volatile solids (VS) is a measurement that may be used to gauge the amount of organic material in the garbage. The amount of influent pumped into the system, as well as the proportion of waste that is VS, determine the organic loading rate of a digester, which is the influent mass per time. The difference in VS concentration between influent and effluent reveals how much waste has been stabilized (destroyed) through digestion. Assuming the influent parameters don't change, more VS stabilization is indicated by smaller particulates in the effluent and a larger reduction in odors. The physicochemical characteristics of the substrate and the design of the system have the most effects on the percentage of organic matter stabilization. In digesters that exclusively use manure, the VS stabilization typically ranges from 30 to 42 percent. For systems that digest manure along with additional high-strength substrates, the amount of waste stabilization varies depending on the co-substrates used. (Labatut, R. A., & Gooch, C. A.2014).

2.4.4 Chemical Oxygen Demand (COD)

The amount of oxygen needed to chemically oxidise the organic molecules in a sample is known as the chemical oxygen demand (COD). COD is widely used to determine the organic content of wastewater or fodder for anaerobic digestion. Higher COD concentrations corresponding to higher organic content may benefit biogas production. The organic matter serves as a substrate for the anaerobic microorganisms, making it easier for them to break down organic compounds into biogas (Strande & Brdjanovic, 2014).

2.4.5 Alkalinity

The digester material's ability to act as a buffer determines how strongly absorption occurs in an anaerobic digester. An increased ability to withstand pH variations is indicated by a greater alkalinity value. An anaerobic digester's alkalinity value might range from 1500 to 5000 mg/L. (Saev et al., 2009).

2.4.6 Volatile Fatty Acids

As a key component of anaerobic digestion, methanogens are significantly impacted by the amount of volatile fatty acids (VFAs) in the reactor. VFAs are byproducts of anaerobic digestion, including acetic acid, propionic acid, butyric acid, and valeric acid. While methanogens are naturally produced during this process, excessive concentrations of VFAs can negatively impact their growth, restricting and slowing down their development. This can lead to stress on the anaerobic digestion system, affecting its overall efficiency and performance. (Magdalena, 2019).

2.4.7 Temperature

The generation of biogas depends critically on the temperature within the digester. Three primary temperature ranges—psychrophilic (below 25 degrees C), mesophilic (25–45 °C), and thermophilic (50–70 °C)—generally have an impact on biogas production. Methane production occurs at these specific temperature ranges, and each range has its impact on the efficiency of the biogas production process. (Kayhanian & Masoud, 1994).

2.5 Gas measurement techniques

2.5.1 Syringe and needle measuring method

To quantify the vaporous byproducts of a process, use a gas syringe. When using the gas syringe to measure gases, it is imperative to maintain it clear of flails. According to Henry's law, gases can decompose in fluids, particularly under subsequent tension, resulting in inaccurate estimates. The response's gas measurement in moles may be estimated using the volume of gas progressed under standard for known) pressure conditions (gas law, PV= nRT). Similarly to this, it is crucial that the syringe barrel slides freely inside the syringe chamber if the targeted gas has the same weight and temperature. (Haseler et al., 2011)

2.5.2 Liquid Displacement Method

On the continuous liquid replacement method (CLRM), the intermittent liquid replacement method, and syringes, there was less variation in the amount of gas that was measured by these setups. On the other hand, the intermittent liquid replacement method measured the maximum amount of biogas because continuous real time monitoring may have had some leakages, and the plunger on syringes was not withdrawn sufficiently to collect all of the gas. The difference, however, is not significant (Parajuli, 2011).

2.5.3 Gas chromatography

The well-known instrument gas chromatography (GC) has a few benefits, such as high aims, speed, great affectability, and excellent quantitative findings. For measuring the gas in contact with its fluid stage, GC is the best instrument available. After performing the predefined CO and CH arrangement rules, tests are immediately implanted into the GC. In anaerobic digesters, biogas levels typically range from 60 to 70 percent. However, the methane content may fluctuate if the system is disturbed (Parajuli, 2011).

Chapter 3

3 Material and Methodology

3.1 Designing of Sampler

The sampler is made of acrylic pipes, which are used to take samples from pits and septic tanks. The main reason why this type of sampler was designed is because it would help to distinguish between the volume of liquid and solid sludge that is collected in the sampler.

The material we selected for our sampler was acrylic pipes due to its transparency, durability, and strength. There was a steel rod to support the sampler and pull it out after it was filled. The design parameters of our sampler are as follow:

Number of acrylic pipes used = 5

Length of each pipe = 2 ft.

Internal Diameter of the pipe = 40mm

External Diameter of the pipe = 50mm

Total length of sampler = 10 ft.

Diameter of the steel rod = 10mm

Length of steel rod = 11.5 ft

Figure 3.1 Picture of Sampler

3.2 Collection of Inoculum, Substrate and Co-Substrate

The first and main component of the process of Bio-Methane Potential (BMP) test is the collection of samples, which includes inoculum, substrate, and co-substrate.

3.2.1 Collection of Inoculum

The inoculum that we selected was digested cow manure of a Biogas plant collected from a local farm situated in Fateh Jang city of district Attock, Punjab. Before storage at a temperature of 35 degrees Celsius in water bath, for removing any waste and big particles, it was sieved using a 5 mm sieve. The purpose of storing the inoculum in water bath is to maintain its temperature at around 35 degree Celsius. In this way, it provides working environment for microbes present in inoculum. Inoculum is the source of microbes. After collection of inoculum, the bottles lids were kept opened for few days for the purpose of degasification.

The digester is BMP Bottle. The operating conditions for inoculum includes mesophilic temperature (35^{o}) and pH of range 6.5-8.5.

Figure 3.2 Collection of Inoculum

3.2.2 Collection of Substrate

The samples of fecal sludge utilized in this investigation were taken from septic tanks and pits located in Muslim colony and I-8 Markaz, Islamabad, respectively. Three collection sites were selected for both pits and septic tank and three samples were collected from each site. The samples from pits were sieved by using a 5mm sieve and then mixed. Similarly, samples from septic tanks were also sieved using 5 mm sieve and then mixed. The samples were then kept cooled, at a temperature of 4°C.

Figure 3.3 Collection of Fecal Sludge from pits and septic tanks

3.2.3 Collection of Co-Substrate

Food waste gathered from the mess of the hostels at the National University of Science and Technology (NUST) was the co-substrate used in the present study. This food waste is comprised of fruit and vegetable peels. The food waste was first grinded then sieved by using a 5 mm sieve and stored in refrigerator at 4^{o} C.

3.3 Characterization of Inoculum, Substrate and Co-Substrate

The experiments listed below were carried out to characterize the samples and examine their behavior:

3.3.1 Determination total solids and Volatile solids (TS, VS):

Using the standard technique, total solids and volatile solids were determined. China dish and weighing balance (Model: Shimadzu UX6200) were used to measure the weight of the samples then dried in oven (Model: WTC Binder) for 24 hours at 105 C^{o} and then ignited in Muffle furnace (Model: NEY M-525 Series 11) for 30 minutes at 550 C^{o}.

Figure 3.4 Oven for TS and VS

3.3.2 Determination of Chemical Oxygen Demand (COD):

The standard technique (Standard Methods for the Examination of Water and Wastewater, 23rd edition, 2017, technique 5220D) was used to calculate chemical oxygen demand (COD).The reagents which were used are potassium dichromate, sulfuric acid reagent and ferrous ammonium sulfate (FAS) solution and ferroin indicator solution. The sample involves simple digestion for 2 hours at 150C°. All samples were diluted 50 to 100 times but in the case of fecal sludge from pits it was diluted 200 times. The apparatus, which was used, is COD Reactor (Model: HACH USA 45600-00) and COD closed Vessel.

Figure 3.5 COD Reactor

3.3.3 Determination of Soluble Chemical Oxygen Demand (SCOD):

The standard technique (Standard Methods for the Examination of Water and Wastewater, 23rd edition, 2017, technique 5220D) was used to calculate soluble chemical oxygen demand (COD). The reagents which were used are potassium dichromate, sulfuric acid reagent and ferrous ammonium sulfate (FAS) solution and ferroin indicator solution. The sample involves simple digestion for 2 hours at 150C°. All samples were diluted 50 to 100 times. The apparatus, which was used, is COD Reactor (Model: HACH USA 45600-00) and COD closed Vessel.

Figure 3.6 COD and sCOD filled tubes

3.3.4 Determination of pH:

The pH was determined using pH meter (model: Hannah HI 8520). Two buffer solutions with established pH values—pH 4.0 and pH 7.0—were used to calibrate the pH metre, and results were given to two decimal places.

3.3.5 Determination of Electrical Conductivity (EC):

Using an EC metre (Model: Hannah HI 2003 EDGE), electrical conductivity was assessed. The conductivity meter was calibrated with two standard solutions of known conductivity and reported to 1 decimal place.

