Anaerobic Treatment and Biogas Production of Municipal Solid Waste Leachate

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Environmental Science

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To my most solid support– my parents.

They have always encouraged me and believed in me.

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List of Abbreviations

Abbreviations	Description
AD	Anaerobic Digestion
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
CH_3COOH	Acetic Acid
CH_4	Methane
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
CSTR	Completely Stirred Tank Reactor
HRT	Hydraulic Retention Time
M	Molar Solution
mg/L	Milligram Per Liter
NH_3	Ammonia
OLR	Organic Loading Rate
ORP	Oxidation reduction potential
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
VFAs	Volatile Fatty Acids
VS	Volatile Solids

Abstract

Around 70% of waste is disposed of in open and dumps and landfills around the world, with the leachate generation containing high concentrations of organic compounds. The generated leachate adversely affects the environment and public health if discharged without proper treatment. An effective method for managing and treating municipal solid waste leachate and producing biogas is anaerobic treatment. In this research, a laboratory scale anaerobic completely stirred tank reactor (CSTR) of 12L volume was used for the treating fresh municipal solid waste leachate having chemical oxygen demand (COD) of 25,000 mg/L. The reactor was inoculated with acclimatized cow dung and biogas plant effluent. Anaerobic CSTR was operated for 171 days under mesophilic condition with batch start-up phase of 37 days followed by continuous phase of 134 days. During continuous phase, the reactor was operated under different organic loading rates (OLR) of 1, 2, 4 and 6 g COD/(L.d) corresponding to hydraulic retention time of 25, 12.5, 6.25 and 4.25 days respectively. The overall performance of anaerobic CSTR was investigated by assessing pH, oxidation reduction potential (ORP), VFA/Alkalinity, ammonia nitrogen, chemical oxygen demand (COD) removal and biogas production. Results revealed that 75% of COD removal and 219 mL/g COD specific biogas production was obtained during batch phase while optimum of 60% & 70% COD removal were achieved under organic loading rates of 2 g COD/(L.d) and 4 g COD/(L.d) respectively. Average specific biogas production of 59.2 mL/g COD was achieved under organic loading rate (OLR) of 4 g COD/(L.d). Thus based on anaerobic treatment, biogas production and stability of anaerobic CSTR, organic loading rate (OLR) of 4 g COD/(L.d) was considered as optimum organic loading rate for anaerobic treatment of municipal solid waste leachate. Overall, the study indicates that fresh municipal solid waste leachate can be treated in anaerobic CSTR under the varying range of organic loading rates with biogas production.

Chapter 1

Introduction

1.1 Background

Since the advent of civilization, many community-based environmental issues have been evolving. The municipal solid waste (MSW) management in a sustainable manner is one of the most pressing environmental issues facing both industrialised and developing nations (Cetrulo et al., 2018). The world generates 2.01 billion tonnes of MSW annually while in Pakistan, it's approximately 0.05 billion tonnes which has been increasing more than 2.4 percent annually (Wijekoon et al., 2022). Pakistan has a population of 207.77 million, making it the fifth most populous nation in the world (Pakistan Beauro of Statistics, 2017). According to the geography and socioeconomic development of the city, the typical rate of municipal solid waste creation is between 0.285 kg/c/d (kg/capita/day) and 0.615 kg/c/d. (PEPA, 2005).

Among worldwide waste management strategies, 11% of waste is incinerated, 19% is recycled, 33% is open dumped and 37% is disposed of in landfill as shown in (Sohoo et al., 2022). Just half of the MSW produced in Pakistan's cities is being collected by the government (PEPA, 2005). While the rest of waste is left as mountains in the streets, on roadsides, in sanitary drains and sewers in the city areas. There is absence of non-engineered landfill amenities for waste disposal. Due to this reason, open dumping is a very collective practice for solid waste disposal (Korai et al., 2017). Open dumping practises have made these sites into breeding grounds for mosquitoes, flies, animals, and birds as well as a source of disease transmission, offensive odours, and unintentional fire hazards. Ineffective disposal of MSW contributes significantly to global climate change by releasing unchecked amounts of greenhouse gases (GHGs), primarily methane. Among total GHG emissions of 408.1 MtCO_2-eq, the contribution of the waste sector is 15.5 MtCO_2-eq. MSW is causing 12.5 MtCO_2-eq. of CH_4 emissions (MoCC, 2015). Above all, due to the interstitial water content of waste through waste stratums, municipal solid waste leachate can undergo a series of biochemical processes inside waste stream, creating very polluted effluent known as leachate. High levels of organic and inorganic pollutants, heavy metals, poisonous substances, ammonia, and inorganic elements are present (Chávez et al., 2019). The main issue with municipal solid waste leachate is that it has the potential to pollute since it can move through the subsurface into surface and groundwater, endangering aquatic life, plants, and people's health. (Mishra et al. 2019).

There are three types of municipal solid waste leachate depending on the leachate age known as young leachate having age less than one year, medium leachate having age in between 1-5 years and old leachate with more than five years of time (Gao et al., 2014). More affordable and comprehensive leachate treatment methods must be developed for developing nations.

MSW leachate can be treated through physicochemical methods and biological methods. Coagulation/flocculation, chemical precipitation, adsorption & ion exchange, ammonium stripping, etc. are examples of physico-chemical processes. while biological methods comprising of natural and artificial systems. Due to the high operational costs and generation of secondary pollutants, physicochemical processes lack over biological system. Biological treatment is done in natural systems as well as in artificial systems. Due to high rate of biological treatment and less intensive land requirement, artificial biological treatment systems are preferred over natural biological systems. Artificial system includes bioreactors which may be anaerobic or aerobic in nature (Gao et al. 2014). Anaerobic biological process is preferred over aerobic process due to low sludge production and energy requirement. Also anaerobic process produce digestate along with biogas which give an edge over aerobic process. (Chan et al., 2009). There are four major type of reactor that have been used for treating municipal solid waste leachate including completely stirred tank reactor (CSTR), up flow anaerobic sludge blanket reactor (UASB), fluidized bed reactor (FBR) & expanded bed reactor (EBR). In completely stirred tank reactor, substrate is constantly fed into the reactor and digested material or stream is continuously removed from the reactor at the same flow rate. Good settleability and low retention time can be achieved by UASB systems. Fluidized bed (FBR) and expanded bed reactor (EBR) are suitable for reactions involving solid reactants. Design organic loading rates (OLR) for AF reactors are often in the range of 9-17 g COD/(L.d)

There are some limitations for anaerobic CSTR process. Few of them are following as,

- It is not good at removing non-degradable organic pollutants
- Methanogenic archea are slow growing microbes so these systems need long startup and retention time
- pH must be controlled along with monitoring of volatile fatty acids

- Needs a high temperature to function properly
- To comply with discharge standards, additional treatment may be needed in an aerobic treatment process.
- Anaerobic CSTR is unable of removing biological nitrogen or phosphorus.

1.2 Objectives

- Treatment of municipal solid waste (MSW) leachate in mesophilic anaerobic CSTR operated under various organic loading rates (OLR)
- Analysis of various operational and monitoring parameters of anaerobic CSTR during leachate treatment

1.3 Scope of study

This study was accompanied to assess the overall performance of anaerobic CSTR operated under various organic loading rates. Its scope can be defined as follows:

- Substrate i.e. municipal solid waste leachate was collected from the transfer station of Albyrak Workshop located near Liaqat Bagh Rawalpindi. After it's characterization, synthetic leachate was prepared at lab scale
- Inoculum was a mixture of cow dung and biogas plant effluent. Cow dung was brought from local animal farm of H-13 Islamabad while biogas plant effluent was collected from National Cleaner Production Centre (NCPC) Rawalpindi. Both inoculum were mixed in 1:1 on volatile solids (VS) basis
- Lab scale anaerobic CSTR of 9 L working volume was operated at 37°C under two phases i.e. startup phase of 37 days followed by 134 days of continuous operation under organic loading rate of 1, 2, 4 and 6 g COD/(L.d) with hydraulic retention time (HRT) of 25, 12.5, 6.25 and 4.17 days respectively
- Lab tests of pH, oxidation reduction potential (ORP), VFA/Alkalinity ratio, ammonia nitrogen, effluent COD and biogas production were carried out to determine the overall performance of anaerobic CSTR under different phases
- This study was limited to applying short range of organic loading rates to anaerobic CSTR due to time restriction of MS study

Chapter 2

Literature Review

This chapter contains information on the production and disposal of MSW, the characteristics of the leachate produced from these wastes, the various methods used to treat the leachate, and prior research on the anaerobic treatment and production of biogas from these wastes.

Municipal solid waste generation and disposal

The management of municipal solid waste (MSW) is one of the most pressing issues facing the world today (Cetrulo et al., 2018). The population of world is expected to increase from 7.6 billion to 8 billion people by the year 2030. Municipal solid waste generation currently stands at roughly 1.9–2.0 billion tonnes annually, with 3.0–4.09 billion tonnes anticipated to be produced by 2050 (Nadeem et al., 2022). Of the various waste management techniques, landfills receive 37% of municipal solid waste, open dumping receives 33%, recycling and composting recovers 19% of garbage, and incineration treats 11% of waste (Sohoo et al., 2022).

With a population of 207.77 million, Pakistan ranks fifth in the world in terms of population (Pakistan Beauro of Statistics, 2017). In Pakistan, population growth, economic expansion, fast urbanisation, and higher communal living standards are the primary drivers of rising solid waste levels. According to the geography and socioeconomic development of the city, Pakistan's average rate of municipal solid waste output is predicted to be between 0.30 kg/c/d (kg/capita/day) and 0.60 kg/c/d (PEPA, 2005).

Pakistan's metropolitan regions currently generate 50,438 tonnes of municipal solid trash per day at a rate of 0.84 kg/c/d; by 2025, it is anticipated that this number will increase to 110,000 tonnes per day at a rate of 1.07 kg/c/d (Sohoo et al., 2021).From collection to disposal, there is a lack of a suitable waste management system for municipal solid waste.

In Pakistan, the local government of each province is in charge of managing municipal solid garbage. In Pakistan, the government offers services that collect at least 50% of the generated municipal solid waste, but the remaining debris is left in mounds in the streets, by the sides of the roads, in storm drains, and on unused land, endangering both human health and the environment. In Pakistan, sanitary landfills, composting, and incineration are infrequently employed as methods of disposing of municipal solid waste. Open dumping is a widespread practise in Pakistan (Sohoo et al., 2021).

Uncontrolled emissions of greenhouse gases (GHGs), which contribute significantly to climate change, the formation of dust and odour, particularly under windy conditions, inadvertent risks from open burning, and breeding grounds for bacteria, mosquitoes, flies, viruses, rodents, and other vermin are just a few of the environmental and health issues caused by improper disposal of municipal solid waste.

Above all, leachate, a byproduct of biochemical reactions in the waste stream, is produced because rainwater percolates through solid waste layers and there is interstitial water content in the waste mass. MSW leachate primary problem is the potential for pollution brought on by the flow of the generated leachate via the subsurface into the surface and groundwater, which has a negative effect on the ecology and general well-being (Mohan and Joseph, 2021).

Characterization and environmental concerns of municipal solid waste leachate

Leachate from municipal solid waste undergoes intricate chemical, physical, and biological degradation. Inorganic chemicals (chlorides, ammonium, phosphates, and nitrates), dissolved organic matter (fatty acids and organic carbon), and heavy metals (copper, zinc, lead, and mercury) are the three main types of contaminants found in municipal solid waste leachate (Lindamulla et al., 2022). Table 2.1 shows typical characteristics of municipal solid waste leachate.

Some of the factors that can alter the composition of the leachate from the transfer station include the degree of compaction, age of the leachate, composition, climate, moisture content, and biodegradable components.

In general, three types of leachate can be distinguished based on their age: young leachate, which is defined as having an age of less than one year and having a chemical oxygen demand (COD) of greater than 15,000 mg/L and a BOD5/COD of 0.5 to 0.1; medium leachate, which is defined as having an age of one to five years and having a COD range of 3100–15,000 mg/L and a BOD_5/COD.

While the NEQS, allow only 150 mg/L of COD and BOD_5/COD of 0.54. Therefore, municipal solid waste leachate treatment needs significant attention to minimize the pollution.

Table 2.1 Characteristics of municipal solid waste leachate

Parameters	Classification of Leachate			NEQS
	Young	Medium	Old	
Age (Year)	< 1	1-5	> 5	--
pH	< 6.5	6.5-7.5	> 7.5	6-9
COD (mg/L)	> 15000	3000-15000	< 3000	150
BOD_5/COD	0.5-1	0.1-0.5	< 0.1	0.53
NH_3-N (mg/L)	< 4000	400	> 4000	40
Heavy Metals (mg/L)	> 2	< 2	< 2	--
Organic Compound	80% Volatile Fatty Acids	5-30% VFA + Humic Acid + Fulvic Acid	Humic Acid + Fulvic Acid	--

Source: (Gao et al., 2014)

Leachate from untreated municipal solid waste may have negative effects on ecosystems, including soil, groundwater, and surface water. The quality of ambient soil and ground water resources may be impacted by leachate's infiltration into the soil and ground water. Leachate contamination may spread over a great distance and contaminate the groundwater aquifer in other locations once it has become a part of the groundwater (Vaverkova et al., 2020). During wet seasons, large amounts of leachate from open garbage dumps may infiltrate neighbouring surface water bodies, such as rivers, streams, irrigation water canals, and tiny irrigation channels, through surface runoff. Leachate contains several contaminants that can bioaccumulate in living tissues via the food chain (Daniel et al., 2021). Leachate generated from open dump sites in Pakistan currently requires effective management and treatment in order to prevent adverse effects on the quality of the surrounding soil, ground & surface water, and human health.

