Performance Evaluation of an Anaerobic-Aerobic SBR System for the Treatment of High Strength Industrial Wastewater

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Dedicated

To

My Beloved

Mother and Brothers

Thanks for your endless love, sacrifices, prayers, supports and advice

And above all,

TO THE ALMIGHTY ALLAH

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ABSTRACT

A lab-scale anaerobic-aerobic SBR system was investigated for the treatment of high strength industrial wastewater. Anaerobic-aerobic SBR system was operated as a coupled system having 12 h HRT in each reactor and decoupled system having 48 h HRT in anaerobic reactor and 6 h in aerobic reactor. Results showed that coupled anaerobic- aerobic system, when used just for the combined organics removal is a suitable treatment but it is uneconomical due to reduction in biogas production. Increasing OLR in anaerobic SBR from 2- 6 kg COD/m^3.d in coupled system caused instability of both reactors, and thus caused decline in biogas production from 11.0 to 10.7 L/d and % COD removal in anaerobic SBR from 59.0 to 43.8 % and combined from 98.5 to 94.5 %. Decoupling of reactors and using anaerobic reactor for organic removal and biogas production, and aerobic just for polishing purpose proved to be a cost effective and stable treatment with overall 98.6, 99.5 % organics and TKN removal, respectively and biogas production of 22.7 L/d was observed in decoupled system. Biogas production was increased up to 50 %, by increasing HRT in anaerobic reactor. Decoupled anaerobic-aerobic system also proved to be an economical and stable treatment, when used for the treatment of textile wastewater, containing reactive and basic dyes with overall organics, color and TKN removal of 99.6, 92.0 and 99.9 % respectively.

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LIST OF ABBREVIATIONS

AnSBR	Anaerobic Sequencing Batch Reactor
ASBR	Aerobic Sequencing Batch Reactor
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
COD: N: P	Chemical Oxygen Demand to Nitrogen to Phosphorus Ratio
HRT	Hydraulic Retention Time
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
OLR	Organic Loading Rate
SBR	Sequencing Batch Reactor
SRT	Sludge Retention Time
VFA	Volatile Fatty Acids

Chapter 1

INTRODUCTION

1.0 Background

One of the major and complicated issues regarding environmental pollution is the treatment of industrial wastewater. Biological treatment of high strength biodegradable, industrial wastewater may be achieved both aerobically and anaerobically. But both biological options have some merits and demerits for example, aerobic treatment is suitable for low strength wastewater having COD less than 1000 mg/L, and requiring high maintenance cost i.e., aeration cost (Chan et al., 2009).While anaerobic reactors are preferable for the treatment of high strength industrial wastewater due to high organics removal, low sludge production and energy generation in terms of biogas production being a substitute of a natural gas (Ramos et al., 2014 ; Heijnen et al., 1990).

However, disadvantage in just providing anaerobic treatment of high strength wastewater includes, slow growth rate of microbes and final effluent containing anaerobically non-degradable ammonia, generated during the treatment of wastewater containing organic nitrogenous compounds such as amino acids, urea and proteins (Bernet, 2000; Heijnen et al., 1990), incomplete removal of soluble organic matter and high concentration of suspended solids due to poor settling of anaerobic sludge. Noxious anaerobic effluent contains ammonium ions, therefore requires post treatment to meet the effluent standards, signifying the potential of using anaerobic–aerobic systems for the treatment of high strength industrial wastewater (Gray, 2005).

Combination of anaerobic-aerobic system for wastewater treatment may be an effective option in terms of low operational cost of treatment while achieving high removal efficiency of organic matter and nutrients, low energy requirements, biogas production and low specific production of excess sludge (Sheng et al., 2003; Heijnen et al., 1990).