3.3.6 Determination of Total Kjeldahl Nitrogen (TKN):

The standard technique (Standard Methods for the Examination of Water and Wastewater, 23rd edition, 2017, technique 4500-N org B.) was used to calculate the total kjeldahl nitrogen (TKN). The following reagents 20%Boric acid solution, standard solution of sulfuric acid, 30%NaOH solution and mixed indicator were used. Each sample was diluted 2 times. TKN distillation assembly (Model: Velp UDK-149) and TKN Digestion assembly (Model: Velp DK-6) were used.

Figure 3.7 TKN digestion assembly

3.3.7 Determination of Total Ammonia Nitrogen Concentration (TANC):

The standard technique (Standard Methods for the Examination of Water and Wastewater, 23rd edition, 2017, technique 4500-NH3 F.) was used to calculate the concentration of total ammonia nitrogen (TAN). The following reagents 20%Boric acid solution, standard solution of sulfuric acid and mixed indicator were used. Each sample was diluted 2 times. TKN distillation assembly (Model: Velp UDK-149) was used.

Figure 3.8 TANC Distillation Apparatus

3.3.8 Determination of Volatile Fatty acids (VFA):

The standard technique (Standard Methods for the Examination of Water and Wastewater, 23rd edition, 2017, technique 5560B) was used to determine the amount of volatile fatty acids (VFA). The material was initially rotated in a centrifuge (Model: Hermle Z206A) for 15 minutes at 5000 rpm. Then supernatant was collected to perform further test.

Figure 3.9 Centrifuge

3.3.9 Determination of Alkalinity:

The standard approach (Standard Methods for the Examination of Water and Wastewater, 23rd edition, 2017, Method 2320B) was used to assess alkalinity. The material was initially rotated in a centrifuge (Model: Hermle Z206A) for 15 minutes at 5000 rpm. Then supernatant was collected to perform further test.

3.4 Bio-methane Potential (BMP) test Setup

We moved on to our second target, which was to set up and conduct the Bio Methane Potential (BMP) test for Mono and Co-digestion of fecal sludge, after characterizing all of the samples that had been collected. BMP bottles with a capacity of 250 ml were utilized for this investigation, but the bottles were only filled to 175 ml before being left with headspace. For 25 days, the experiment was conducted in batch mode. It was decided to use the Substrate to Inoculum Ratios (SIRs) of 0.25, 0.5, 1, 2, and 4. The experiment was carried out in triplicates for each ratio with a fourth bottle for weekly analysis.

3.4.1 Nitrogen Purging

Nitrogen purging was performed using nitrogen cylinder, two needles and a pipe. On Sealed BMP bottles, two needles were inserted one for the removal of O2 and other for addition of N2 connected with pipe which is carrier of N2 from nitrogen cylinder. To eliminate any oxygen, the BMP bottles were flushed with nitrogen gas for 90 seconds.

Figure 3.10 Nitrogen Purging using Nitrogen Cylinder

3.4.2 Different Mixtures of samples for BMP Test

These bottles were then submerged in a water bath that was heated to 35°C, or mesophilic temperature. BMP test was performed for following:

i. Mono Digestion of fecal sludge from pits
ii. Co-digestion of fecal sludge from pits with food waste
iii. Mono digestion of fecal sludge from septic tanks

Figure 3.13 Filled BMP Bottles

Figure 3.11 BMP Bottles in Water Bath

The basic experimental setup of a BMP test is shown in the figure below.

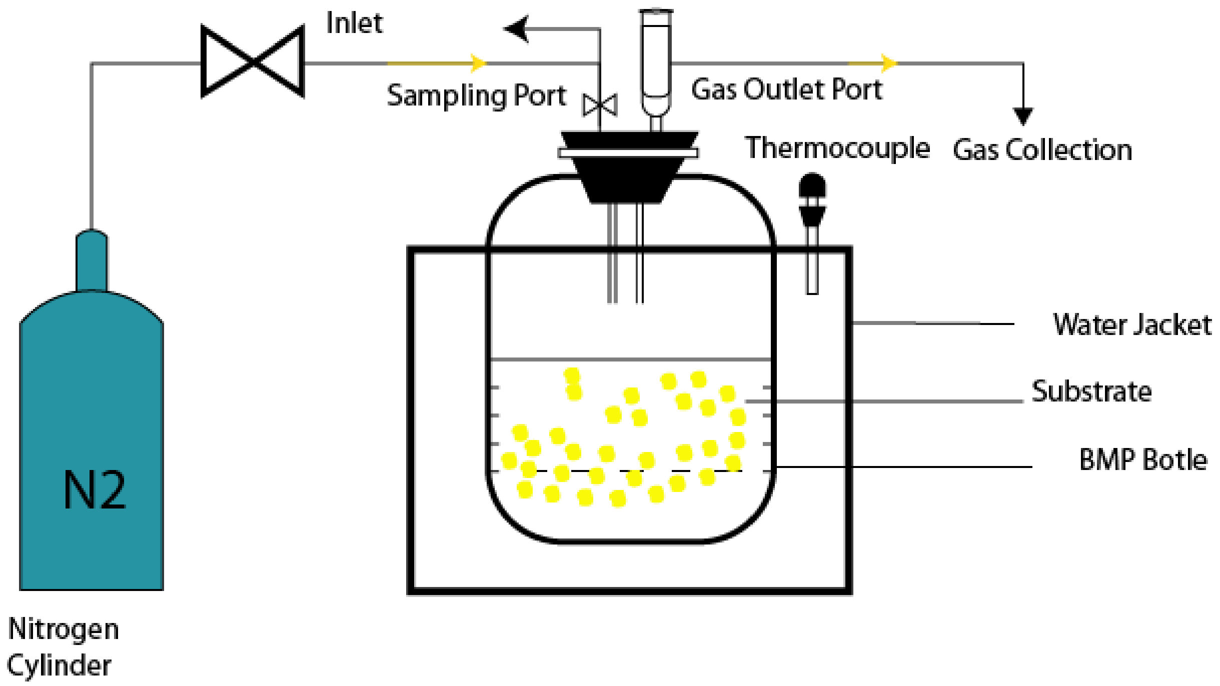

Figure 3.12 Experimental Setup for BMP

3.5 Calculations of Mixing Ratio

Based on the Volatile Solid (VS) content of the inoculum, fecal sludge, and food waste, the mixing ratios for these three experiments were determined. The BMP bottles' entire working capacity was 175 ml. The tables for the SI ratio calculations are given below.

3.5.1 SIRs for Mono digestion of fecal sludge from pits

Table 3.2 SIRs for Mono-digestion of fecal sludge from pits

S/I ratio	VS(g) Inoculum	Volume Required ml (Inoculum)	VS(g) Substrate	Volume Required ml (Substrate)	Volume of Inoculum in 175 ml bottle	Volume of Substrate in 175 ml bottle
1	1	11.62	1	16.26	72.94	102.06
0.25	1	11.62	0.25	4.07	129.60	45.40
0.5	1	11.62	0.5	8.13	102.96	72.04
2	1	11.62	2	32.52	46.07	128.93
4	1	11.62	4	65.04	26.53	148.47

3.5.2 SIRs for Co-digestion of fecal sludge from pits with Food waste

Table 3.3 SIRs for Co-digestion of fecal sludge from pits with food waste

S/I ratio	VS(g) Inoculum	Volume Required ml (Inoculum)	VS(g) Substrate	Volume Required ml (Substrate)	VS(g) FW	Volume Required ml (FW)	Volume of Inoculum in 175 ml bottle	Volume of Substrate in 175 ml bottle	Volume of FW in 175 ml bottle
1	1	15.29	0.5	11.13	0.5	5.68	83.36	60.68	30.97
0.25	1	15.29	0.125	2.7825	0.125	1.42	137.27	24.98	12.75
0.5	1	15.29	0.25	5.565	0.25	2.84	112.92	41.10	20.97
2	1	15.29	1	22.26	1	11.36	54.71	79.65	40.65
4	1	15.29	2	44.52	2	22.72	32.42	94.40	48.18

3.5.3 SIRs for Mono digestion of fecal sludge from septic tanks

Table 3.4 SIRs for Mono-digestion of fecal sludge from septic tanks

S/I ratio	VS(g) Inoculum	Volume Required ml (Inoculum)	VS(g) Substrate	Volume Required ml (Substrate)	Volume of Inoculum in 175 ml bottle	Volume of Substrate in 175 ml bottle
1	1	15.29	1	34	54.29	120.71
0.25	1	15.29	0.25	8.5	112.47	62.53
0.5	1	15.29	0.5	17	82.87	92.13
2	1	15.29	2	68	32.13	142.87
4	1	15.29	4	136	17.69	157.31

Chapter 4

4 Results and Discussion

4.1 Characteristics of Inoculum, Feed and Co Feed

When compared to the feed (fecal sludge), the inoculum, cow dung, had a high Total Solid (TS) and Volatile Solid (VS) value. The fecal sludge from pits has a higher TS (6.25 ± 1.03) and VS (6.01± 0.7) as compared to the fecal sludge from septic tanks having a value of TS (4.15 ± 0.02) and VS (2.93 ± 0.1) as shown in Table 4.1. The values of pH of all the samples collected were in the suitable range of 6.5-7.5 except for the fecal sludge collected from the septic tanks having a pH of 6.45, which is slightly lower than the required.