Methods for treatment of MSW leachate

Municipal solid waste leachate can be treated through physicochemical methods having coagulation and flocculation, chemical precipitation, adsorption & ion exchange, ammonium stripping etc.; biological methods involving activated sludge, biological filters and aerobic as well as anaerobic lagoons and advanced treatment methods like Fenton process, solar photo-Fenton processes (Costa et al., 2019).

The choice of an appropriate leachate treatment method is influenced by a variety of elements, such as current waste disposal methods, the kind and location of dump sites, regional weather patterns, the composition of waste and leachate, and financial considerations of leachate management and treatment. Leachate treatment techniques that are commonly utilized worldwide are shown in Figure 2.1.

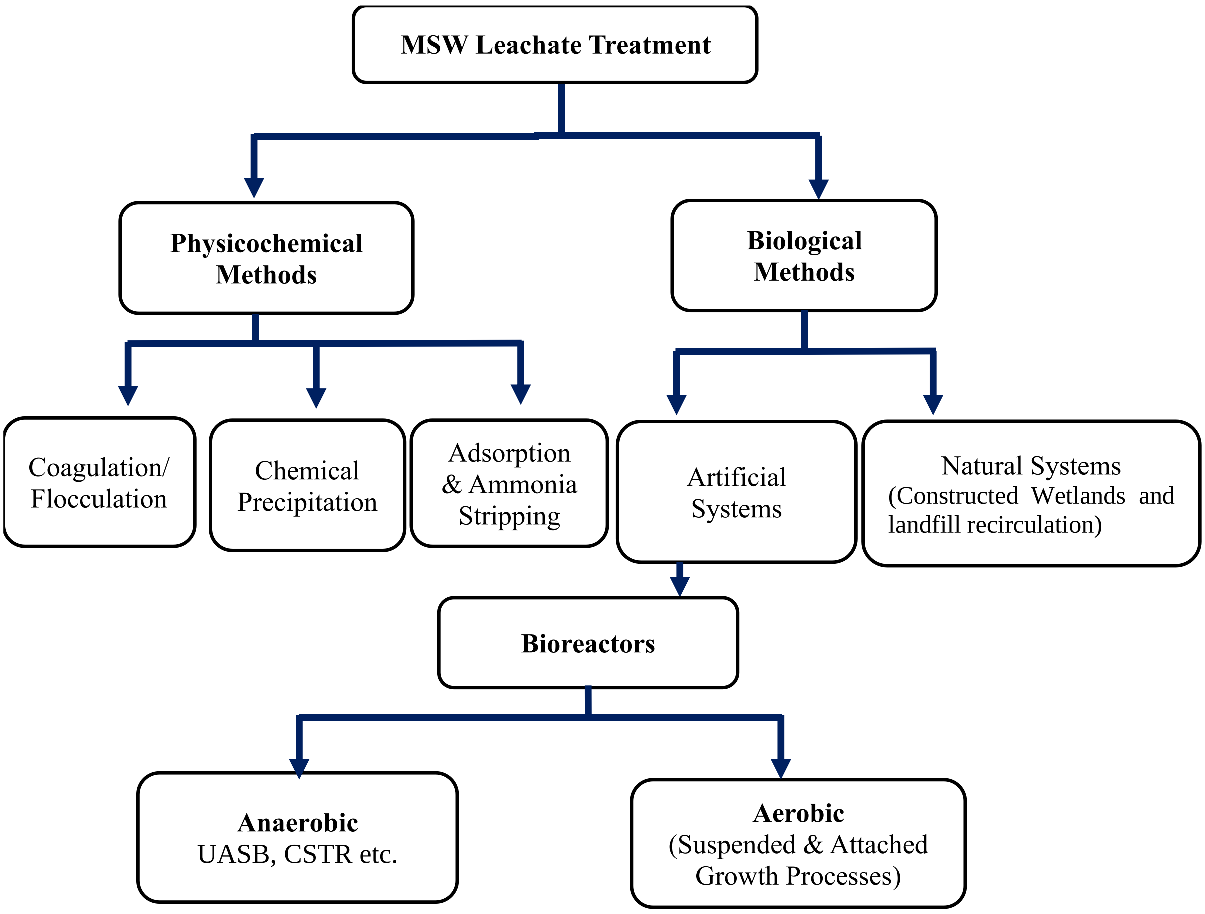

Figure 2.1 Different methods of municipal solid waste leachate treatment

2.1.1 Physicochemical methods

These methods involve reduction of suspended & colloidal and floating material through adsorption, chemical precipitation, ammonium stripping and coagulation with flocculation (Renou et al., 2018). Due to the high operational costs and generation of secondary pollutants, these methods are mainly used as pretreatment and post-treatment to supplement the biological treatment processes.

Coagulation & flocculation involving destabilization of colloidal particles brought about by adding reagent like aluminum sulphate, ferrous sulphate, ferric chloride or ferric sulphate called as coagulant is known as coagulation while flocculation is the accumulation of destabilized particles into flocs which can be settled under the action of gravity. Dolar et al (2016) studies application of coagulation/flocculation as pretreatment methods for nanofiltration (NF) and reverse osmosis (RO) of stabilized leachate resulted in COD removal of 65%. Turbidity removal of 62% and COD removal of 34% from wastewater was measured using Salvia hispanica (Tawakkoly et al., 2019). Ishaq et al (2018) examined COD removal of 90% using coagulation-flocculation together with ultraviolet based sulfate radical process. Using tannin-based natural coagulant for treating landfill leachate for coagulant dose of 0.73 g at a pH of 6 resulted in COD removal of 53% and ammonia nitrogen removal of 60% respectively (Banch et al., 2021).

Heavy metals and non-biodegradable organic components in wastewater are often removed using chemical precipitation technique. The leachate is mixed with a precipitation reagent in the chemical precipitation procedure. Filtration can be used to get rid of the particles from the precipitated mixture. Along with non-biodegradable organic pollutants and heavy metals, it can be used to remove ammonia, nitrogen, phosphorus, and other inorganic pollutants from various types of leachate. Metal removal efficiency by chemical precipitation methods depends on amount and concentration of precipitation reagent and metal concentration of leachate (Siddiqi et al., 2022). The initial ammonia nitrogen concentration in the landfill leachate was in the range of 610–640 mg/L. Chen et al. (2013) worked on chemical precipitation handling landfill leachate for treating its nitrogenous compounds and observed 88% of ammonia removals for optimal conditions of pH 10, contact time 30 min, Mg/N/P molar ratio 1:1:1. Another investigation into chemical precipitation using biomass ash for landfill leachate removed 99% of the colour and 74% of the COD (Viegas et al., 2021).

Adsorption is the process by which chemical species that are dissolved in a leachate and are referred to as adsorbates take up space on solid materials such as adsorbent at the liquid-solid interface. Activated carbon in granular and powdered form is the most often used adsorbent. When silica nanoparticles were utilised as an adsorbent for the treatment of MSW leachate, 70% COD reduction was accomplished (Pavithra and Shanthakumar, 2017).

COD removal of 90% for landfill leachate was achieved using activated carbon under pH 4 at 20 °C and activated carbon dose of 20 g/L (Ferraz and Youn., 2020). Soubh et al. (2018) studied the effect of nanofibers of zero-valent iron adsorbent fixed on the surface of reduced large graphene oxide for treatment of fresh leachate. Under pH of 3, adsorbent dose of 1.7 g/L and retention time of 45 minutes. Optimized conditions of COD and ammonia removal efficiencies for the fresh leachate were 81 and 73%, respectively.

Ammonium stripping is a simple desorption method used for lowering ammonia content in wastewater stream. Different chemicals are being added to convert ammonium ions into free ammonia. The leachate is then poured down through a column thus allowing the ammonia and other gases to come out of wastewater escaping into air. Smaoui et al. (2020) worked on landfill leachate treatment using air stripping accompanied with biological treatment. 78% of COD removal and 80% ammonia removal was achieved under OLR of 3.2 g COD/L/d respectively.

In order to remove NH_3-N from landfill leachate at high pH values, ammonium stripping is frequently utilised. Using ammonium stripping to treat landfill leachate, 95% of the nitrogen from ammonia was removed at the optimal temperature of 60°C (Campos et al., 2013). When air flow rate of 1.2 L/min and a 71% recovery in sulphuric acid were utilised, a stripping efficiency of around 94% was obtained to ultimately produce ammonium sulphate.

2.1.2 Biological methods

These methods treat the high strength wastewater including leachate using biological degradation under the metabolic activities of microorganisms. These methods are usually hired to remove nutrients like NH_3 and degradable organic chemicals because they are cost-effective; however, these techniques may not be effective for removing heavy metals and non-biodegradable substances (Miao et al., 2019). The two main categories of biological leachate treatment techniques are artificial treatment techniques using various kinds of bioreactors and natural treatment techniques using manmade wetlands and landfill leachate recirculation. Artificial biological treatment systems are preferred over natural biological systems due to their high rate of biological treatment and less intense land demand. Aerobic and anaerobic processes are divisions of artificial biological methods, including bioreactors (Dabaghian et al., 2019). Table 2.2 provides comparison of aerobic and anaerobic biological processes.

Table 2.2 Comparison of aerobic and anaerobic biological treatment methods

Sr. No	**Parameters**	**Aerobic**	**Anaerobic**
1	Organic Loading Rate	Moderate	High
2	Nutrients Removal	High	Low
3	Sludge Production	High	**Low**
4	Energy Requirement	High	**Low**
5	Sensitive towards temperature	Low	High
6	Start-up Phase	3-5 weeks	1.5-2 months
7	Odor Problems	Low	High
8	Effluent Quality	Good	Moderate
9	Bio-Energy Production	No	**Yes**

Source: (Chan et al., 2009)

Continuous oxygen supply is necessary for aerobic treatment. Microorganisms in wastewater utilise the organic material as a source of energy. Biomass with suspended growth and biomass with fixed growth are the two main categories of aerobic treatment. Aerated lagoons and the activated sludge process are two examples of suspended growth systems. Fixed growth biomass is mostly utilized in trickling filters and rotating biological contactors.

In the absence of oxygen, anaerobic treatment transforms the organic content in leachate into CH_4, CO_2, and other metabolites. Due to the high COD and high BOD_5/COD ratio of municipal solid waste leachate, anaerobic treatment approaches perform better at treating leachate than aerobic treatment methods (Azreen and Zehrim, 2018).

Anaerobic treatment techniques produce biogas and generate little sludge, making them extremely energy-efficient and environmentally friendly (Gamon et al., 2019). Leachate can be treated anaerobically to considerably lower ammonia and COD levels.

For the reasons stated above, anaerobic biological treatments are preferred to aerobic ones.

Leachate classification and treatment options

Figure 2.2 shows which parameters should be evaluated in the choice of a treatment for a municipal solid waste leachate. If a raw municipal solid waste leachate is having 15,000 mg/L COD and ratio of BOD_5 /COD is between 0.4-0.8, then leachate will be classified as young while leachate having COD of 3000-15,000 with BOD_5 /COD ratio of 0.1-0.4 would be categorized as mature leachate. Young leachate, requiring biological treatment and mature leachate needs combined biological and physicochemical or just physicochemical process of treatment (Costa et al., 2019).

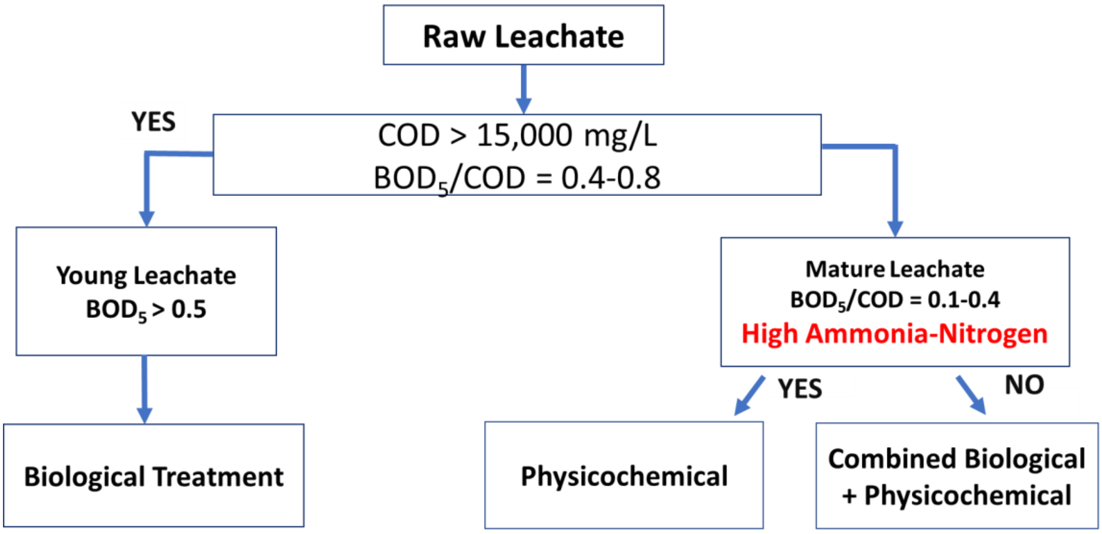

Figure 2.2 Treatment options for municipal solid waste leachate treatment

Leachate has been treated using a combination of physicochemical treatment approaches to increase removal efficiency while reducing energy use. Advanced oxidation processes with membranes, adsorption; membrane filtration combined with coagulation or adsorption, and advanced oxidation processes with coagulation are among the integrated approaches being employed for combined physical/chemical methods (Xiang et al., 2019).