1.1 Problem Statement

Different studies have been conducted to treat high strength industrial wastewater such as cotton textile, paper and pulp, pharmaceutical, municipal, synthetic and palm oil mill effluent by different kinds of high rate anaerobic reactors such as upflow anaerobic sludge blanket (UASB) reactor (Oktem et al., 2008), upflow anaerobic filter (UAF), upflow fluidized bed (UFB) and down-flow fluidized bed (DFB) (Sahinkaya and Gungor, 2010) systems under different organic loading rates (OLRs) and hydraulic retention times (HRTs). Above mentioned technologies have some disadvantages like lower methane emission due to foaming, clogging of filter media at high OLRs, high media and support cost, high power requirements for bed fluidization, high cost of carrier media, poor sludge settleability, foaming and sludge floatation at high OLRs, long startup period if granulated, seed sludge is not used. Parawira et al., (2006) observed foaming and sludge floatation in the UASB reactor when operating at higher OLRs (> 6.1kg COD/m^3 day). Treatment efficiency of anaerobic filters is affected by clogging of filter media due to solids accumulation at high OLRs, high media and support cost (Krishna, 2013; Parawira et al., 2006; Jawed and Tare, 2000).

Solution to the above-mentioned problems of different high rate anaerobic reactors for the treatment of high strength wastewater is anaerobic continuously stirred sequencing batch reactor, having advantages of simple design, easy operation, low capital costs; independent of type of biomass. Other advantages include sufficient contact between substrate particles and microbial communities through mixing, increased gas production by reduction in scum build up through proper mixing and elimination of temperature stratification. The major advantages of combining anaerobic SBR with aerobic SBR includes elimination of aeration cost due to reduction in volume of aerobic reactor as most of the organics will be removed by anaerobic sequencing batch reactor,

recovery of methane, and the production of significantly less excess biomass (Firozjaee et al., 2013; Abdurehman et al., 2011).

1.2 Significance

Previous studies were conducted on optimization of parameters in integrated systems of high rate anaerobic reactor with aerobic SBR in a single reactor configuration with no objective of biogas production. This study was conducted to remove organics, nutrients and to investigate biogas production as well.

1.3 Objectives

Therefore, the objectives of this study were to

1. Establish anaerobic – aerobic SBR system to treat high strength industrial wastewater at lab scale.
2. Evaluate the performance of anaerobic SBR when coupled and decoupled with aerobic sequencing batch reactor in terms of biogas production, organic and nutrients removal.
3. Optimize HRT in both reactors by keeping in view the stability of each reactor, maximum biogas production, nutrients and organics removal.

1.4 Scope

1. Lab – scale anaerobic and aerobic SBR setups.
2. Treatment of synthetic wastewater of different COD concentrations of 1000, 2000 and 3000 mg/L.
3. Addition of 6 mg/L of dyes to COD of 3000 mg/L in the last stage under optimum conditions.

Chapter 2

LITERATURE REVIEW

2.0 Biological wastewater treatment processes

With suitable examination and environmental control, practically all wastewaters containing biodegradable elements with a BOD/COD ratio of 0.5 or greater can be treated simply by biological means (Metcalf, 2002). The goal of biological treatments is the breakdown of soluble biodegradable organic material within the wastewater stream (Grady et al., 2011).

During biological digestion, a group of microorganisms utilizes the soluble organic material as a food (Grady et al., 2011). Microorganisms that are involved in biological wastewater treatment are aerobic, anaerobic or facultative in nature. Aerobic microbes require oxygen to live while anaerobic bacteria live and grow in environments that are deprived of dissolved oxygen (DO). Facultative microbes may live and grow in aerobic or anaerobic environment. (Spellman, 2003). Apart from the aerobic and anaerobic divisions, bioreactors can be separated into suspended-growth bioreactors and attached-growth bioreactors (Mihelcic et al., 2010; Grady et al., 2011). In attached growth process biofilm, which is an active thin layer of microorganisms is developed on the solid support. Organic matter, nutrients and oxygen diffuse into the biofilm where they are utilized and reacted by the microorganisms, whereas the products diffuse out from biofilm.

2.1 Aerobic treatment

Aerobic wastewater treatment processes results into the oxidation of organic material present in wastewater (Hansen & Cheong, 2007). The aerobic microorganisms utilize the organic matter as a carbon and energy source and oxygen as an electron acceptor (Doble & Kumar, 2005). The

organic material is disintegrated to carbon dioxide, nitrates, water, sulphates and biomass (Doble & Kumar, 2005).