Table 4.1 Characterization of all Samples

Sr. No.	Tests	Inoculum (cow manure)	Fecal Sludge (Pits)	Fecal Sludge (Septic Tanks)	Food waste
1	pH	7.12	7.38	6.45	6.95
2	Total Solids(%)	10.60 ± 0.45	6.25 ± 1.03	4.15 ± 0.02	7.02± 0.89
3	Volatile Solids(%)	8.55 ± 0.30	6.01 ± 0.7	2.93 ± 0.1	6.35± 1.1
4	COD (mg/l)	27200 ± 3200	22400 ± 2100	19200 ± 2100	74600 ± 3200
5	Soluble COD (mg/l)	9600 ± 100	8100 ± 754	8533 ± 320	12260 ± 1508
6	NH_4^+-N	406.9 ± 17	1079.8 ± 54	280 ± 17	270.6 ± 19
7	TKN (mg/l)	352.3 ± 65	950 ± 70	90 ± 12	119.3 ± 36

4.2 Biogas production from anaerobic digestion at different substrate to inoculum ratio

The Bio Methane Potential (BMP), which was operational for 25 days, was created for both mono- and co-digestion. The water displacement method was used each day to measure the amount of biogas generated. The results obtained are discussed below.

4.2.1 Biogas production from Mono-digestion of fecal sludge from pits

In this experiment, five different ratios were chosen to examine the generation of biogas. On the first day following incubation, biogas generation began instantly. The maximum biogas output was noticed in SIR 0.25 with a value of 45.20 ± 1.13 ml/g VS of the substrate, and the biogas production declined as the substrate-to-inoculum ratio (SIR) increased. The other ratios achieved a highest value of 35.56 ± 0.82, 29.46 ± 0.81, 25.37 ± 0.72, 26.76 ± 0.81 ml/g VS of substrate for SIR 0.5, 1, 2 and 4 respectively. As there are very limited studies on fecal sludge so this research article by Fagbohungbe et al, (2015) is particularly pertinent to our study as it also utilized fecal sludge as a substrate. We found that there was an inverse relation between biogas production and substrate-to-inoculum ratio (SIR) when different types of waste materials were digested e.g. rice straw by Zhou et al, (2017), Sheep paunch by Lawal et al, (2016) and fecal sludge by Fagbohungbe et al, (2015). The research done by Fagbohungbe et al. (2015) also showed a comparable trend in biogas generation, with the maximum amount produced at 0.5 SIR being 254.4 ±12.6 ml g/VS. With a value of 110 ±1.3 ml g/VS, the 4.0 SIR's maximum organic loading, however, had the lowest methane production. According to Zhou et al. (2017), the study they conducted, the highest methane yield was attained at a lower substrate-to-inoculum ratio of 4, with a methane production of 193.4 0.4 ml CH4/gVS, and the lowest methane yield was at a higher S/I ratio of 10, with a methane production of 36.7 ± 5.5 ml/gVS. Lawal et al.'s (2016) study also revealed that the largest biogas generation occurred at a lower S/I ratio or 1.46784 Nm3/kg VS, and the lowest at the highest S/I ratio, or 0.57195 Nm3/kg VS.

Indeed, based on the findings of this project and supported by Zhou et al. (2017), it is evident that lower SIR (substrate to inoculum ratio) results in higher biogas production compared to higher SIR. In lower S/I ratios, there is less competition among microbial populations for nutrients and space, promoting better microbial growth and activity. This improved microbial activity leads to more efficient hydrolysis and methane production. Additionally, lower S/I ratios provide a higher liquid phase (inoculum) relative to the solid phase (substrate), which enhances the mass transfer of nutrients and metabolites. The enhanced mass transfer allows microorganisms better access to the substrate, further contributing to increased methane production. As a result, the methane production is more significant in lower SIR conditions.

According to (Zhou et al., 2011), the most efficient SIRs for AD operation and methanogenesis were those between 0.3 and 1. The quantities of methane generated under the 0.5 and 1 SIR conditions were equivalent to those reported by them. The cumulative gas production over the period of 25 days for all the SIRs used is given in the Figure 4.1.

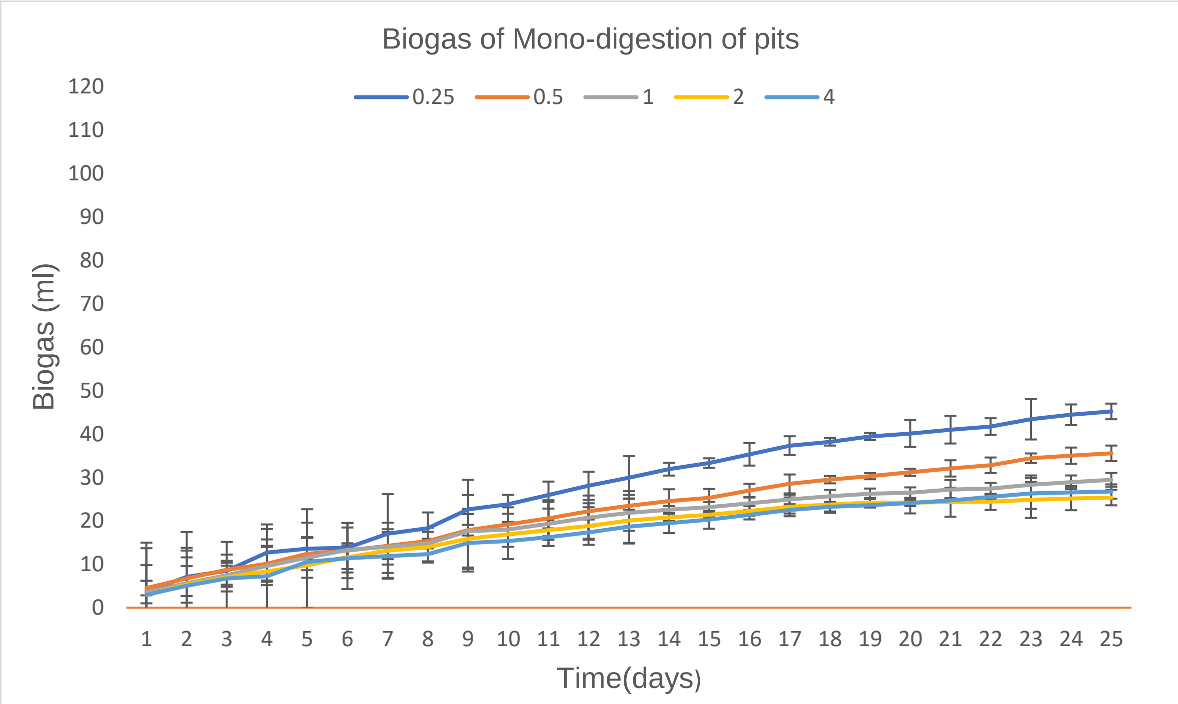

Figure 4.1 Biogas of Mono-digestion of pits

4.2.2 Biogas production from Co-digestion of fecal sludge from pits with food waste

On the first day following incubation, biogas generation began instantly. SIR 0.25 produced the highest biogas, with a value of 121.09 3.10 ml/g VS of substrate. The other ratios achieved a highest value of 86.34 ± 1.50, 90.25 ± 2.91, 40.44 ± 1.10, 41.5 ± 1.04 ml for SIR 0.5, 1, 2 and 4 respectively. The cumulative gas production over the period of 25 days for all the SIRs used is given in the Figure 4.2. Co-digestion increased biogas generation by 1.5 to 3 times when compared to mono-digestion of pit fecal sludge. The same thing was noted by Burka et al. in 2021. Co-digestion of fecal sludge has been proven to considerably enhance biogas output when compared to fecal sludge of mono-digestion, according to Burka et al. (2021). According to the study, co-digestion with additional organic matter such as sewage sludge, cow manure, and poultry manure increased the biogas generation by 1.2 to 2 times. The biogas output from the mono-digestion of fecal sludge alone was 13 ml/g VS in 14 days. However, the biogas generation rose to 18 ml/g VS after 14 days when these extra organic elements were introduced to the fecal sludge. The highest biogas yield of 28 ml/g VS in 14 days was achieved when only poultry manure was added to the fecal sludge. This demonstrates the significant positive impact of co-digestion on biogas production.

According to Cucina et al. (2021), co-digestion is a process that harnesses the synergistic effects of combining different substrates in anaerobic digestion. This combination of diverse organic compositions leads to an improved biomethane potential and more efficient degradation of organic matter, resulting in enhanced biogas production. Co-digestion helps in maintaining a balanced nutrient composition within the digester. The inclusion of various substrates like brewery trub (BT), fruit waste (FW), fecal sludge (FS), and slaughterhouse waste (SW) enhances microbial activity in the digester. These substrates contain exo-cellular enzymes and abundant microbial populations that contribute to the breakdown of complex organic matter, leading to higher biogas yields. Overall, the co-digestion process enhances the stability of anaerobic digestion, improves nutrient balance, and promotes a consistent and increased production of biogas (Afifah & Priadi, 2017).