Similarly, biological methods used for treatment of municipal solid waste leachate alone are not sufficient enough to remove refractory contaminants. For that, various combined biological methods along with physical & chemical methods are being used to improve biodegradability ratio and enhancement in performance. These includes combined adsorption and biological methods, integrated advance oxidation process with biological methods, integrated coagulation and biological methods (Mojiri et al., 2016).

Types of anaerobic reactor

Four major type of reactor are mostly used for treating municipal solid waste leachate (Ersahin et al., 2011).

2.1.3 Completely stirred tank reactor (CSTR)

Here, feedstock is continuously supplied into the reactor, where an impeller ensures effective mixing. In addition, digested material or stream is constantly withdrawn from the reactor for a predetermined hydraulic retention period at the same flow rate (HRT). Hydraulic retention time (HRT) and sludge retention time (SRT) for anaerobic CSTR may range from 14 to 45 days (Siciliano et al., 2019).

CSTR is appropriate anaerobic reactor for running wastewater at high volumetric loading rates. COD range of 9000-55000 mg/L of different wastewater is used. For completely mixed anaerobic digesters, the typical organic loading rate (OLR) is between 2 g COD/(L.d) to 5 g COD/(L.d).

2.1.4 Upflow anaerobic sludge blanket reactor (UASB)

With a reduction in up-flow velocity and an improvement in solids retention, the influent flow dispersed at the bottom of the UASB reactor which ultimately travels in an up-flow mode through the sludge blanket before exiting along the edges of the funnel, providing a larger area for effluent (Gotvajn and Pavko. 2015).

The entire digestion of the substrates is carried out by the dense mixed population of microorganisms that composes the granules that spontaneously develop after several weeks of reactor operation. Systems using UASB technology can operate at very high OLR levels while yet having excellent settleability and short retention durations. 6–15 g COD/(L.d) is the typical design organic loading rate (OLR).

2.1.5 Fluidized bed reactor (FBR) & Expanded bed reactor (EBR)

In FBR and EBR, usually a greater area for effluent provided by the influent flow which is distributed at the bottom of the UASB reactor. Influent flow travels in an up-flow mode through the sludge blanket and flows out along the borders of the funnel with a reduction in up-flow velocity and an improvement in solids retention (Gotvajn and Pavko. 2015). Typically, its design organic loading rate (OLR) ranges from 8 to 20 g COD/(L.d).

2.1.6 Anaerobic filter reactors (AFR)

It is a fixed-bed anaerobic reactor with more than one filtering compartments which are connected in series. Here surface of the filter material is attached with the active biomass. The filter material traps particles as wastewater passes through it and degrade organic materials (Márquez et al., 2021). In AFR, probability for loosing biosolids in the down flow systems is very high in AFR reactors.

Owing to the simple configuration, cheaper fabrication, it’s easier cleaning and maintenance activity, efficient stirring function, excellent treatment efficiency under low OLR, CSTR are favorable than other discussed anaerobic reactors.

Anaerobic treatment process

The process of anaerobic digestion uses a population of bacteria to break down organic material without the use of oxygen, resulting in biogas and digestate as the final products (Gong et al., 2021). The process occurs in four stages i.e. hydrolysis, acidogenesis, acetogenesis,and methanogenesis. Each step is carried out with different groups of bacteria producing different end products. The steps in anaerobic digestion is shown in the Figure 2.3 (Chew et al., 2021).

2.1.7 Hydrolysis

In anaerobic digestion, hydrolysis is the first step in which a group of bacteria converts organic matter (which are in the form of polymers) into simple and soluble form (oligomers and monomers).

It is an essential step prior to acidogenesis as the consortia of bacteria in acidification cannot intake complex form of organic matter. In other words, hydrolysis provides substrate to the group of bacteria involves in acidogenesis. Enzymes like cellulase, amylase and xylanase converts carbohydrate into monosaccharides i.e. simple sugars, protease degrades proteins into amino acids and lipase converts lipids into fatty acids and glycerol.

Temperature is major factor affecting the enzymes activity and ultimately, the hydrolysis rate. The optimum temperature for hydrolysis is reported to be 30ºC-60ºC (Nie et al., 2021)

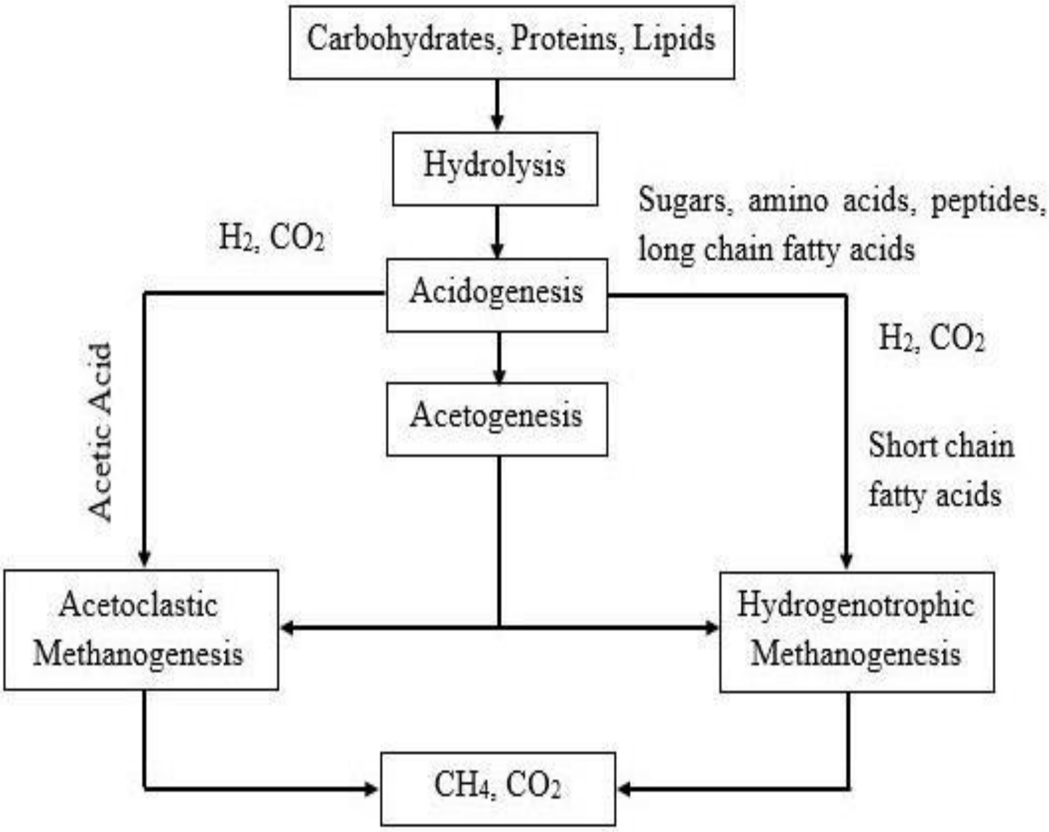

Figure 2.3 Steps in anaerobic treatment

2.1.8 Acidogenesis

Here, acidogenic bacteria convert the products of hydrolysis into acetate, butyrate etc., aldehydes, carbon dioxide, hydrogen, nitrogen and sulfur compounds etc. This process releases a high amount of energy for microorganisms. Products of this phase is utilized in further steps of anaerobic digestion especially acetic acid which is majorly utilized in methanogenesis. In acidogenesis, pH falls below 5.2 because of the acid production (Feng et al., 2021a).

2.1.9 Acetogenesis

Acetogens convert organic acids (which are produced in acidogenesis) into acetic acid, carbon dioxide, hydrogen etc. The main product of acetogenesis is acetic acid, which will be utilized by methanogens. Mostly, acetogens have rapid growth rate than methanogens (Amin et al., 2021).

2.1.10 Methanogenesis

Here methanogens convert acetic acid, carbon dioxide and hydrogen into methane. In this stage, the production of methane occurs in two paths. In first, acetoclastic methanogens convert acetic acid in to methane. While, on the other hand, carbon dioxide and hydrogen is converted in to methane by hydrogenotrophic methanogens. A large amount of methane is produced by acetic acid. Whereas, very few amount of CH_4 is produced by hydrogenotrophic methanogens using CO_2 and H_2. Optimum pH for methanogenic bacteria is 6.5–7.5 (Li et al., 2019).

Factors affecting anaerobic treatment

As anaerobic digestion is performed by different groups of bacteria, they require favorable conditions to work at optimum level. Many parameters affect their growth and working which ultimately affects the biogas yield. These parameters are described below:

2.1.11 Temperature

Temperature is one of the most key factors in anaerobic digestion as it directly effects the microbial growth and plays a vital in separation of gases like methane and carbon dioxide from liquid phase. Depending on temperature conditions, there are three type of microorganism groups known as psychrophiles, mesophiles and thermophiles, respectively. The working range of temperature for psychrophiles, mesophiles and thermophiles are 4–15°C, 20–40°C and 45–70°C respectively. In the previous studies it has been seen that mesophilic and thermophilic digestion gives better results as compared to others. Thermophilic digestion requires high energy and maintenance cost that is why mesophilic digestion is preferred over it. The mesophilic temperature in the range of 35–37°C is usually preferred for the hydrolysis/acidogenesis of organic waste (Van et al., 2020).

2.1.12 pH

pH is also one of the most important parameter whose slight change can highly effect the microbial growth and performance. The optimum pH for methanogenesis is reported to be 6.8-7.2. During acidogenesis and acetogenesis the pH falls due to the excessive VFA's productions but if the pH drops from 6 it will cause inhibition in the biogas production. Same is the case with methanogenesis, as methanogens cannot work in the environment with low pH. As a result of which hydrogen and acetic acid starts to accumulate, inhibiting the biogas production (Kumar and Samadder, 2020).

2.1.13 VFA and alkalinity

Volatile fatty acids (VFA) leads to the production of methane during anaerobic digestion. But their accumulation lowers the pH of mixture effecting the performance and growth of methanogens (Eryildiz and Taherzadeh, 2020). Whereas, formation of ammonia increases the pH and also acts as the buffer (alkalinity) in response to VFA. The optimum VFA/TA ratio that shows stable anaerobic digestion is less than 4.

2.1.14 Water content

Water is very important in AD as it provides medium for biochemical reactions, microbial activities, nutrient absorbance and locomotion (Heiske et al., 2015). Different moisture content is required for different substrates. Usually, 75-80 % of moisture content is maintained in the reactors. The maximum cumulative biogas production for cattle dung and vegetable waste were found to be 11.25 and 4.09 L respectively at feedstock to water ratio of 3:2 (Basumatary et al., 2022).

2.1.15 Inoculum

Inoculum is very essential to start the anaerobic digestion as it contains rich consortia of microbes. In the absence of inoculum, the process takes too much time to start. It reduces the lag phase and increases the biogas and methane yield. It is one of important factor affecting biogas production and stability of anaerobic digestion process. In maximum of studies regarding anaerobic digestion of organic waste, inoculum to substrate ratio of 1.0 is used. Digested cattle manure, sewage sludge, Fresh cow dung, organic wastes, swine manure, and anaerobic sludge, are the commonly used inoculum types in anaerobic digesters (Bella and Rao, 2022).

2.1.16 Ammonia-nitrogen as inhibitory and toxic compounds

A number of substances can be a source of inhibition reactions like heavy metals, cations, sulfide, and ammonia and light metals. During reactions sulphates restricts methane generation by giving a substitute as an electron receiver. Sulfide causes requirement of oxygen which decreases mound of chemical oxygen demand treated. For methanogens there are many compounds of carbon which cause inhibition. Toxicity by ammonia is one of the key factors in anaerobic reactions and treatments if wastewaters including elevated amounts of NH_3-N and TAN. Free ammonia is reflected as toxic for methanogenic microorganisms. Minimum amount of ammonia that is considered as toxic for methanogenic bacteria in anaerobic digestion is 600 mg/L (Grady et al., 2016). Ammonia nitrogen acts as an inhibitory element when it is above a threshold concentration i.e. greater than 1450 mg/L ultimately causing a significant reduction in microbial growth Free ammonia nitrogen levels up to 200 mg/L are sufficient for provision of nitrogen regarding microbial growth and improving the digester buffer capacity (Yellezuome et al., 2022).

2.1.17 Organic loading rate

It represents the quantity of organic feedstock added on daily basis in the digester. For wastewater case mostly it is mentioned in g COD/(L.d). The gas generated on daily basis in a reactor actually rests on its organic loading rates. Gou et al. (2017) reported higher destabilization and loss in COD removal at higher OLR's for wastewater. Anaerobic digestion as well as gas generation rate relies on OLR. It has been observed that anaerobic treatment destabilizes at higher organic loading. Ahmad et al., (2021) considered the effect of organic loading rate for treatment of leachate and found that maximum COD removal was achieved at low OLR.

2.1.18 Hydraulic retention time (HRT)

HRT is the period spent by feedstock in reactor for anaerobic treatment. HRT rests on the thermal conditions in system. For high temperature the hydraulic retention time is less as compared to the lower temperatures. For thermophilic environment, the required hydraulic retention time is about 15 to 25 day while that required mesophilic conditions ranges between 30-50 days. Low hydraulic retention time increase the hydrolysis rate consequently reducing duration of the hydrolytic phase. Bi et al. (2020) found anaerobic treatment gives maximum biogas yield when the system shifted from low HRT to high HRT.