The aerobic biological process is presented as:

Organic matter + O_2 + (microorganisms/nutrients) ----------→ H_2O + CO_2 + biomass

According to Mosse et al. (2011) aerobic biological processes are generally easy to implement, it generates a high COD reduction efficiency and it is very versatile in terms of the size of the operation. Although aerobic wastewater treatment has excellent treatment performance, high operational and capital cost effects substantial financial limitations. This lead to rising of further research to look for cost-effective and reliable technology (Kassab et al., 2010).

2.1.1 Aerobic treatment process technology

Aerobic biological treatment processes can be divided into two major groups, suspended- and attached-growth processes. Both processes perform similar oxidation reaction but they differ in the way biomass is retained inside the reactor. Suspended growth processes include stabilization ponds, activated sludge process, sequencing batch reactor, membrane bioreactors. While, attached growth processes include trickling filters, rotating biological contractor, expanded or fluidized bed reactors.

2.1.1.1 Suspended growth process

Stabilization ponds are also known as aerated lagoons and oxidation ponds (Mihelcic et al., 2010). This treatment method is considered as one of an economical and cheapest in which wastewater is just stored in a pit through which oxygen is bubbled. Sedimentation of solids occurs in lagoons as no manual or mechanical mixing is provided (Hansen & Cheong, 2007; Doble & Kumar, 2005).

Major operational parameters for stabilization pond includes temperature of a surrounding environment, light penetration, oxygen concentration and pond geometry.

Aerated lagoons are easy to construct and manage, but they need longer time for aeration compared to other aerobic processes. They also have a high-energy demand **(**Montalvo et al., 2010**).** (Laginestra, 2009). Disadvantages of stabilization pond includes large area requirement, production of bad odours and effluent contains high concentrations of TSS due to algal growth and bad odours.

Activated sludge process is one of the most common aerobic biological process that involves the suspended growth of bacteria (Hansen & Cheong, 2007). The solid retention time (SRT) is kept longer than the hydraulic retention time (HRT) to ensure an improved treatment process (Hansen & Cheong, 2007). Generally, the effluent of a primary treatment process (such as a sedimentation tank) is taken to an aeration tank. The wastewater is then mixed with a consortium of microorganisms comprising of bacteria, fungi, and protozoa (Mihelcic et al., 2010). Air is supplied in aeration tank for microbes to degrade organic matter present in wastewater. After treating wastewater in aeration tank, sludge is separated from the mixed liquor coming out of aeration tank and separated sludge is then concentrated in a concentrator to produce a concentrated sludge and separated water.

The activated sludge processes are simple, flexible and economical in terms of BOD, COD and nutrients removal; however, the process has a high-energy demand and requires additional nutrients **(**Fumi et al., 1995). A Fumi et al. (1995) study revealed that the process itself is flexible to tolerate the large variations in the organic material loadings.

Aerobic membrane bioreactor is a combination of a suspended-growth process and the membrane process. The mixed liquor (mixture of liquid, waste solids and microorganisms) in the suspended

growth tank is filtered through a membrane to separate the biomass/sludge from the water. A vacuum of less than 50 kPa is applied. A pump is used to force the mixed liquor at less than 100 kPa, for an external membrane system. The water is then filtered through membrane and biomass is sent back to the aeration basin **(**Mihelcic et al., 2010).

The advantage of membrane bioreactors includes better effluent quality, smaller foot print and can operate at higher organic loading rates. Main disadvantages of membrane bioreactors are the high operational and capital costs, short life span, and membrane requires frequent cleaning or be replaced (EPA, 2007).

2.1.1.2 Attached growth processes

Rotating biological contractors involves a use of biological film to rotate within the wastewater (Hansen & Cheong, 2007). The discs are covered with a biofilm which degrades the organic material in wastewater. The rotating disc allows the film to alternate between the wastewaters and the air. The available oxygen is used by aerobic microorganisms to degrade the organic matter (Doble & Kumar, 2005).