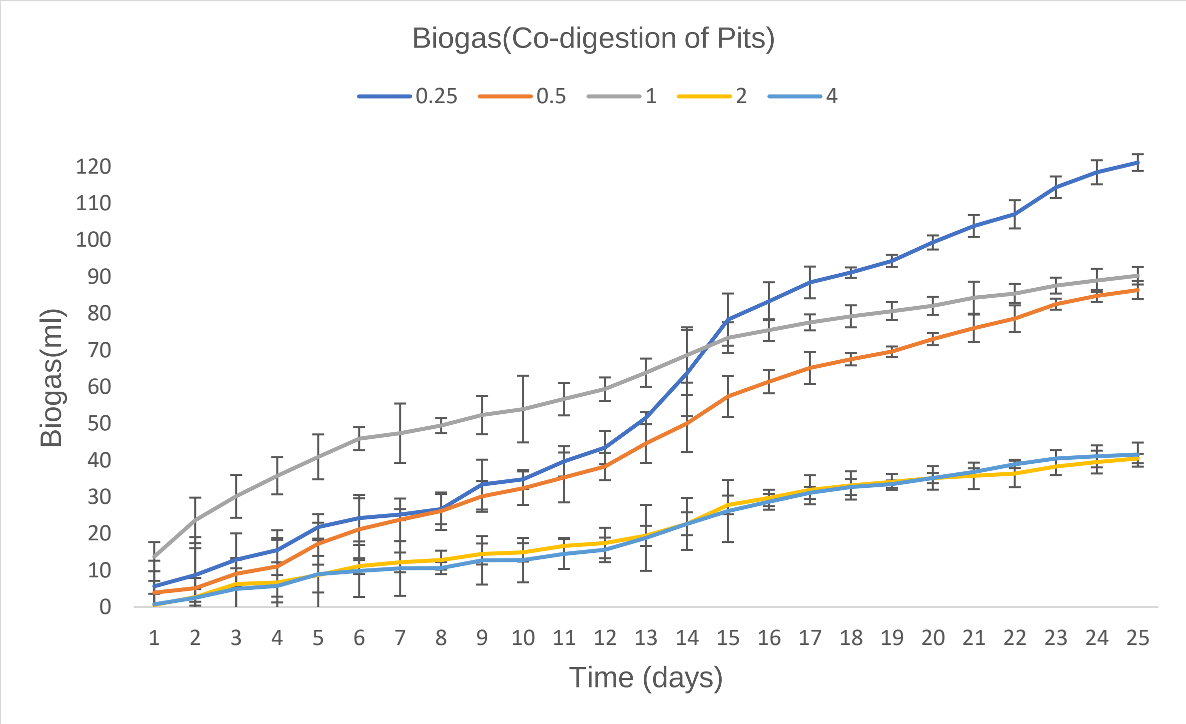

Figure 4.2 Biogas of Co-digestion of pits

4.2.3 Biogas production from Mono-digestion of fecal sludge from septic tanks

This BMP test was performed to have a comparison of biogas production for different type of fecal sludge having different characteristic and composition. The fecal sludge was removed from a septic tank in a commercial area, such as a restaurant. On the first day, following incubation, biogas generation began promptly, as in the prior two studies. The SIR 0.5 dominated and produced maximum biogas with a value of 104.7 ± 1.27 ml/g VS of substrate. The other ratios achieved a highest value of 64.3 ± 1.14, 83.96 ± 1.19, 12.06 ± 0.32, 18.86 ± 0.64 ml/g VS of substrate for SIR 0.25, 1, 2 and 4 respectively. Similarly, Fagbohungbe et al, (2015), observed maximum biogas production for SIR 0.5 anaerobic mono-digestion process. According to Raposo et al. (2009), among the factors that affect how much inoculum affects biogas output are the type of biomass and the inoculum-to-substrate ratio. To overcome digester inhibition and accelerate the start of active methanogenesis, the addition of inoculum must be done correctly (Raposo et al., 2009). The amount of volatile fatty acids (VFA) present and each substrate's capacity to buffer the accumulated VFAs during digestion define the appropriate S/I (substrate-to-inoculum) ratio. Lower S/I ratios can help with the generation of enzymes necessary for anaerobic digestion, but higher ratios can be harmful (Feng et al., 2013). Lower SIR produces more biogas than greater SIR, as was previously described in section 4.2.1. Figure No. 4.3 shows the total gas output for all of the SIRs utilized throughout a 25-day timeframe.

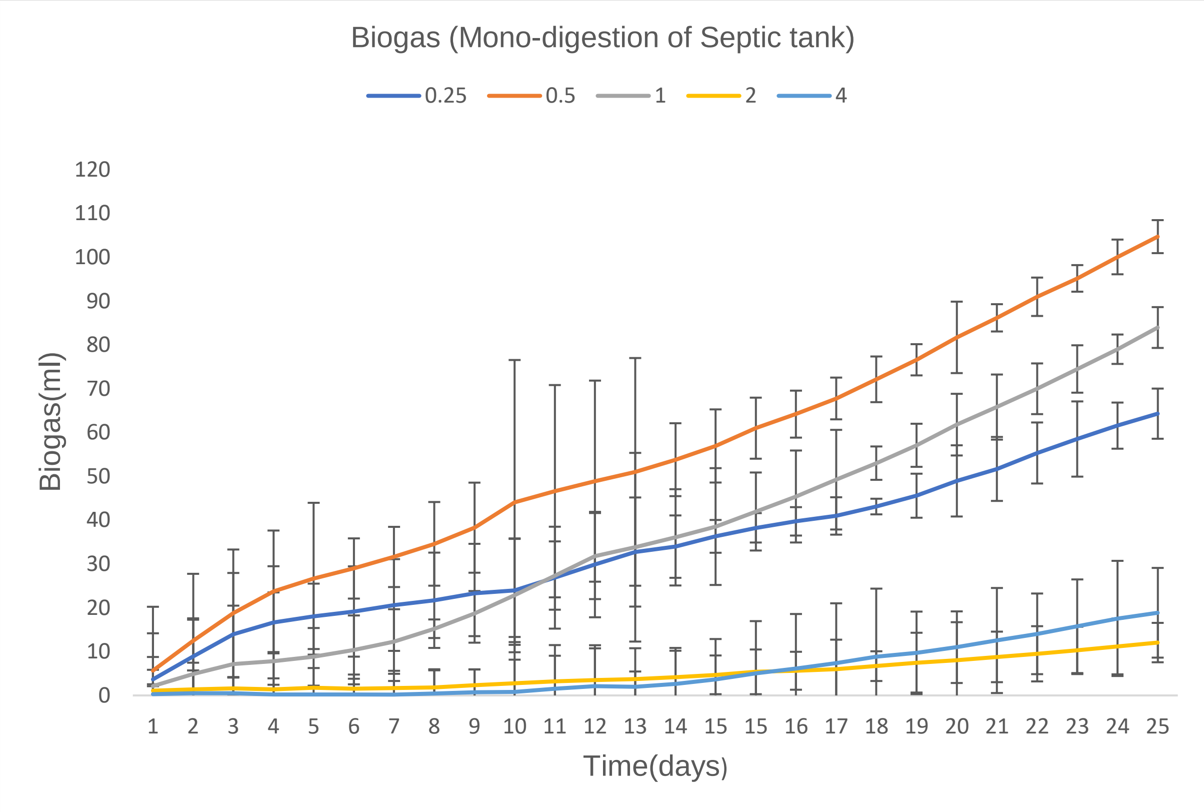

Figure 4.3 Biogas of Mono-digestion of Septic Tank

4.3 Process Performance

To analyze biodegradation during anaerobic condition, weekly analysis of BMP bottles sample was also performed for six parameters.

The following parameters were measured on the weekly basis to check their trend as the BMP test proceeded.

i. Total Solids (TS)
ii. Volatile Solids (VS)
iii. Chemical Oxygen Demand (COD)
iv. Soluble Chemical Oxygen Demand (sCOD)
v. NH_4^+-N
vi. pH
vii. Electrical Conductivity

The following two parameters were checked at the start and completion of BMP test in each case.

i. Volatile Fatty Acids (VFA)

ii. Alkalinity

4.3.1 Total and Volatile Solids

The Total solids and Volatile solids showed decreasing trend as the days passed in all the three BMP tests performed. The maximum removal of TS and VS in mono-digestion of fecal sludge from pits was observed for SIR 0.25 as 39 % and 39 % respectively. In the co-digestion of fecal sludge from pits with food waste, the highest removal of TS and VS was reported for SIR 0.25 with values of 45% and 52%. Similarly, maximum removal of TS and VS in mono-digestion of fecal sludge from septic tank was observed for SIR 0.5 as 32 % and 60 % respectively. Kilucha et al. (2022) similarly noted a higher removal of VS compared to TS during the anaerobic digestion of fecal sludge, which they speculated was due to a greater absorption rate of the organic part of TS by methanogenic bacteria. As in this study, it was observed that max biogas production was on those SIR than max removal of TS and VS. Maximum biogas production was observed on that ratio which had higher removal rate of TS and VS as studied by Deepanraj et al, (2021). As higher removal means more organic content is being converted into methane so more production of methane in anaerobic digestion occur.