Types of anaerobic treatment based on mode of operation

On the basis of operational mode, anaerobic digestion can carry out in two different ways i.e. batch mode digestion and continuous mode digestion. Both modes are described below:

2.1.19 Batch mode digestion

In batch mode digestion, the reactor is fed with inoculum, substrate and water at the start with the buffer solution to control the pH change during the anaerobic digestion. In batch mode operation, the digestion of substrate is higher than continuous as it spends more time in the digester. The production of biogas is low in the start but slowly increases to the maximum and then decreases at the end (Franca and Bassin Carrere, 2016).

2.1.20 Continuous mode digestion

In continuous mode digestion, the reactor is fed with substrate on daily basis around 1- 8 times. The old one is replaced by the freshly added substrate. As, the substrate has less retention time in the digester hence, most of the substrate remained undigested. The biogas production in continuous mode is constant as compared to batch mode (Wei et al., 2018). For attaining thermophilic temperature, it is preferred that bioreactors should be operated in batch phase before running the bioreactor in continuous mode operation.

Research studies on anaerobic treatment of different types of leachate

Table 2.3 shows various studies that have been accompanied on different types of leachate treatment under various conditions.

Table 2. 3 Summary of studies on leachate treatment through various bioreactors

Substrate	Reactor	Conditions		COD Removal (%)	Reference
		OLR g COD/(L.d)	HRT (days)		
Landfill leachate	MABR (5600 mg/L)	1.4-5.6	4-1	76	Ahmad et al.,2021
Compost leachate	CSTR (66500 mg/L)	20 & 30	--	62	Siciliano et al., 2021
Landfill leachates	UASB (53400 mg/L)	27-86	1.5-0.5	83	Moujanni et al.,2020
Compost leachate	CSTR	4.25 & 38.5	15.5-1.8	90	Siciliano et al., 2019
Incineration leachate	UASB (40,000 mg/L)	4.4- 28.6	9.3-1.4	89	Lei at al., (2018)
Compost leachate	AMBR-ASBR (25000-98000 mg/L)	1.04-19.65	1	97	Eslami et al., 2018

All of studies regarding the leachate treatment have been conducted at mesophilic condition. Anaerobic bioreactors of various sorts have been employed in a variety of OLR and HRT.

COD range for most of leachate are in range of 5000-98000 mg/L. OLR and HRT were varied in different types of anaerobic bioreactor to check COD removal. COD removal of 60%-97% can be achieved using different types of reactors. Details for some other studies are as:

- Shin et al. (2022) worked on the start-up of a demonstration-scale anaerobic digestion facility handling leachate from food waste. They treated food waste leachate with a 600 m^3 working capacity anaerobic digester with 96,000 mg/L COD. During the digester's initial starting, it was being watched. System was operated throughout the course of three stages for a total of 89 days. Volatile fatty acid (VFA) content increased during the first phase of 0.45 days, causing the pH of the system to decline with an OLR > 2.7 g COD/(L.d). The pH level was held at 7.8. A sequencing batch operation was used to target rapid system recovery during the second phase, which lasted 30 days. One batch cycle of 2–5 days included a combined drawing and feeding step lasting 5 hours, a responding step lasting 91–17 hours, and a settling step lasting 24 hours. The biogas yield was gradually increased over the course of 11 cycles, reaching 0.55 L/g CODin without causing any reactor instability. Phase 3 involved running the system for 15 days with an OLR of 4.1-0.3 g COD/ (L.d). They came to the conclusion that a successful recovery technique for biogas plants experiencing system instability can be the sequencing batch operation with configurable cycle duration. Phases 1, 2, and 3 each showed a methane output of 0.20, 0.23, and 0.34 L/g COD_{in}.
- Siciliano et al. (2019) investigated the production of biogas through the anaerobic treatment of composting plant leachate with COD of 65000 mg/L in semi-continuous CSTR. During the reactor's beginning phase, it was operated at an OLR of 1-2 g/(L.d) with a HRT of 66 to 33 days, respectively. While for the operational period, OLR ranged for HRT of 15.5 to 1.8 days with an OLR of 4.25 to 38.5 g COD/(L.d). 91% of the COD was removed from OLRs up to 14.5 g COD/(L.d), and 80% was removed from an OLR of 24.5 g COD/. (L.d). Methane production yield of 0.34–0.38 L /g $COD_{removed.}$ were achieved.
- Research on the anaerobic treatment of landfill leachate utilizing anaerobic digester was done by Kheradmand et al. (2010). Activated sludge units operating at ambient temperature and two-stage lab scale reactors operating at 32°C were utilised to treat landfill leachate with an oxygen demand of 975-50534 mg/L. OLR was changed from 0.065 to 3.369 g COD/(L.d). At an OLR of 2.247 g COD/(L.d), maximum COD removal of 73%, 69%, 92%, 30%, and 94% was reached for the first and second digesters combined, the activated sludge unit, and the entire system,

respectively. The methane generation rates for the first digester, second digester, and the combination of the two digesters were 0.03-0.05, 0.05-0.08, and 0.03-0.05 L/g COD_{rem}, respectively.

- Govahi et al. (2012) used a UASB and an aerated lagoon with a COD concentration of 1000–40,000 mg/L to study the treatment of landfill leachate. For a total of 160 days, OLR adjustments of 0.5 g COD/(L.d) to 20 g COD/(L.d) were made. COD elimination increased from 59% to 87% as the OLR was increased from 1 g/(L.d) to 7.1 g/(L.d). The effluent from the second (CSTR) reactor had COD reductions ranging from 35 to 70%. The COD elimination efficiency for the entire system varied from 66 to 94%. The greatest COD removal of 71% was achieved at an OLR of 6 g/L/day for the UASB reactor, 75% for the aerated lagoon, and 94% for the overall system. Biogas produced at an OLR at a rate of 22.5 L/day.
- Ye et al. (2011) used a UASB reactor in a mesophilic environment to treat fresh leachate from incinerator that had calcium having COD in the range of 70,390-75,480 mg/L. 4000 mg/L COD was added in UASB under an HRT of 3 days. After 12 days of use, the COD elimination effectiveness climbed to 93%. Maximum of 82 % COD removal was achieved under an OLR of 12.5 kg COD/(L.d) with run time of 170 days. 88.6% of the COD removed was converted to methane under an OLR of 12 g COD/L, yielding biogas of 0.34 L/ g $COD_{removed}$
- Nayono at al. (2010) studied the anaerobic breakdown of leachate that was squeezed out of municipal solid waste's organic component. "Press water" was used to feed an anaerobic digester that was maintained at a mesophilic temperature for five months. OLR was gradually increased to 27.7 g COD/(L.d) from 5.9 g VS/(L.d) to 15.3 g VS/(L.d) during HRT of 7.7 days. A maximum value of 81.5 L/(L.d) of biogas generation was attained at an OLR of 10.7 g COD/L d. At an OLR of 17 g COD/(L.d), COD elimination was 70%; at an OLR of >25 g COD/, it was 60%. (L.d)
- HE et al. (2009) studied the ability of the upflow blanket filter reactor for treating fresh leachate from incinerator with COD contents varying from 3130 to 63100 mg/L. For HRT of 9 days to 3.6 days, the reactors were subjected to a range of OLRs from 0.51 to 14.56 g COD/(L.d). At an OLR of 2.55 g COD/(L.d) and a methane production of 0.2 L/(L.d) with a maximum of 90% of the COD removal was achieved.

- Zayen et al. (2010) worked an anaerobic membrane bioreactor for treating landfill leachate. By changing the feed's COD from 14000 mg/L COD to 41000 mg/L, the AnMBR's OLR was uniformly enhanced from 2.24 g COD/(L.d) to 6.27 g COD/(L.d). A maximum biogas output of 0.45 L/g $COD_{removed}$ was achieved with 92% COD removal at an OLR of 2.24 g COD/(L.d).

Summary

Discussed literature proves that leachate treatment along with biogas production through anaerobic treatment is a viable option to sustainably decrease the impacts of municipal solid waste leachate. Apart from various types of biological treatments methods, anaerobic CSTR have proven to be comparatively economic and environmental friendly due to its simple configuration, cheaper fabrication, easier cleaning and maintenance activity, efficient stirring function and excellent treatment efficiency. Besides that, biogas has recognized as renewable source of energy which hampers dependence on conventional fuels and greenhouse emissions.

Chapter 3

Materials and Methods

This chapter is focused on the materials and methods followed throughout the study which has been conducted in Environmental Chemistry Teaching Lab, IESE-NUST. The section includes

1. Collection as well as characterization of substrate and inoculum
2. Design of experimental setup
3. Operation of experimental setup
4. Analysis of various analytical parameters

Experimental work has been summarized as shown in Figure 3.1

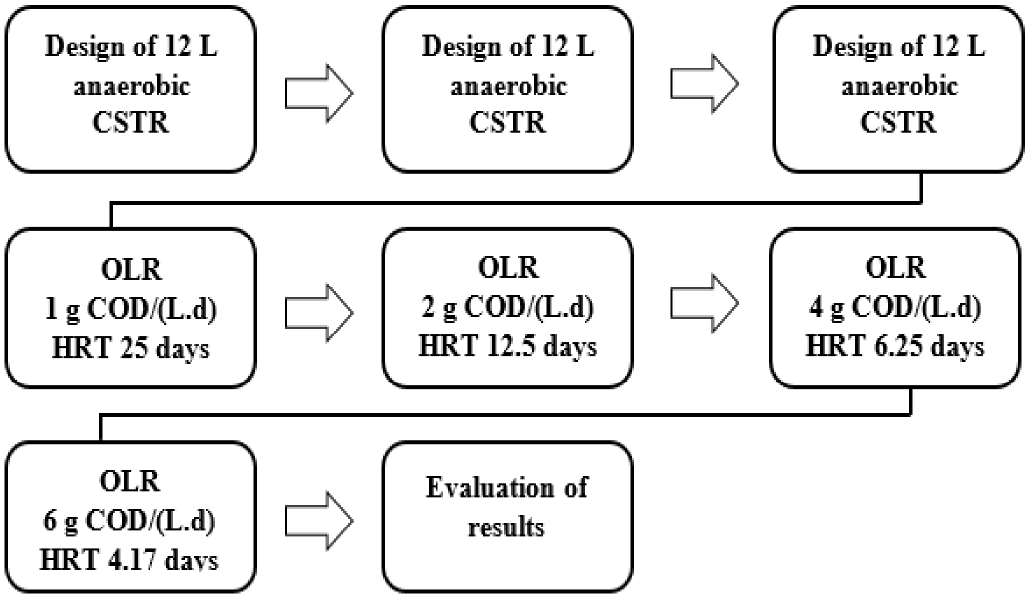

Figure 3.1 Methodology of experimental work carried out during the operation

3.1 Substrate & inoculum

3.1.1 Substrate

Fresh municipal solid waste (MSW) Leachate was obtained from the transfer station of Albyrak Workshop located near Liaqat Bagh Rawalpindi. The leachate was firstly settled for 24 hours and then it was sieved with a mesh size of 2mm. After its characterization, synthetic MSW leachate of 25,000 mg/L COD was prepared at the lab. The composition of synthetic MSW leachate (Vo et al., 2016) is as followed.

Table 3.1 Recipe of synthetic MSW leachate

Sr. No	Chemicals	Unit	Value
1	Sodium Chloride (NaCl)	mg/L	4683
2	Sodium hydrogen Carbonate ($NaHCO_3$)	mg/L	8436
3	Sodium Nitrate ($NaNO_3$)	mg/L	887
4	Potassium Hydrogen Carbonate ($KHCO_3$)	mg/L	21720
5	Ammonium Hydrogen Carbonate (NH_4HCO_3)	mg/L	907
6	Ammonium Sulphate $(NH_4)_2SO_4$	mg/L	173
7	Magnesium Sulphate ($MgSO_4$)	mg/L	20560
8	Calcium Chloride ($CaCl_2$)	mg/L	7259
9	Ferrous Tetrachloride ($FeCl_4$)	mg/L	93
10	Dextrose ($C_6H_{12}O_6$)	mg/L	25000
11	Dipotassium Phosphate (K_2HPO_4)	mg/L	203

Source: (Vo et al., 2016)

Dextrose ($C_6H_{12}O_6$) was added as a substrate for microbes. Magnesium sulfate ($MgSO_4$) and dipotassium phosphate (K_2HPO_4) were added as macro-nutrients. Calcium chloride ($CaCl_2$), and ferrous tetrachloride ($FeCl_4$) were added to serve as micro-nutrients. To maintain the pH of feed between 7.0-7.5, sodium hydrogen carbonate ($NaHCO_3$) was used.

3.1.2 Inoculum

The effluent of biogas plant from National Cleaner Production Centre (NCPC) Rawalpindi was used as inoculum. Fresh cow dung that was taken from cattle farm located in Sector H-13 of Islamabad was also added as second inoculum. Both of inocula were characterized and added in anaerobic CSTR with 50% ratio on basis of volatile solids (VS). TS & VS of the inoculum were measured and used for this purpose.

3.2 Design of experimental setup

In order to treat municipal solid waste leachate, an CSTR system at mesophilic temperature of 37°C was used.

The lab scale anaerobic CSTR having dimensions of 20 cm (length) x20 cm (width) x30 cm (height) is shown in Figure 3.2. It is made of acrylic with three influent as well as effluent ports. The volume of anaerobic CSTR was designed to be 12 liters consisting of 9 liters of working volume.

The temperature of anaerobic CSTR was raised with the help of a heater installed inside of the reactor which was maintained by a digital temperature controller device. A temperature sensor was provided for checking the current temperature inside the reactor. A mechanical mixer was provided for slow mixing for interaction of inoculum with leachate. The mixing speed was controlled by a mixer timer.

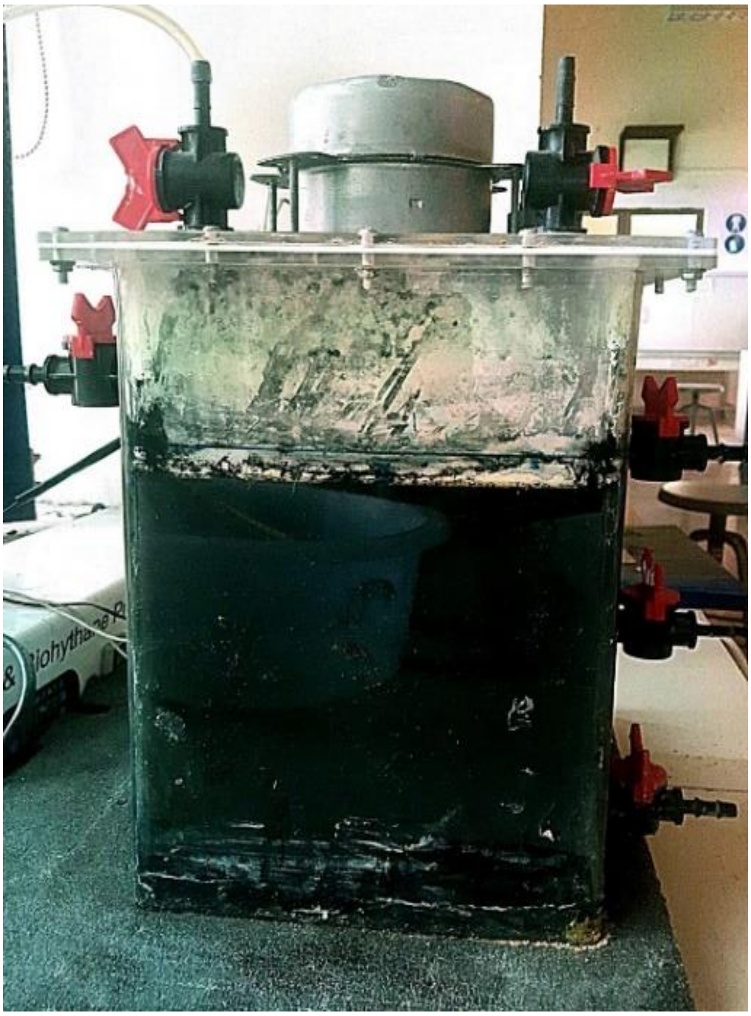

Figure 3.2 Diagram of anaerobic CSTR

The experimental setup is shown in Figure 3.3 starts with a leachate tank having COD of 25,000 mg/L. Feed was added to the anaerobic CSTR with the help of a feed pump (Model: Lead Fluid BQ80S). Anaerobic CSTR was run under various organic loading rates (OLR) and effluent was withdrawn for checking different monitoring and operational parameters to check the performance of the system and biogas was measured with the water displacement method.

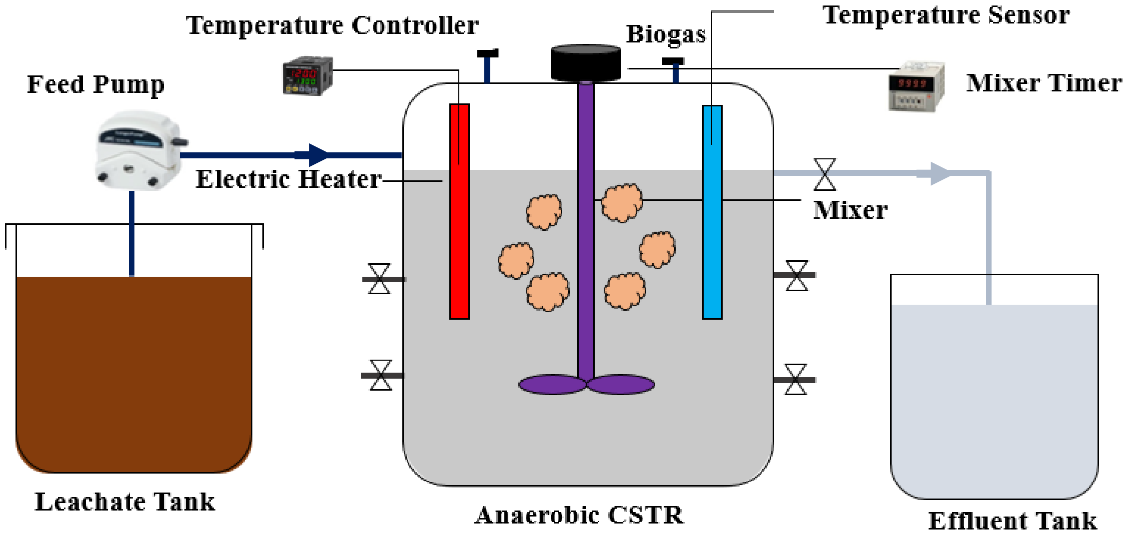

Figure 3.3 Schematic diagram of experimental setup

3.3 Operation of experimental setup

3.3.1 Startup phase operation

For the batch experiment, anaerobic CSTR was fed with 25000 mg/L leachate along with mixed inoculum in 1:2 on volatile solids (VS) basis. Nitrogen gas was flushed in anaerobic CSTR for providing anaerobic conditions. The inoculum was acclimatized by rising 0.5°C day from ambient conditions of 25°C temperature till attainment of the mesophilic temperature of 37°C. pH adjustment was performed manually by 0.02 N NaOH when it dropped to value of below 6.90.

Slow mixing for 1 minute after 3 minutes of off-time through mixer timer with speed of 20 revolutions per minute (RPM) was provided with an impeller installed inside of the reactor. Biogas volume was measured with the water displacement method and effluent was withdrawn to analyze various parameters of pH, VFA/Alkalinity, ammonia nitrogen, oxidation reduction potential (ORP) and effluent COD. The reactor was operated for 37 days till 75% COD removal was obtained (Feng et al., 2021b). After that, the reactor got acclimatized and shifted towards the continuous operation.

3.3.2 Continuous operation

After the acclimatization phase, anaerobic system was operated under various organic loading rates (OLR) in two different stages as shown in Figure 3.4. It includes working organic loading rate (OLR) of 1 g COD/(L.d), 2 g COD/(L.d), 4 g COD/(L.d) and 6 g COD/(L.d) and transition stage of 3 g COD/L.d and 5 g COD/L.d organic loading rate (OLR). Transition stages are provided in order to avoid instability of system due to direct shifting from one to higher working organic loading rates.

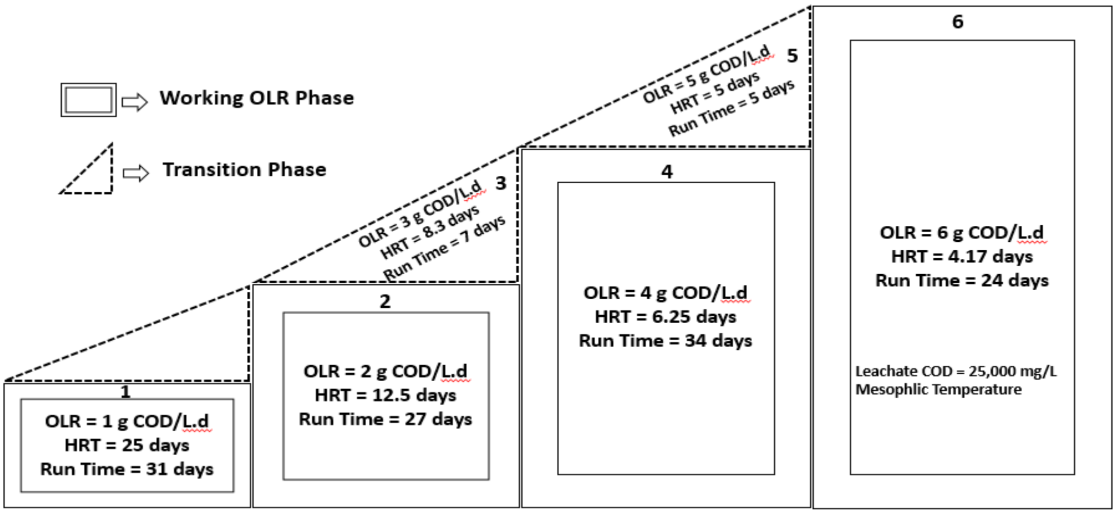

Figure 3.4 Operation of anaerobic CSTR in continuous phase

The HRT for working OLR were 25 days, 12.5 days, 6.25 days and 4.17 days for 1 g COD/(L.d), 2 g COD/(L.d), 4 g COD/(L.d) and 6 g COD/(L.d) respectively. Similarly, HRT for transitional organic loading rate were 8.3 days and 5 days for of 3 g COD/(L.d) and 5 g COD/(L.d) organic loading rate respectively.

For working OLR, the system was run for a period of at least of two times of respective HRT's (Siciliano et al., 2019). Slow mixing for 1 minute after 3 minutes of off-time through mixer timer with speed of 20 revolutions per minute (RPM) was provided with an impeller installed inside of the reactor. Biogas volume was measured with the water displacement method.

Effluent was withdrawn to analyze various parameters of pH, VFA/Alkalinity, ammonia nitrogen, oxidation reduction potential (ORP) and effluent COD to check the performance of anaerobic CSTR. The reactor was operated for 134 days at temperature of 37°C.

3.4 Analysis of various analytical parameters

Table 3.2 shows various analytical parameters along with units and their standards methods.

Effluent was withdrawn on daily basis for analyzing pH and oxidation reduction potential (ORP) while VFA/Alkalinity, ammonia nitrogen and COD of effluent sample were measured thrice a week. Biogas volume was measured on daily basis with the water displacement method.

Table 3.2 Analytical parameters and standard methods

Sr. No	Parameter	Unit	Instrument	Standard Methods (Reference)
1	Total Solids	%	Oven Memmert (Model: 100-800)	2540-G (APHA, 2017)
2	Volatile Solids	% of TS	Muffle Furnace (Model: 270 H) JS Korea	2540-G (APHA, 2017)
3	pH	--	pH Meter with ORP Electrode (Model: pH-700) EUTECH Instruments	Electrode Method
4	Oxidation Reduction Potential	mV	pH Meter with ORP Electrode (Model: pH-700) EUTECH Instruments	Electrode Method
5	Chemical Oxygen Demand	mg/L	COD Digester (Model: 45600) HACH	5220-C (APHA, 2017)
6	Ammonia Nitrogen	mg/L	TKN Apparatus (UDK 129) Velp Scientifica	4500-B (APHA, 2017)
7	Volatile Fatty Acids	mg/L	Centrifuge (Model:Z 206A) HERMLE	2310 B Titration Methods (APHA, 2017)
8	Alkalinity	mg/L	Centrifuge (Z 206A) HERMLE	2320 B Titration Methods (APHA, 2017)
9	Volume of Biogas	mL	--	Water Displacement Method

3.4.1 Total solids (TS)

The gravimetric method was used to determine the total solids. TS is a term referred to the residues left in the dishes after 24 hours of drying at 105°C, calculated by putting the values in the following formula:

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

Where,

A = weight of dried out residue in china dish + china dish after 24 hours at 105°C (g)

B = weight of china dish (g)

C = sample size (g)

3.4.2 Volatile solids (VS)

The pre-dried sample used for the determination of total solids was weighed (A) and ignited at 550° C in a muffle furnace for 30 minutes. The crucible was then transferred to a desiccator for half an hour. Then it was weighed (D) and the acquired values were used to calculate the volatile solids in the sample as

$$\text{Volatile Solids (\%)} = \frac{A - B}{A - C} \times 100$$

Where,

A = weight of dried out residue in china dish + china dish after 24 hours at 105°(g)

B= weight of china dish after ignition at 550°C (g)

C = weight of china dish (g)

D = sample size (g)

3.4.3 Chemical oxygen demand (COD)

COD was determined by using closed reflux titrimetric method. COD vial was prepared by adding 2.5 ml volume of sample, 1.5 ml of 0.02 M $K_2Cr_2O_7$ and 3.5 ml volume of H_2SO_4 reagent. Then, for the purpose of digestion, these vials were placed in a preheated COD digester for a period of two hours at a temperature of 1500 °C. Vials were allowed to cool to room temperature after digestion. Using a ferroin indicator, the vials were titrated against 0.25 M ferrous ammonium sulphate (FAS) until the end point, or yellow to brown, was reached. The following equation was used for calculating the COD of each sample:

$$\text{COD (mg/L)} = \frac{(B-A) x Normaity\ of\ Titrantx\ Equivalent\ Weight\ x1000}{Sample\ Size\ (ml)}$$

Where,

A = Volume of titrant used for titration of sample (ml)

B = Volume of titrant used for titration of Blank (ml)

N = Normality of titarnt

3.4.4 Ammonia-Nitrogen

Ammonia-Nitrogen was determined by firstly distilling the sample using the distillation unit of TKN apparatus and then titration of sample with sulphuric acid. Following formula is used to compute the Ammonia-Nitrogen of effluent sample.

$$\text{Ammonia-Nitrogen (mg/L)} = \frac{(A-B) x Normaity\ of\ Titrantx\ Equivalent\ Weight\ x1000}{Sample\ Size\ (ml)}$$

3.4.5 Alkalinity

- 10 ml sample from anaerobic CSTR was collected and its pH was measured by using a pH meter.
- Samples with pH above 6.5 were titrated with 0.02 N H_2SO_4 and the pH was adjusted to 6.5, then it was further titrated with H_2SO_4 until the pH reached to a value of 3.5
- Volume of acid consumed was noted and alkalinity of the effluent was calculated as:

$$\text{Alkalinity (mg/L)} = \frac{(\text{Volume of acid used }) x Normaity\ of\ Acidx\ Equivalent\ Weight\ x\ 1000}{Sample\ Size\ (ml)}$$

3.4.6 Volatile fatty acids (VFA's)

- For VFA quantification, the samples used for alkalinity determination were heated on the hot plate by placing the beakers on temperature around 80-100 °C for 15 minutes.
- Then, it was cooled down at room temperature and titrated against 0.02 N NaOH, to bring back the pH up to 6.5.
- Volume of NaOH was noted and following equation was used to measure the VFA concentration of effluent.

$$\text{Volatile Fatty Acids (mg/L)} = \frac{(\text{Volume of base used }) x Normaity\ of\ Basex\ Equivalent\ Weight\ x\ 1000}{Sample\ Size\ (ml)}$$

Chapter 4

Results and Discussion

This chapter is classified into three parts. 1st part includes results obtained during the characterization of leachate and inoculum, 2nd part comprising of results during operation of batch phase operation of mesophilic anaerobic completely stirred-tank reactor (CSTR), and 3rd part covers the results when anaerobic CSTR was run in continuous phase under varying organic loading rates (OLR's).