4.3.2 COD and sCOD

The COD and sCOD also showed decreasing trend as the days passed period in all the three BMP tests performed. The maximum removal of COD and sCOD in mono-digestion of fecal sludge from pits was observed for SIR 0.25 as 69.99 % and 81.82 % respectively. The maximum removal of COD was observed in co-digestion process having fecal sludge and food waste for SIR 0.25 as 35.48 % and maximum removal of sCOD was observed for SIR 1 as 73.71 %. But the maximum biogas yield was obtained for SIR 0.25. Similarly, maximum removal of COD in mono-digestion of fecal sludge from septic tank was 71.43 % at SIR 0.5 and maximum removal of sCOD was 80.01 % at SIR 2. Maximum biogas production was observed on that ratio which had higher removal rate of COD as studied by Deepanraj et al, (2021). As COD indicates that total organic content but sCOD indicates only soluble organic content. As in this project, max biogas is produced on those SIRs, which have max removal of COD but not sCOD. As sCOD is portion of COD, which might have less soluble organic content on those SIRs, which have max biogas production. Therefore, it can be suggested that it does not have major impact on the generation of biogas (Jimenez et al, 2005).

This demonstrates that lower SIR results in greater COD removal and increased biogas output. Similar findings were reported (Kilucha et al., 2022) where the decrease varied from 41.7% to 66.9%. The COD elimination varies from 35.48% to 71.43%.

4.5 Effect of VFA and NH_4^+-N on biogas Production

More than 70% of the methane produced during anaerobic digestion comes from VFAs (volatile fatty acids), which serve as important intermediate products. However, VFAs have a tendency to rise and may inhibit methanogenesis at greater organic loading levels. The study assessed residual VFAs and NH4-N along with COD, SCOD, and other operational parameters in order to evaluate the stability of anaerobic digestion under various SIRs (Solid-Inoculum Ratios) (Table 4.2c and d). All SIR AD systems had VFA values < 500 mg/L, according to the results, which are consistent with those of prior research by Li et al. (2013). All SIR AD systems have low residual VFA concentrations, which means that VFA generation is below their consumption rate and that biogas production is not inhibited by VFA concentrations.

With an increase in organic loading, the NH_4^+-N concentration also rises. According to a study by Liu and Sung (2002), elevated ammonium concentrations often lead to inhibition of aceticlastic methanogens, which are responsible for 70% of methane production. While ammonium concentrations above 10,000 mg/L have been found to limit methanogenic activity (Katukiza et al., 2012), methanogens can adapt to greater ammonium concentrations in certain situations. However, in all three cases studied here, the NH4+-N concentration remains below 10,000 mg/L, indicating that there is no inhibition of methanogenesis due to NH_4^+-N concentration.

4.6 Effect of EC and pH on Biogas Production

As organic loading rate increases along with SIR, the pH decrease due to accumulation or increase of VFA as VFA increment causes acidogenesis in the process which decreases the pH (Münch & Greenfield, 1998). As indicated in Table 4.2(e), a rise in pH has no adverse effects on the generation of biogas since VFA buildup is within the permissible range. According to Tanko et al. (2018), as shown in Table 4.2 (d), electrical conductivity (EC) rises when organic matter is broken down during anaerobic digestion because this process produces different ions that raise EC.

Table 4.2: (a): Removal percentage of parameters BMP Test

	Cum Biogas (ml/gVS)						**TS**					**VS**				
S/I	**0.25**	**0.5**	**1**	**2**	**4**		**0.25**	**0.5**	**1**	**2**	**4**	**0.25**	**0.5**	**1**	**2**	**4**
M.P	45	36	29	25	27	**Week 0**	10.5	8.39	8.35	5.85	9.06	4.52	4.97	4.92	4.45	4.64
						Week 4	6.41	6.24	7.1	4.18	7.74	2.76	3.11	4	3.14	3.03
						Removal	39	26	15	29	15	39	37	19	29	35
M.S	64	105	84	12	19	**Week 0**	8.02	7.03	6.99	4.96	8.41	5.08	3.33	3.9	4.38	3.95
						Week 4	6.36	4.79	4.92	3.36	6.87	3.25	1.32	1.78	2.96	2.17
						Removal	21	32	30	31	18	36	60	54	32	45
C.P	121	86	90	40	42	**Week 0**	11.4	9.76	10.8	9.73	9.79	8.39	7.72	8.35	8.66	8.32
						Week 4	6.27	6.12	7.2	6.52	6.16	4.01	3.84	4.82	5.49	4.04
						Removal	45	37	33	33	37	52	50	42	37	51

Table 4.2: (b): Removal percentage of parameters BMP Test

	Cum Biogas (ml/gVS)					**COD(mg/L)**					**SCOD(mg/L)**				
S/I	**0.25**	**0.5**	**1**	**2**	**4**	**0.25**	**0.5**	**1**	**2**	**4**	**0.25**	**0.5**	**1**	**2**	**4**
M.P	45	36	29	25	27	21333	25600	36266	29866	23466	11733	9600	12800	7466	8533
						6400	8533	14933	17066	12800	2133	2133	3200	4266	3200
						70	67	59	43	45	82	78	75	43	62
M.S	64	105	84	12	19	17066	14933	29866	23466	17066	8533	4266	6450	5333	5333
						8533	4266	12800	8533	6400	4266	2133	2133	1066	1066
						50	71	57	64	62	50	50	67	80	80
C.P	121	86	90	40	42	66133	68266	72533	68266	76800	11733	12800	16000	13866	14933
						42667	46933	49066	51200	55466	5333	6400	4206	5333	7466
						35	31	32	25	28	55	50	74	62	50

Table 4.2: (c): Removal percentage of parameters BMP Test

	Cum Biogas (ml/gVS)					**VFA**					**Alkalinity**				
S/I	**0.25**	**0.5**	**1**	**2**	**4**	**0.25**	**0.5**	**1**	**2**	**4**	**0.25**	**0.5**	**1**	**2**	**4**
M.P	45	36	29	25	27	40	53	83	46	60	986	1110	1653	1400	1196
						23	36	60	26	43	843	966	1506	1206	1076
						43	32	28	43	28	15	13	9	14	10
M.S	64	105	84	12	19	536	573	646	676	713	1713	1833	1923	1960	2026
						410	453	496	580	620	1526	1680	1673	1810	1846
						24	21	23	14	13	11	8	13	8	9
C.P	121	86	90	40	42	163	196	136	233	246	1233	1360	1740	1623	1533
						106	93	63	173	190	1016	1116	1473	1440	1363
						35	53	54	26	23	18	18	15	11	11

Table 4.2: (d): Removal percentage of parameters BMP Test

	Cum Biogas (ml/gVS)					**NH_4^+-N(mg/L)**					**EC(mS/cm)**				
S/I	**0.25**	**0.5**	**1**	**2**	**4**	**0.25**	**0.5**	**1**	**2**	**4**	**0.25**	**0.5**	**1**	**2**	**4**
M.P	45	36	29	25	27	364	532	714	518	640	4.73	4.73	5.17	5.28	5.31
						420	462	546	644	1288	5.12	5.31	5.69	5.45	6.23
						-15	13	24	-24	-101	-8	-12	-10	-3	-17
M.S	64	105	84	12	19	280	406	448	686	728	2.78	2.69	3.12	3.27	3.22
						238	294	434	490	574	3.49	3.17	3.21	3.86	3.23
						15	28	3	29	21	-26	-18	-3	-18	0
C.P	121	86	90	40	42	546	630	700	1106	1470	5.21	5.11	5.43	5.47	5.53
						462	420	336	1134	1302	4.62	4.35	3.82	4.11	5.27
						15	33	52	-3	11	11	15	30	25	5

	C.P					**121**					8 6	90	4 0	4 2	54 6	63 0	70 0	11 06
							46 2	**42 0**	**33 6**	**11 34**								
							15	33	52	-3								
							VFA											
							0. 25	0. 5	1	2								
							40	53	83	46								
							23	36	60	26								
							43	32	28	43								
							53 6	57 3	64 6	67 6								

							410	453	496	580

4.7 VFA/Alkalinity Ratio

The VFA (volatile fatty acids) to alkalinity ratio, which is divided into three essential values by Feng et al. (2013), is used to assess the stability of anaerobic digestion. The process is regarded as stable when the ratio is lower than 0.4. There may be some instability between 0.4 and 0.8. However, there is a major danger of instability in the anaerobic digestion process if the ratio is more than 0.8. In all three cases, anaerobic digestion is in stability level. Comparing M.P and C.P, M.P has less VFA/Alkalinity ratio, which means having less VFA concentration; biogas production would be less than C.P, which have more VFA concentration. However, in both cases, ratio is less than 0.4, which indicates that there will be production of biogas unless it crosses stability level then inhibition would start (Filer et al, 2019).