4.1 Characteristics of leachate and inoculum

Table 4.1 shows characteristics of various parameters of leachate, cow dung and biogas plant effluent. pH of synthetic leachate, cow dung and biogas plant was found to be 7.50, 7.10 and 7.46 respectively.

Table 4.1 Characteristics of leachate and inoculum

Parameters	**Syn. MSW Leachate (Substrate)**	**Cow Dung (Inoculum)**	**Biogas Plant Effluent (Inoculum)**
pH	7.50	7.10	7.46
Total Solids (%)	7.73	18	1.03
Volatile Solids (% of TS)	23	76	0.50
Chemical Oxygen Demand (mg/L)	25,000	ND	ND
Total Kjeldahl Nitrogen (mg/L)	1497	ND	ND
Ammonia-Nitrogen (mg/L)	226	ND	ND
Oxidation Reduction Potential (mV)	351	-173	-257

ND, not determined

Cow dung has comparatively higher volatile solids than substrate. Maximum TS and VS content of raw cow-dung were 18% and 76%, respectively. The COD of substrate i.e. municipal solid waste leachate found to be 25,000 mg/L. Nandi et al. (2020) used cow dung as inoculum for anaerobic digestion and found that pH, TS and VS were 7.08, 17.30% and 14.51% and respectively. Momayez et al. (2018) used biogas plant effluent for biogas production and found pH of 8.3, TS of 4% and VS of 2.3%.

4.2 Batch phase of the mesophilic anaerobic completely stirred-tank reactor (CSTR)

4.2.1 pH and oxidation reduction potential (ORP)

As shown in figure 4.1, trend for pH of anaerobic CSTR started from 7.54 at 1st day to 7.30 till 30th day. Initially the pH of reactor was not controlled during batch phase. Minimum pH of 6.92 was observed at 31st day indicating the maximum production of volatile fatty acids. After that, pH of anaerobic CSTR was adjusted to 7.12 by adding 4 drops of 0.02 N NaOH after which the reactor showed stable trend till the end of batch phase. Anaerobic processes are tolerant to pH values between 6.5 and 8.0 however pH less than 6.7, the metabolic rate of methanogenic bacteria remains ceased (Laiq Ur et al., 2019).

At start of batch phase, nitrogen gas was purged into the anaerobic CSTR for providing anaerobic condition. -403 mV of oxidation reduction potential (ORP) was observed at 1st day with the slight decreasing trend till 15th day. Anaerobic CSTR fluctuated between -384 mV to -523 mV showing that ORP value of anaerobic reactor decrease

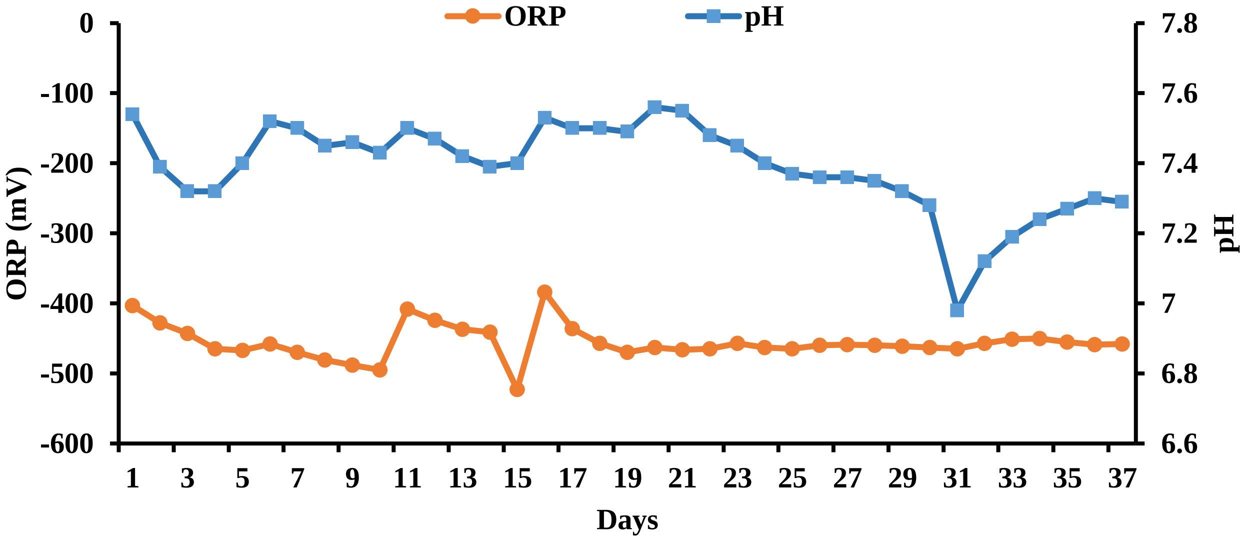

Figure 4.1 Variation of pH and ORP during batch phase

with increase in HRT. An increase in volatile fatty acids productions might be the possible reason for more reduced condition in anaerobic reactor. Papirio et al. (2022) found that ORP less than -300 mV is most suitable for the methane-producing bacteria (MPB) because methanogens require an extremely reducing environment for production of methane production.

4.2.2 Ammonia-Nitrogen (NH_3-N) & VFA/Alkalinity ratio

Both ammonia-nitrogen and VFA/Alkalinity ratio were measured from 11th day and onward as shown in Figure 4.2. At start of batch phase, ammonia-nitrogen in anaerobic CSTR was found to be 49 mg/L which increased to 57 mg/L till 15th day. This is due to degradation of nitrogenous compounds present in inoculum particularly in cow dung.

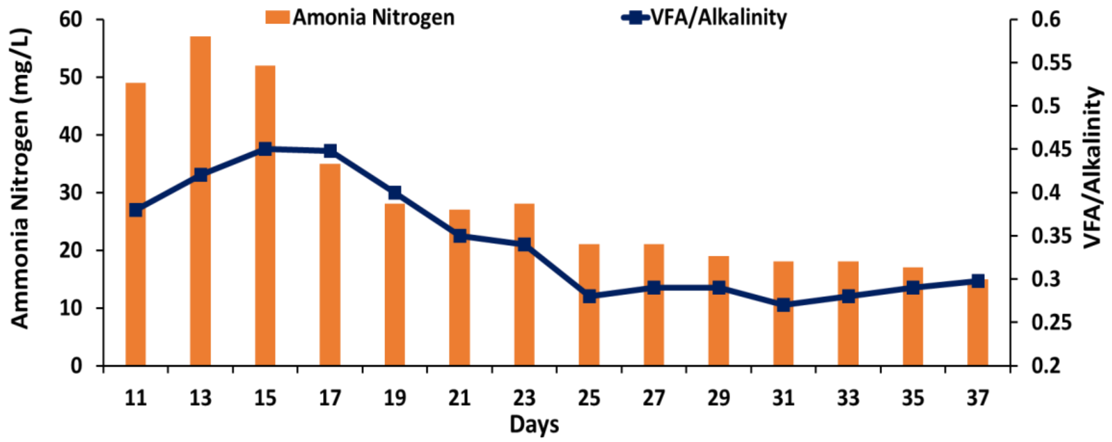

Figure 4.2 Variation of ammonia-nitrogen & VFA/Alkalinity ratio of during batch phase

From 15th day to 37th day, 71% decrease in ammonia was found. It remained within range of 20-27 mg/L from 17th to 37th day which were well below concentrations leading to cause inhibitory effects. However small amount of NH_3-N is constantly consumed by microorganisms as nutrient (Babson et al., 2013). It was reported that NH_3-N of more than 600 mg/L concentrations is causing significant inhibition of methanogenic activity (Jo et al., 2018).

At start, more organic matter was present in anaerobic CSTR. VFA/Alkalinity ratio of 0.38 was found at 11th day. Due to anaerobic treatment, volatile fatty acids produced with the passage of time thereby increasing VFA/alkalinity ratio to 0.46 at 17th day. Microorganism being living things took some time for acclimatization, so as they acclimatized, they start consuming volatile fatty acids thereby decreasing VFA/alkalinity ratio to 0.28 at 25th day. After that a stable trend of VFA/alkalinity of 0.28-0.29 was observed from 25th day to 37th day. Wang e al. (2021) found that VFA/Alkalinity ratio between 0.29-0.31 is most suitable for smooth operation of anaerobic treatment.

4.2.3 Effluent chemical oxygen demand (COD) & COD removal

Fig. 4.3 shows the performance of anaerobic CSTR for effluent COD and COD removal of anaerobic CSTR during batch phase. COD variation is important in order to assess microorganism activities toward environmental changes during acclimatization phase.

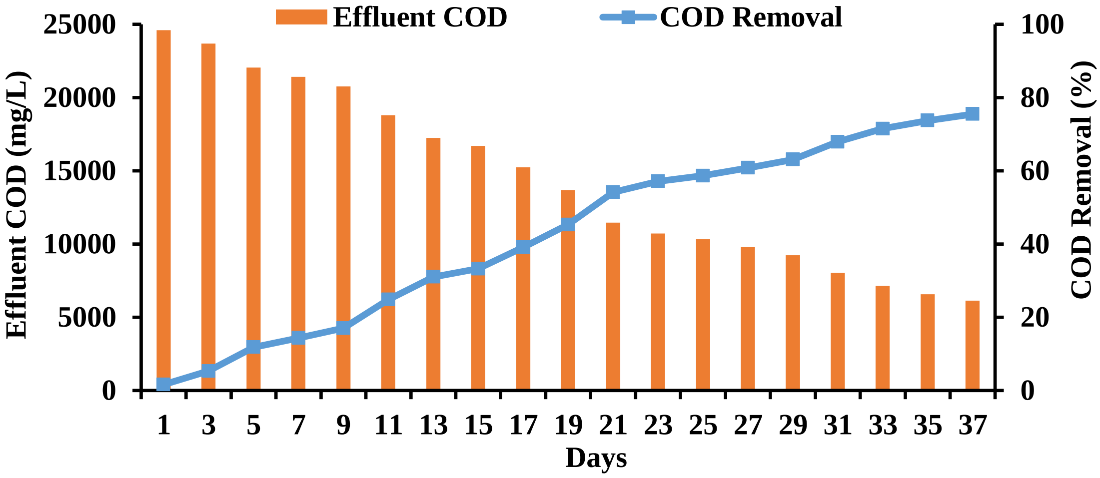

Figure 4.3 Variation of effluent COD & COD removal of during batch phase

At the initial stage of batch operation, substrate and nutrients were present in excess. COD concentration was decreased from initial value of 25000 mg/L to 21000 mg/L till 9th day, a phase of low COD degradation (lag phase) followed by COD degradation from 11th day to 29th day (exponential phase). As no significant difference in terms of COD reductions was observed after 35th day so it was decided to stop batch phase operation after 37th day. Overall a decreasing trend for effluent COD observed from 1st day to 37th day of anaerobic CSTR showing most of organic matter of leachate is consumed by microorganism as their feed.

Similarly, COD removal at start of batch phase operation was less because microorganisms just began to adopt to leachate. Till 17th day, COD removal was less than 40%. Anaerobic inoculum got acclimatized thus degrading leachate COD with maximum removal of 76% after which the system was shifted from batch to continuous mode of operation. Batch mode operation was shifted to continuous phase when 75% of COD removal was achieved for time duration of 40 days (Feng et al., 2021b).

4.2.4 Biogas production and cumulative biogas production

Figure 4.4 shows biogas production of anaerobic CSTR during batch phase operation. From 3rd day, biogas production started. An increasing trend for biogas production rate was observed from 3rd day to 17th day giving 1st peak of 36.53 mL/g COD at 15th day.

At start more organic matter as food was present in anaerobic CSTR for microorganisms thereby converting degradable matter into biogas. Daily biogas production was lesser in the start possibly due to undesirable environment i.e. high VFA/alkalinity ratio and un-acclimatized condition of anaerobic CSTR. (Srisowmeya et al., 2021). Though, biogas generation rate initiated to progress gradually as the system advanced to constancy as pH and alkalinity enhanced.

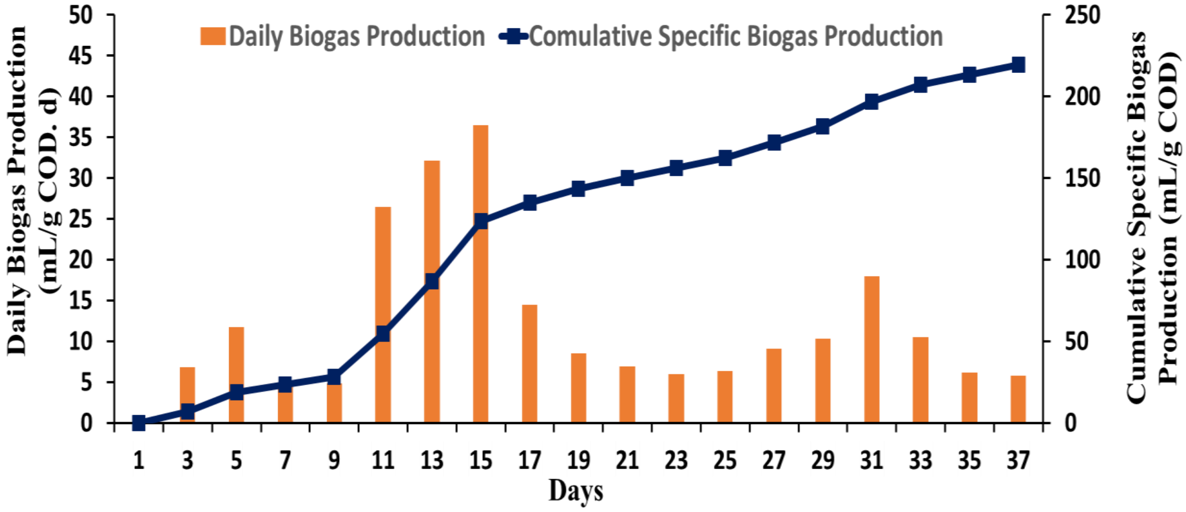

Figure 4.4 Variation of biogas production during batch phase

2nd peak of biogas production rate was observed at 31st day. This is possible due to the enhanced metabolic activity of microorganisms during exponential phase of bacterial growth. Also, the inoculum was acclimatized and microbes were working at their best. After that, less biogas production rate was observed till 37th day as most of substrate was used up by microorganisms.

An increasing trend for cumulative biogas production rate was observed from start to end of batch phase. Cumulative biogas production was exponentially increased from 28 mL/g COD at 9th day to 135 mL/g COD at 17th day. After that a relatively stable trend for cumulative biogas production rate was observed. Overall, 219 mL/g COD of cumulative biogas production rate was measured till the end of acclimatization stage.

4.3 Results summary for batch phase

Table 4.2 shows an overall performance of anaerobic CSTR during batch phase was observed as all the stability parameters were in suitable range of anaerobic treatment. The pH range of the anaerobic microorganisms' responses in the reactor varies from 5.0 to 7.5. However, methanogenesis occurs in the neutral range, or between 7.00 and 7.40 (Ye et al., 2013). Anaerobic CSTR's batch phase had an average oxidation reduction potential of -455 mV, making it the most appropriate for anaerobic processes (Papirio e al., 2022).

Table 4.2 Results for batch phase operation of anaerobic CSTR

Sr. No	Parameter	Unit	Value
1	pH	--	7.38
2	Oxidation Reduction Potential	mV	-455
3	VFA/Alkalinity	--	0.32
4	Ammonia	mg/L	28.92
5	Effluent COD	mg/L	6134
6	COD Removal	%	76
7	Cumulative specific biogas production	mL/g COD	219.28

The ratio of VFA/Alkalinity for anaerobic CSTR was within optimum range i.e. less than 0.5 (Wang et al., 2016). COD removal of 76% was obtained after which the system was shifted from batch to continuous mode of operation (Feng et al., 2021b).

4.4 Effect of organic loading rate on various parameters during continuous phase

4.4.1 pH and oxidation reduction potential (ORP)

Figure 4.4 shows pH and oxidation reduction potential (ORP) of anaerobic CSTR under varying conditions of organic loading rate.

Continuous operation of anaerobic CSTR started from 39^{th} day under OLR of 1 g COD/(L.d). pH of anaerobic CSTR was 7.26 at 39^{th} day which increased to 7.65 at 55^{th} day. Due to leakage issues, continuous operation was stopped at 57^{th} day, due to which production of fatty acids production enhanced thereby dropping pH from 7.65 to 6.92 under OLR of 1 g COD/(L.d). However, anaerobic CSTR stabilized itself till 59^{th} day when continuous operation again started after fixing leakage issue.

As the OLR was increased to 2 g COD/(L.d), system effluent shifted from pH 7.45 to 8.20. A stable pH trend was observed in the range of 7.52-7.59 from 122^{th} day to 171^{th} day under an OLR of 4-6 g COD/(L.d).

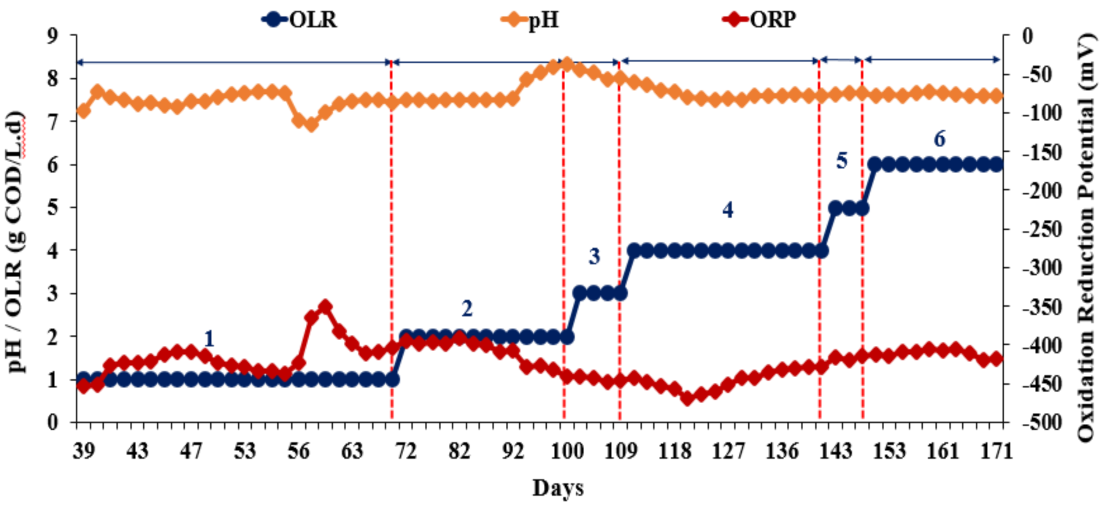

Figure 4.4 pH & ORP of anaerobic CSTR during continuous phase

The averaged pH of 7.47, 7.58, 7.63 and 7.62 was achieved for 1 g COD/(L.d), 2 g COD/(L.d), 4 g COD/(L.d) and 6 g COD/(L.d) OLR respectively. pH lower than 6.5 are considered unsuitable for methanogenic archaea. The anaerobic process can handle pH values between 6.5 and 8.0, however 6.8 to 7.2 is the ideal range for the anaerobic reactor (Shahzad et al., 2020).

ORP of -453 mV to -423 mV was measured from 39th to 56th day under OLR of 1 g COD/(L.d). On 57th day, owing to aerobic condition due to leakage issue, system ORP increased to -351mV. However, the ORP of effluent was range of anaerobic condition. Average ORP of -408, -445 and -411 mV were observed under organic loading rate (OLR) of 2 g COD/(L.d), 4 g COD/(L.d) and 6 g COD/(L.d) which are most suitable for the anaerobic process (Papirio e al., 2022). Martin et al. (2018) reported ORP in range of -310 mV to -384 mV in AnMBR for treating synthetic wastewater.

4.4.2 VFA/Alkalinity ratio at various organic loading rates

Figure 4.5 shows VFA/Alkalinity ratio of anaerobic CSTR under different organic loading rates. VFA/Alkalinity is considered as first warning parameter for digester failure. At start, VFA/Alkalinity ratio of 0.33 was found under OLR of 1 g COD/(L.d). Volatile fatty acids start increasing that were consumed by microorganisms thereby reducing VFA/alkalinity ratio to 0.27 till 55th day. After that, a sudden increase was observed due to leakage issue found at 57th day. As no substrate was added during that time, so VFA/Alkalinity ratio got increased to 0.57. When the leakage issue was fixed and reactor feeding started, it showed a decrease in VFA/ alkalinity ratio to 0.48 at 70th day.

For each run of anaerobic CSTR under OLR of 2, 4 and 6 g COD/(L.d), firstly an increase in VFA/alkalinity ratio was observed followed by a decreasing trend (Wang e al., 2021). This is due to reason that microbes usually took some time to adjust new condition of OLR, as they acclimatized for that particular condition, they started to consume VFA's thus decreasing VFA/alkalinity ratio. An average VFA/Alkalinity of 0.39, 0.45, 0.46 and 0.30 were measured under working OLR of 1, 2, 4 and 6 g COD/(L.d)

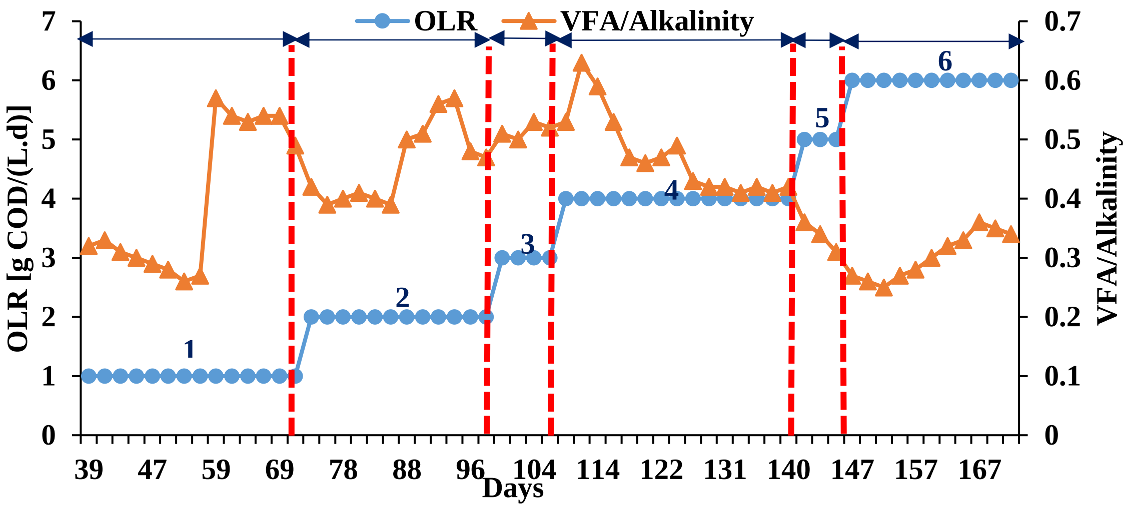

Figure 4.5 VFA/Alkalinity of anaerobic CSTR during continuous phase

A VFA/Alkalinity ratio of higher than 0.50 resulted risk of acidification or reactor failure (Aramrueang et al., 2016).

4.4.3 Ammonia-Nitrogen at different organic loading rates

Figure 4.6 shows variation in ammonia nitrogen under different organic loading rates. Under OLR of 1 g COD/(L.d), 29 mg/L of ammonia nitrogen was obtained in effluent sample till 49th day. It abruptly increased from 29 mg/L to 74 mg/L during 59th day possibly due to unstable condition during leakage issue found in the reactor.

A decreasing trend of ammonia nitrogen from 65 mg/L at 70th to 41 mg/L at 86th day After that a stable value in range of 37-30 mg/L till end of 6 g COD/(L.d). Small amount of NH_3-N is constantly consumed by bacteria as nutrient (Babson et al., 2013). Average of 48 mg/L, 46 mg/L, 35 mg/L and 30 mg/L ammonia nitrogen was measured during organic loading rate (OLR) of 1g COD/(L.d), 2g COD/(L.d), 4g COD/(L.d) and 6g COD/(L.d) respectively.

Average of 29 mg/L ammonia nitrogen was observed for higher OLR's. Even with greater OLRs, ammonia-nitrogen levels in the reactor did not dramatically change and remained relatively in a stable range of 38-29 mg/L throughout the experimental period which were well below the concentration at which ammonia inhibits the anaerobic process.

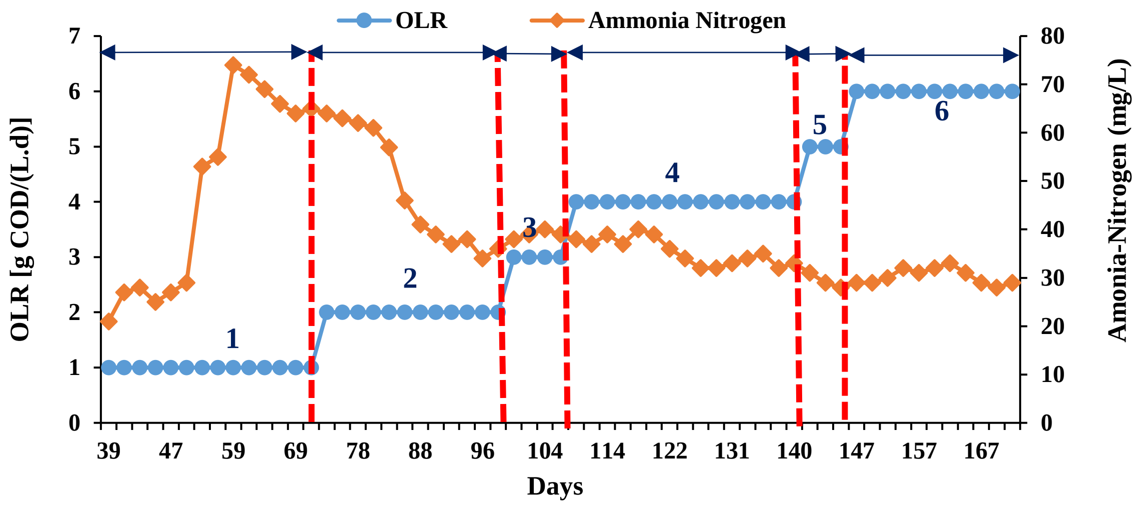

Figure 4.6 Ammonia-Nitrogen of anaerobic CSTR during continuous phase

It was reported that NH_3-N of more than 600 mg/L concentrations is causing significant inhibition of methanogenic activity (Jo at al., 2018).