Table 4.3 VFA/Alkalinity ratio

S/I		**0.25**	**0.5**	**1**	**2**	**4**
M.P	Week 0	0.04	0.05	0.05	0.03	0.05
	Week 4	0.03	0.04	0.04	0.02	0.04
M.S	Week 0	0.31	0.31	0.34	0.34	0.35
	Week 4	0.27	0.27	0.29	0.32	0.34
C.P	Week 0	0.13	0.14	0.07	0.14	0.16
	Week 4	0.10	0.08	0.04	0.12	0.14

4.8 Comparison of Biogas Production in all Three BMP tests

In order to investigate the variations in gas output between sludge from domestic pit latrines and sludge from commercial septic tanks, the study first compared the biogas generation through mono-digestion of fecal sludge derived from pits and septic tanks. The findings showed that mono-digestion of septic tank sludge produced 1.4–2.9 times more biogas than mono-digestion of pit sludge at lower substrate-to-inoculum ratios (SIRs) of 0.25, 0.5, and 1. The SIRs of 2 and 4 in pit sludge mono-digestion showed slightly higher gas production, but the difference was not significant. This indicates that sludge from septic tanks yields more biogas and is more suitable for anaerobic digestion. The findings suggest that septic tanks have greater biogas production potential during mono-digestion, highlighting the need for studying co-digestion of fecal sludge from septic tanks for further comparison.

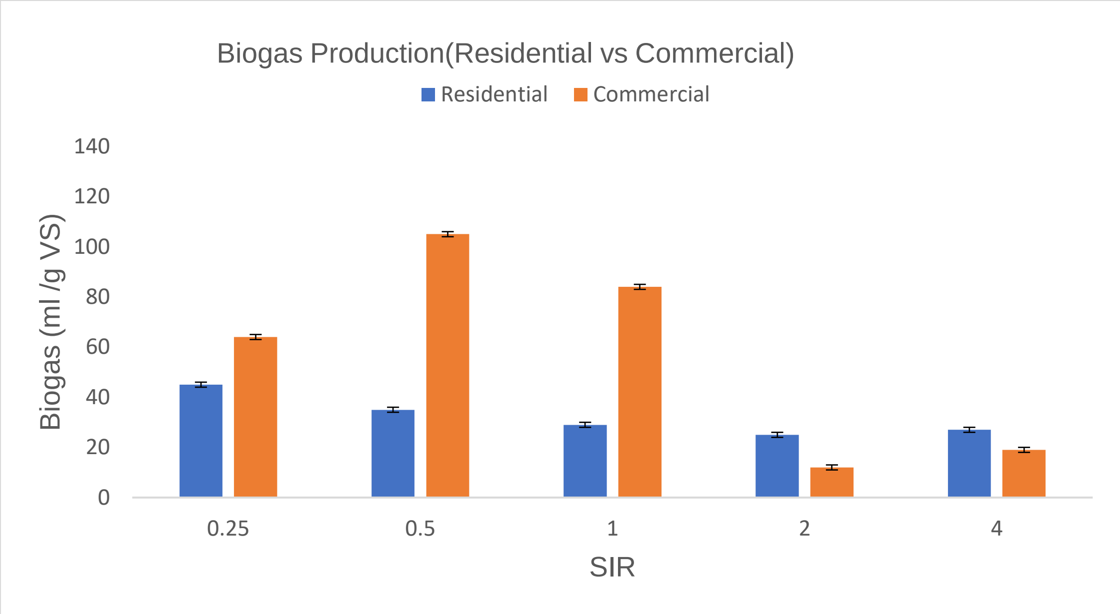

Figure 4.4 Biogas Comparison (Residential vs Commercial)

The process of anaerobic digestion depends critically on the source of the fecal sludge. Household fecal sludge tends to have a higher solid content, whereas restaurant fecal sludge primarily consists of food waste that is abundant in protein, lipids, and carbohydrates (Prabhudessai et al., 2014). This composition can significantly impact the overall digestibility and potential for biogas production. Food waste is abundant in organic matter, making it simple for anaerobic processes to convert it to methane.. However, it often lacks essential minerals, micronutrients, and macronutrients (Esteves & Devlin, 2010). On the other hand, residential fecal sludge, which is mostly made up of fecal matter, has lower amounts of biodegradable organic matter but is richer in nitrogen and trace elements (Lee et al., 2013; Kim et al., 2011). Due to this, residential fecal sludge often has less potential to produce biogas than fecal sludge from restaurants.

Secondly, to further understand the differences between anaerobic mono and co-digestion, the study compared the generation of biogas from pit sludge of mono-digestion with pit sludge and food waste of co-digestion, as illustrated in Figure 4.5. Comparing mono-digestion of pit sludge alone to co-digestion of pit sludge with food waste, roughly 1.5–3 times more biogas was produced. These findings are also supported by the study paper by Matheri et al. (2017), which shows that co-digestion enhances biogas generation by stabilising digestion process variables such the carbon-to-nitrogen ratio, nutrients, pH, and hazardous substances.. This suggests that co-digestion enhances biogas yield and offers potential benefits for anaerobic digestion systems.

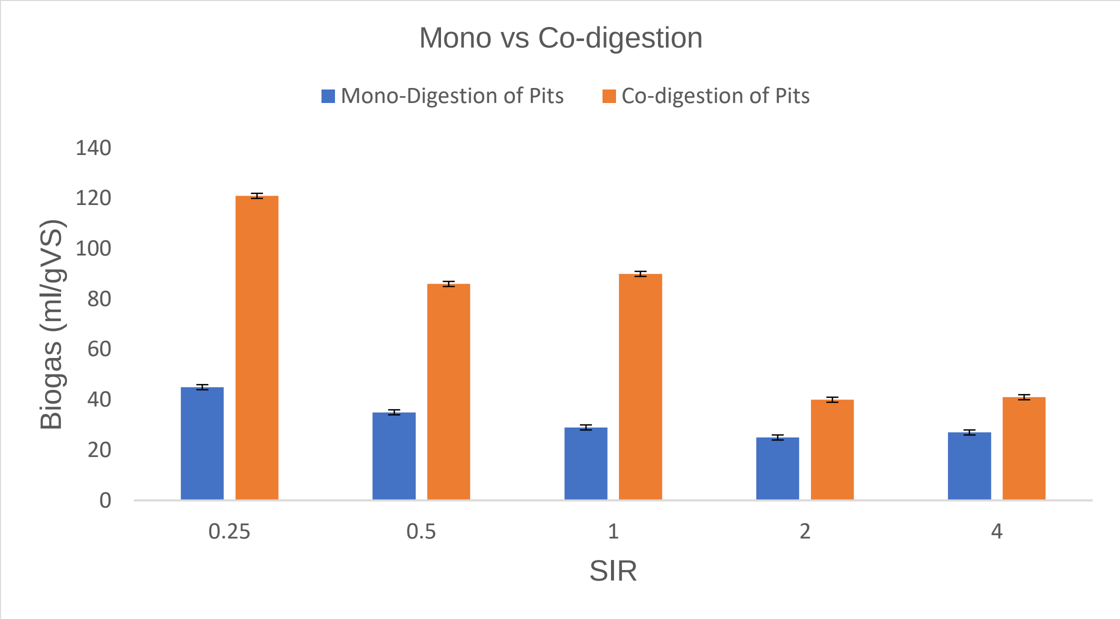

Figure 4.5 Biogas Comparison (Mono vs Co-digestion)

Chapter 5

5 Conclusion

This research was done to examine the biomethane potential of fecal sludge and determine how the Substrate to Inoculum Ratio (SIR) affects the generation of biogas. Some key findings of this study are as follow;

- Using a correct SIR is very important in anaerobic digestion to achieve optimum results and biogas production.
- The yield of biogas decreased with increase in SIR. Mono and co-digestion of fecal sludge from pits has the highest yield at 0.25 SIR with value of 45.20 ± 1.13 ml/g VS of substrate and 121.09 ± 3.10 ml/g VS of substrate respectively. Mono-digestion of fecal sludge from septic tank has the highest biogas production in SIR 0.5 with a value of 104.7 ± 1.27 ml/g VS of substrate. This means more amount of inoculum is required for good anaerobic digestion.
- Co-digestion is more suitable to digest and stabilize fecal sludge because it produced about 1.5-3 times more biogas as compared to mono-digestion of fecal sludge from pits.
- Fecal sludge from septic tanks (commercial) has a better potential to produce biogas as compared to fecal sludge from pits (residential) because it produced a highest value of 104.7±1.27 ml/g VS as compared to the pits which produced only 45.2±1.13 ml/g VS.