4.4.4 Effect of organic loading rate on effluent COD

Figure 4.7 shows an effluent COD under varying OLR's. At start of OLR of 1 g COD/(L.d), low effluent COD of 4000 mg/L was found in effluent sample. A little bit increase in effluent COD was observed till 55th day as microbes being living things take time to adjust themselves in new condition of organic loading rate. After that, a sudden increase in effluent COD at 59th day because of leakage issue found at 57th day corresponding to decrease in temperature and unstable anaerobic condition. Temperature is major factor that affects biological treatment. As leakage issue was fixed and reactor feeding stated, reactor showed a decrease in COD of effluent sample. For each run of anaerobic CSTR under OLR of 2, 4 and 6 g COD/(L.d), firstly an increase in effluent COD was observed followed by a decreasing trend. This is due to reason that microbes usually take some time to acclimatize in new condition of organic loading rate, as they adjust themselves for particular loading rate, they started to consume COD thereby decreasing effluent COD in the sample (Musa et al., 2018).

Continuous operation was ended with effluent COD of 14000 mg/L at 170th day at an OLR of 6 g COD/(L.d)

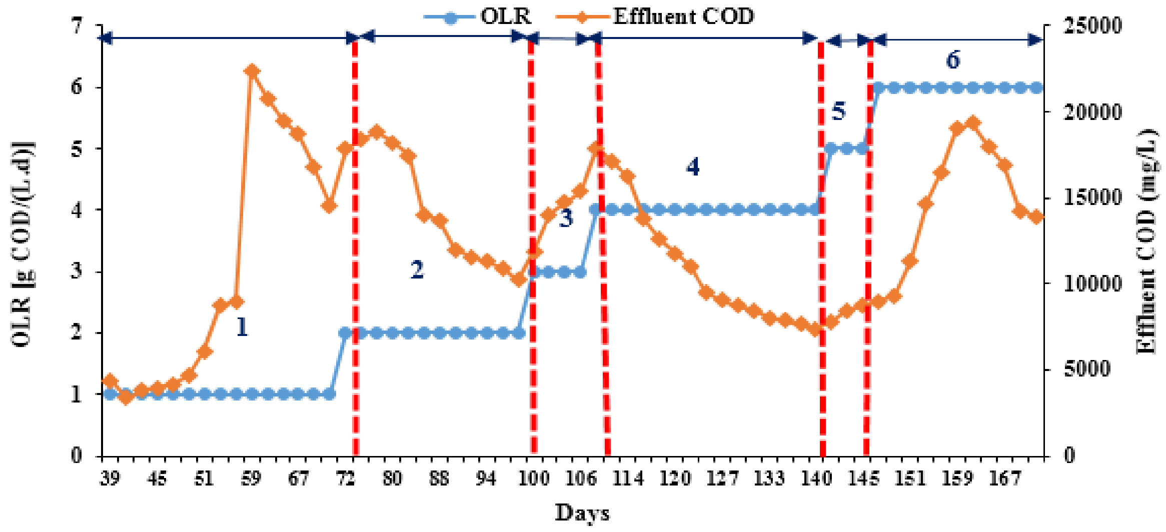

Figure 4.7 Effluent COD of anaerobic CSTR during continuous phase

4.4.5 COD removal at various organic loading rates

Figure 4.8 shows COD removal of anaerobic CSTR under varying organic loading rates during continuous phase operation. COD is a crucial operational criterion for evaluating the effectiveness of biological treatment.

At start of 1 g COD/(L.d) OLR, maximum of 82% COD removal was obtained which decreased to 64% at 55th day as microbes were trying to adjust new condition of organic loading rate. A sudden decrease of 11% in COD removal was found at 59th day owing to leakage issue found during 57th day due to provision of uncontrolled temperature and unstable anaerobic condition. Temperature is major factor that affects biological treatment (Nie et al., 2021). When the continuous operation again started after fixing the reactor's issue, it showed an increase in COD removal. OLR of 4 g COD/(L.d) was considered as optimal at which an overall of 56% COD removal was observed with maximum of 70% at 138th day.

For each run of anaerobic CSTR under OLR of 2, 4 and 6 g COD/(L.d), firstly a decrease in COD removal was observed followed by an increasing trend (Musa et al., 2018). This is due to reason that microorganism took some time to adjust themselves in new condition of organic loading rate. Once they were able to absorb that organic loading rate, proper degradation of organic matter was started thereby increasing COD removal (Thiyagu and Sivarajan, 2018).

Overall, maximum COD removal of 60%, 70% and 54% during organic loading rate (OLR) of 2 g COD/(L.d), 4 g COD/(L.d) and 6 g COD/(L.d) respectively. Continuous operation was ended with COD removal of 44% at 170th day under an OLR of 8 g COD/(L.d).

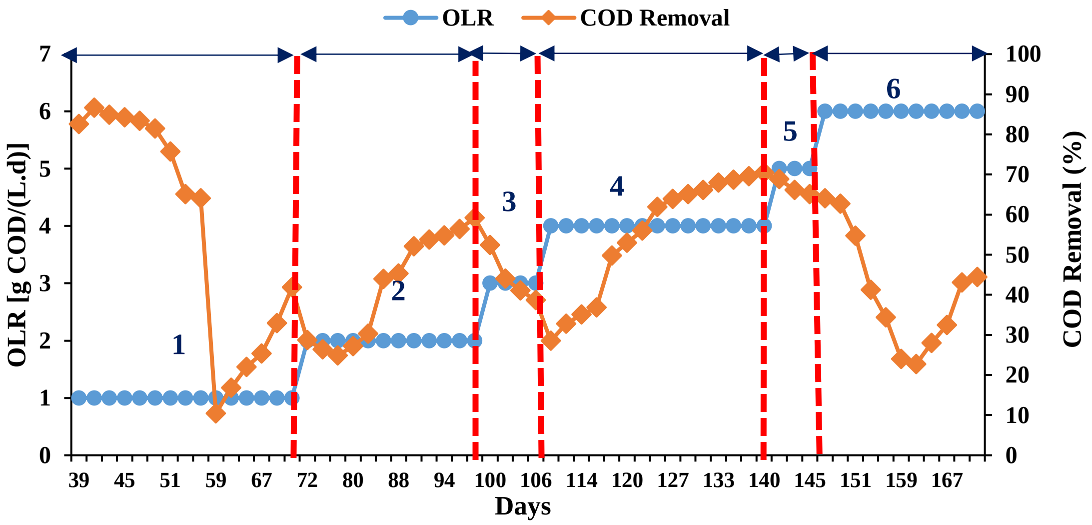

Figure 4.8 COD removal of anaerobic CSTR during continuous phase

4.4.6 Biogas production & cumulative biogas production

Figure 4.9 shows specific biogas production of anaerobic CSTR under varying organic loading rates during continuous phase operation. During OLR of 1 g COD/(L.d) to 3 g COD/(L.d), no biogas was produced possibly due to low organic loading and low VS content of synthetic MSW leachate.

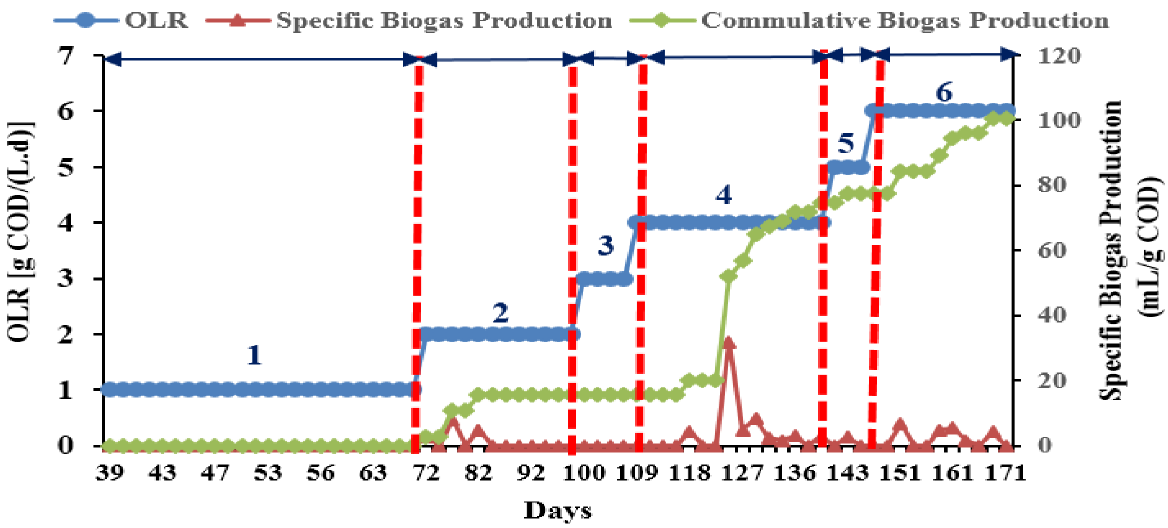

Figure 4.9 Biogas production of anaerobic CSTR during continuous phase

Also there were marked accumulation of VFAs after 57th day, resulting in an unfavorably high VFAs/Alkalinity ratio of over 0.5 (Paula et al., 2021). During OLR of 4 g COD/(L.d), maximum of 32 mL/g COD daily specific biogas was produced. With increase in organic loading rate (OLR), trend for cumulative specific biogas production also increased. (Micolucci et al., 2016). Maximum of 100 mL/g COD_{feed} biogas produced during an organic loading rate (OLR) of 6 g COD/(L.d). Specific biogas production was decreased when OLR was increased from 4 g COD/(L.d) to 6 g COD/(L.d). This could be as a result of rapid VFA production but could not be consumed as quickly as it were produced, which could have led to less biogas production (Rajput and Sheikh, 2019).

This might be due to rapid production of VFAs which could not be consumed as quickly as produced and might have resulted in lower biogas production (Rajput and Sheikh, 2019).

4.5 Summary of results for continuous phase

Results for continuous operation are summarized in Table 4.3. The stability parameters like pH, ORP and All of the OLR's had VFA/Alkalinity levels that were within a reasonable range for anaerobic treatment. Ammonia nitrogen were below 600 mg/L which were well below the conditions under which inhibitory effects seems prominent. Conclusion for overall study of conducted research shows maximum of 70% COD removal and cumulative biogas production of 59.2 mL/g COD under an OLR of 4 g COD/(L.d).

Table 4.3 Results for continuous phase operation of anaerobic CSTR

Organic Loading Rate (OLR) [g COD/(L.d)]	pH	ORP (mV)	VFA/Alkalinity	Ammonia (mg/L)	COD Removal (%)	Cumulative Biogas Production (mL/g COD)
1	7.45	-415	0.39	81.14	57	0
2	7.71	-408	0.45	68.66	42	15.6
4	7.62	-445	0.46	31.93	56	59.2
6	7.62	-411	0.30	28.72	41	23.2

Chapter 5

Conclusions & Recommendations

This study investigated the treatment of municipal solid waste (MSW) leachate in mesophilic anaerobic CSTR operated under various organic loading rates (OLR). Various operational and monitoring parameters of anaerobic CSTR during leachate treatment were analyzed under different OLR's. Anaerobic CSTR was run in batch and continuous phases. The drawn conclusion from these phases are presented in this section.

21.1 Conclusions

21.1.1 Batch phase

- The pH of 7.38 and oxidation reduction potential (ORP) of -455 mV was observed
- An average of 0.32 of VFA/Alkalinity ratio was found which shows system stability
- 75% COD removal and 219 mL/g COD biogas was obtained

21.1.2 Continuous phase

- The pH of anaerobic CSTR found in range of 7.45-7.71 during working organic loading rates (OLR)
- Average value of -451 mV, -440 mV, -469 mV and -419 mV were observed at OLR of 1 g COD/(L.d), 2 g COD/(L.d), 4 g COD/(L.d) and 6 g COD/(L.d) respectively
- Average of 0.39, 0.45, 0.40 and 0.30 of VFA/Alkalinity was observed during organic loading rate (OLR) of 1 g COD/(L.d), 2 g COD/(L.d), 4 g COD/(L.d) and 6 g COD/(L.d) which shows the stability of anaerobic system even at higher organic loading rates
- Maximum of 70% & average of 56% COD removal were obtained during organic loading rate (OLR) 4 g COD/(L.d)
- Optimum of 59.2 mL/g COD biogas was obtained under OLR of 4 g COD/(L.d)

21.2 Recommendations

- Substrate having more volatile solids should be used for more biogas production
- Effect of mono-digestion and co-digestion on leachate treatment and biogas production should be assessed
- For the maximum organic removal of leachate, combined process i.e. physico-chemical and biological treatment should be studied
- Two stage anaerobic digestion should be studied to assess biohythane production

- Anaerobic CSTR should run on higher organic loading rates for checking system stability, high organic removal and biogas production

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