Chapter 6

6 Recommendations

A proper and well-planned Fecal Sludge Management (FSM) is of great significance in achieving the sanitation requirements so it should be highlighted at the national level to develop policies. In this way Pakistan will be able to meet the Sustainable Development Goals and will achieve financial benefits due to improved public health. Treatment methods like anaerobic digestion can bridge the existing gap in the current fecal sludge management system.

Now if we talk about this study and especially BMP test;

- Co-digestion of fecal sludge from septic tanks should be studied as it showed a better potential in mono-digestion as compared to mono-digestion of pits.
- Fecal sludge is relatively a new substrate in the field of anaerobic digestion so more studies should be carried out regarding effect of its different parameters like COD, sCOD, TS, VS, VFA and Alkalinity on the Biogas Production.
- The next step after these studies should be the prototype scale anaerobic digestion.
- Cost benefit analysis should be done so that it can be analyzed whether it can be implemented on industrial or not.
- Perform continuous lab scale study before implementing AD at community level.

Reference

- Afifah, U., & Priadi, C. R. (2017, March). Biogas potential from anaerobic co-digestion of faecal sludge with food waste and garden waste. In *AIP Conference Proceedings* (Vol. 1826, No. 1). AIP Publishing.
- ALI, M. M., DIA, N., BILAL, B., & NDONGO, M. (2018). Theoretical models for prediction of methane. International Journal of Physical, 364-480.
- Anjum, M., Al-Makishah, N. H., & Barakat, M. A. (2016). Wastewater sludge stabilization using pre-treatment methods. *Process Safety and Environmental Protection*, *102*, 615-632.
- Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in energy and combustion science*, *34*(6), 755-781.
- Arthur, R., Baidoo, M.F. (2011). Biogas generation from sewage in four public universities in Ghana: A solution to potential health risk. Biomass and Bioenergy 35(7), p.3086-3093
- Basamykina, A., Kharlamova, M., Burka, M., Lukanin, A., & Savenkova, E. (2021, March). The efficiency improvement of faecal sludge management with rate increase of biodegradation in septic tanks and pit latrines. In *IOP Conference Series: Earth and Environmental Science* (Vol. 689, No. 1, p. 012013). IOP Publishing.
- Brito, L.M.; Coutinho, J.; Smith, S.R. Methods to Improve the Composting Process of the Solid Fraction of Dairy Cattle Slurry. Bioresour. Technol. 2008, 99, 8955–8960.
- Burka, M. S., Basamykina, A. N., & Kharlamova, M. D. (2021, March). Technological features of biogas production while anaerobic co-digestion of faecal sludge, sewage sludge and livestock. In *IOP Conference Series: Earth and Environmental Science* (Vol. 666, No. 4, p. 042052). IOP Publishing.
- Christiane, Q. N., Amankwah, E., Awafo, E. A., & Sodre, S. (2023). Valorization of faecal sludge and organic waste in Bobo-Dioulasso, Burkina Faso to improve sanitation, enhance soil and improve agricultural productivity. *Energy Reports*, *9*, 4951-4959.
- Cucina, M., Pezzolla, D., Tacconi, C., & Gigliotti, G. (2021). Anaerobic co-digestion of a lignocellulosic residue with different organic wastes: Relationship between biomethane yield, soluble organic matter and process stability. *Biomass and Bioenergy*, *153*, 106209.
- Deepanraj, B., Senthilkumar, N., & Ranjitha, J. (2021). Effect of solid concentration on biogas production through anaerobic digestion of rapeseed oil cake. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *43*(11), 1329-1336.
- Dixon, M., Gallop, J. R., Lambert, S. C., Lardon, L., Healy, J. V., & Steyer, J. P. (2007). Data mining to support anaerobic WWTP monitoring. *Control engineering practice*, *15*(8), 987-999.
- Dubrovskis, V., Plume, I., Kotelenecs, V., & Zabarovskis, E. (2010, May). Anaerobic digestion of sewage sludge. In *9th International Scientific Conference Engineering for Rural Development Proceedings*.
- Dubrovskis, Vilis, Plume, I., & Kotelenecs, V. (2010). Anaerobic digestion of sewage sludge. Jelgava, Latvia: Latvia University of Agriculture.
- Eawag/Sandec (2008). Fecal Sludge Management. Sandec Training Tool 1.0 – Module
  5. Eawag/Sandec (Department of Water and Sanitation in Developing Countries,

Swiss
Federal institute of Aquatic Science and Technology (Eawag). Switzerland

- Elsayed, M., Diab, A. & Soliman, M. Methane production from anaerobic co-digestion of sludge with fruit and vegetable wastes: effect of mixing ratio and inoculum type. Biomass Conv. Bioref. 11, 989–998 (2021). https://doi.org/10.1007/s13399-020-00785-z
- Fagbohungbe, M. O., Herbert, B. M., Li, H., Ricketts, L., & Semple, K. T. (2015). The effect of substrate to inoculum ratios on the anaerobic digestion of human faecal material. Environmental Technology & Innovation, 3, 121-129. https://doi.org/10.1016/j.eti.2015.02.005
- Fatima A, Urooj S, Mirani ZA, Abbas T, Khan MN (2021) Fecal Coliform Contamination of Drinking Water in Karachi, Pakistan. PSM Microbiol 6(2): 42–48
- Feng, L.; Li, Y.; Chen, C.; Liu, X.; Xiao, X.; Ma, X.; Zhang, R.; He, Y.; Liu, G. Biochemical Methane Potential (BMP) of Vinegar Residue and the Influence of Feed to Inoculum Ratios on Biogas Production. Bioresources 2013, 8, 2487–2498.
- Filer, J., Ding, H. H., & Chang, S. (2019). Biochemical methane potential (BMP) assay method for anaerobic digestion research. *Water*, *11*(5), 921.
- Giribabu, D., Bharadwaj, P., Sitiraju, R., Burra, M., Rao, P. P., & Reddy, C. S. (2019). Combating open defecation through community-led sanitation. *Dr Sulaiman Al Habib Med J*, *1*(3-4), 45-51.
- H. W. Kim, J. Y. Namb, H. S. Shin, A comparison study on the high-rate co-digestion of sewage sludge and food waste using a temperature-phased anaerobic sequencing batch reactor system, Bioresource Technology 102 (2011) 7272–7279
- Haseler, L. J., Sibbitt, R. R., Sibbitt, W. L., Michael, A. A., Gasparovic, C. M., & Bankhurst, A. D. (2011). Syringe and needle size, syringe type, vacuum generation, and needle control in aspiration procedures. *Cardiovascular and interventional radiology*, *34*, 590-600.
- Hawkins, P., & Muxímpua, O. (2015). Developing business models for fecal sludge management in Maputo. *World Bank*.
- https://www.epa.gov/agstar/benefits-anaerobic-digestion
- Hu, S., Zuo, X., Lv, Z., He, J., Wu, Y., Liu, H., Chen, Z., 2020. Drained water quality in sludge treatment wetlands: effects of earthworm densities and plant species.
- J. Clean. Prod. 247, 119128. https://doi.org/10.1016/j.jclepro.2019.119128.
- Jain, M., Upadhyay, M., Gupta, A. K., & Ghosal, P. S. (2022). A review on the treatment of septage and faecal sludge management: A special emphasis on constructed wetlands. *Journal of environmental management*, *315*, 115143.
- Jimenez, J. A., La Motta, E. J., & Parker, D. S. (2005). Kinetics of removal of particulate chemical oxygen demand in the activated-sludge process. *Water Environment Research*, *77*(5), 437-446.
- K. Lee, P. Chantrasakdakul, D. Kim, H. S. Kim, K. Park, Evaluation of methane production and bomass degradation in anaerobic co-digestion of organic residuals, International journal of biological, ecological and environmental sciences (IJBEES) 2 (3) (2013) 2277 – 4394.
- Katukiza, A. Y., Ronteltap, M., Niwagaba, C. B., Foppen, J. W. A., Kansiime, F. P. N. L., & Lens, P. N. L. (2012). Sustainable sanitation technology options for urban slums. *Biotechnology advances*, *30*(5), 964-978.
- Kayhanian, & Masoud. (1994). Performance of a high-solids anaerobic digestion process under various ammonia concentrations. Journal of Chemical Technology and Biotechnology, 349-352.

- Kilucha, M., Cheng, S., Minza, S., Nasiruddin, S. M., Velempini, K., Li, X., ... & Li, Z. (2022). Insights into the anaerobic digestion of fecal sludge and food waste in Tanzania. Frontiers in Environmental Science, 1294
- Kim, B., Bel, T., Bourdoncle, P., Dimare, J., Troesch, S., Molle, P., 2018. Septage unit treatment by sludge treatment reed beds for easy management and reuse: performance and design considerations. Water Sci. Technol. 77, 279–285. https:// doi.org/10.2166/wst.2017.461.
- Labatut, R. A., & Gooch, C. A. (2014). Monitoring of anaerobic digestion process to optimize performance and prevent system failure.
- Lawal, A. A., Dzivama, A. U., & Wasinda, M. K. (2016). Effect of inoculum to substrate ratio on biogas production of sheep paunch manure. *Research in Agricultural Engineering*, *62*(1), 8-14.
- Li, Y., Hua, D., Zhang, J., Gao, M., Zhao, Y., Xu, H., ... & Zhang, X. (2014). Influence of inoculum to substrate ratios (ISRs) on the performance of anaerobic digestion of algal residues. *Annals of microbiology*, *64*(3), 955-960.
- Liu, T., & Sung, S. (2002). Ammonia inhibition on thermophilic aceticlastic methanogens. *Water Science and Technology*, *45*(10), 113-120.
- Magdalena, J. A., Greses, S., & González-Fernández, C. (2019). Impact of organic Loading Rate in Volatile fatty Acids production and population Dynamics Using Microalgae Biomass as Substrate. Scientific reports, 9(1), 1-11.Rojas, C., Fang, S., & Uhlenhut.
- Magri, M.E., Francisco, J.G.Z., Sezerino, P.H., Philippi, L.S., 2016. Constructed wetlands
for sludge dewatering with high solids loading rate and effluent recirculation: characteristics of effluent produced and accumulated sludge. Ecol. Eng. 95, 316–323.
https://doi.org/10.1016/j.ecoleng.2016.06.085.
- Manga, M. The Feasibility of Co-Composting as an Upscale Treatment Method for Faecal Sludge in Urban Africa; School of Civil Engineering, University of Leeds: Leeds, UK, 2017; Available online: https://etheses.whiterose.ac.uk/16997 (accessed on 11 October 2022).
- Maqbool, N., Shahid, M. A., & Khan, S. J. (2022). Situational assessment for fecal sludge management in major cities of Pakistan. *Environmental Science and Pollution Research*, 1-12.
- Matheri, A. N., Ndiweni, S. N., Belaid, M., Muzenda, E., & Hubert, R. (2017). Optimising biogas production from anaerobic co-digestion of chicken manure and organic fraction of municipal solid waste. Renewable and Sustainable Energy Reviews, 80, 756-764.
- Münch, E. V., & Greenfield, P. F. (1998). Estimating VFA concentrations in prefermenters by measuring pH. *Water Research*, *32*(8), 2431-2441.
- Nakasaki, K.; Tran, L.T.H.; Idemoto, Y.; Abe, M.; Rollon, A.P. Comparison of Organic Matter Degradation and Microbial Community during Thermophilic Composting of Two Different Types of Anaerobic Sludge. Bioresour. Technol. 2009, 100, 676–682.
- Panuvatvanich, A., Koottatep, T., Kone, D., 2009. Influence of sand layer depth and percolate impounding regime on nitrogen transformation in vertical-flow constructed wetlands treating faecal sludge. Water Res. 43, 2623–2630. https://doi. org/10.1016/j.watres.2009.03.029.
- Parajuli, P. (2011). Biogas measurement techniques and the associated errors.
- PBS (2021) Pakistan Social And Living Standards Measurement Islamabad. Pakistan Bureau of Statistics, Islamabad

- Peal, A., Evans, B., Ahilan, S., Ban, R., Blackett, I., Hawkins, P., ... & Veses, O. (2020). Estimating safely managed sanitation in urban areas; lessons learned from a global implementation of excreta-flow diagrams. *Frontiers in Environmental Science*, *8*, 1.
- Raposo, F., Borja, R., Martín, M. A., Martín, A., De la Rubia, M. A., & Rincón, B. (2009). Influence of inoculum–substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: process stability and kinetic evaluation. *Chemical engineering journal*, *149*(1-3), 70-77.
- Ricardo, E.; Fernando, L.; Ricardo, O.E.; Fernando, M.L.; Valle, U.; Patricia, T.; Valle, U. Avances En Investigación Sobre El Compostaje de Biorresiduos En Municipios Menores de Países En Desarrollo. Lecciones Desde Colombia. Ing. Investig. Tecnol. 2017, XVIII, 1–13.
- Ritchie, H., & Roser, M. (2021). Clean water and sanitation. *Our World in data*.
- S. Esteves, D. Devlin, Food waste chemical analysis. Final Report. The wales centre of excellence for anaerobic digestion, (2010). http://www.wrapcymru.org.uk/sites/files/wrap/Technical_report_food_waste_characterisation_Wales_2009x2.9086.pdf.
- Saev, M., Koumanova, B., &., & Iv. (2009). Anaerobic Co-Digestion of Wasted Tomatoes and Cattle Dung for Biogas Prodcution. Journal of the University of Chemical Technology and Metallurgy, 55-60.
- Sri Bala Kameswari, K., Kalyanaraman, C., Porselvam, S., & Thanasekaran, K. (2012). Optimization of inoculum to substrate ratio for bio-energy generation in co-digestion of tannery solid wastes. Clean Technologies and Environmental Policy, 14, 241-250.
- Strande, L., & Brdjanovic, D. (Eds.). (2014). *Faecal sludge management: Systems approach for implementation and operation*. IWA publishing.

- Strande, L., & Brdjanovic, D. (Eds.). (2014). *Faecal sludge management: Systems approach for implementation and operation*. IWA publishing.

- Stuart, P. (2006). The advantages and disadvantages of anaerobic digestion as a renewable energy source. *Loughborough University*.
- Sun, X.; Zhang, H.; Cheng, Z.; Wang, S. Effect of Low Aeration Rate on Simultaneous Nitrification and Denitrification in an Intermittent Aeration Aged Refuse Bioreactor Treating Leachate. Waste Manag. 2017, 63, 410–416
- Tanaka, S., Kobayashi, T., Kamiyama, K. I., & Bildan, M. L. N. S. (1997). Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. *Water Science and Technology*, *35*(8), 209-215.
- Tang, B., Yu, L., Huang, S., Luo, J., & Zhuo, Y. (2010). Energy efficiency of pre-treating excess sewage sludge with microwave irradiation. *Bioresource technology*, *101*(14), 5092-5097.
- Tanko, S. F., Ajibola, V. O., Agbaji, E. A., Mukailu, A. S., Putshaka, J. D., & Amana, B. S. (2018). Effect of anaerobic treatment on sulphide concentration, TSS, TDS and electrical conductivity parameters of sulphide-lime unhairing wastewater. *Bayero Journal of Pure and Applied Sciences*, *11*(2), 260-266.
- Tilley, E., Lüthi, C., Morel, A., Zurbrügg, C., Schertenleib, R. (2014). Compendium of Sanitation Systems and Technologies. Swiss Federal Institute of Aquatic Science and Technology (EAWAG) and WSSCC. Dübendorf, Switzerland. 2nd revised edition. Available from www. sandec.ch
- Tirado, S.M.; Michel, F.C. Effects of Turning Frequency, Windrow Size and Season on the Production of Dairy Manure/Sawdust Composts. Compost Sci. Util. 2010, 18, 70–80.

- Tyagi, V. K., & Lo, S. L. (2011). Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: an up to date review. *Reviews in Environmental Science and Bio/Technology*, *10*, 215-242.
- UNICEF. (2021). Water, sanitation and hygiene (WASH)
- UNICEF. WHO (2021) Progress on Household Drinking Water, Sanitation and Hygiene 2000–2020: Five Years into the SDGs. *Geneva: World Health Organization (WHO)*.
- V. Prabhudessai, B. Salgaonkar, J. Braganca, S. Mutnuri, Pretreatment of Cottage Cheese to Enhance Biogas Productio, BioMed Research International 374562 (2014) pages 6 http://dx.doi.org/10.1155/2014/374562
- Vikhareva, I. N., Aminova, G. K., & Mazitova, A. K. (2022). Resource Cycling: Application of Anaerobic Utilization Methods. *Sustainability*, *14*(15), 9278.
- Waqas, M.; Nizami, A.S.; Aburiazaiza, A.S.; Barakat, M.A.; Rashid, M.I.; Ismail, I.M.I. Optimizing the Process of Food Waste Compost and Valorizing Its Applications: A Case Study of Saudi Arabia. J. Clean. Prod. 2018, 176, 426–438.
- WaterAid. (2021). Annual Report 2020-2021, Pakistan
- WHO/UNICEF (2012). Progress on Drinking Water and Sanitation 2012. Joint Monitoring Programme for Water Supply and Sanitation
- World Bank. (2020). Pakistan - Karachi Water and Sewerage Services Improvement Project
- Zhou, Y., Li, C., Nges, I. A., & Liu, J. (2017). The effects of pre-aeration and inoculation on solid-state anaerobic digestion of rice straw. *Bioresource technology*, *224*, 78-86.
- Zhou, Y., Zhang, Z., Nakamoto, T., Li, Y., Yang, Y., Utsumi, M., & Sugiura, N. (2011). Influence of substrate-to-inoculum ratio on the batch anaerobic digestion of bean curd refuse-okara under mesophilic conditions. *Biomass and Bioenergy*, *35*(7), 3251-3256.