RBC has some advantages: easy to operate; short start-up period and little maintenance is required. Biofilm systems have the capacity to operate at high biomass concentrations and no recirculation is required (Malandra et al., 2003). RBC have the drawbacks of low surface area and hence have low biomass content. Therefore, RBCs have the shortcomings of long start-up time and low treatment capacity. Malandra et al. (2003) investigated a small-scale RBC to treat winery wastewater. The conclusion was made that the RBC process was effective to reduce the COD content of winery wastewater. A COD reduction of 43% was obtained and was recommended to use the process in conjunction with other processes.

In Moving bed biofilm reactors, whole tank volume is used for the biomass. No recycling of the sludge is needed. The biomass grows on carriers which are submerged in the water. The biofilm moves in the wastewater through agitation which is caused by the air. Sieve arrangements keep the biofilm within the wastewater. The biofilm thickness is of importance in the process, therefore the biofilm must be thin and evenly dispersed over biofilm carrier (Rusten et al., 2006).

In trickling filter of fixed bed biofilm reactor, biofilm is formed on a fixed medium and uses immobilized microorganisms. The tank is typically filled with stone media and the biological growth forms on the stone media. The organic material is absorbed by the fixed biofilm containing microorganisms. The biofilm thickness may range from 0.07 mm to 4.0 mm (Mihelcic et al., 2010). The FBBR is mostly used to remove soluble organic matter and for oxidation of ammonia (Hansen & Cheong, 2007). The wastewater is pumped from the top to flow down through the fixed bed and air is pumped from the bottom of the bioreactor (Doble & Kumar, 2005). The advantages of the system are easy to manage, small volume required volume as compared to the activated sludge systems, reduction in clogging of solid media, elimination of return flow step. The reactor process is easier to manage (Doble & Kumar, 2005).

2.1.2 Aerobic Sequencing Batch Reactor (SBR)

SBR is a fill and draw activated sludge process. In SBR, wastewater is filled in a reactor and then operated as a batch. After achieving desired treatment, sludge is allowed to settle and after that supernatant is withdrawn from reactor. SBR is found to be low cost, well-organized and flexible technology which can be used to treat different industrial wastewater. The SBR's have several advantages: an external clarifier is not required as the process occurs in a single vessel, which is a cost saving aspect of this process. A study done by Torrijos & Moletta (1997) revealed that the process is simple to operate, it has a low capital cost and a moderate operational cost.

2.2 Anaerobic treatment

To treat wastewater, anaerobic technology has been practical from the 19th century. In 1970s oil crises and demand for effective and efficient treatment of industrial wastewater lead to the rapid progress of the anaerobic process (Gavrilescu, 2002). It is one of the most effective and efficient treatment of wastewater. Organic matter present in wastewater is anaerobically digested, degraded and stabilized by anaerobic microbes under anaerobic conditions. Biogas production and biomass formation are the end products of anaerobic treatment of wastewater containing organic matter (Kelleher et al., 2000).

Being a most efficient and effective treatment of wastewater by reducing pollution and compensating fuel utilization during its process through biogas production; anaerobic digestion has been widely used for the handling of municipal and industrial sludge and treatment of wastewater as well such as municipal, textile, cotton, paper and pulp, winery, palm oil mill effluent, food processing and pharmaceutical industries. (Parkin and Miller, 1983).

As compared to other methods of wastewater treatment it has benefits of lower treatment costs with no secondary pollution (Sponza & Ulukoy, 2006). Aerobic biological processes are normally used for the treatment of organic wastewaters to achieve high degree of treatment efficiency, whereas in anaerobic treatment, significant advancement has been attained in anaerobic biotechnology for wastewater treatment based on the concept of resource recovery and utilization whereas still attaining the objective of pollution control (Seghezzo et al., 1998).

2.2.1 Anaerobic microbiology process description

In anaerobic reactors, conversion of complex organic matter in wastewater to methane and carbon dioxide is carried out by a unique ecosystem of diverse groups of anaerobic bacteria. Anaerobic degradation of complex organic matter is a complex microbial procedure involving several

interdependent successive and corresponding reactions. Several groups of bacteria play vital role in anaerobic digestion includes:

1. Hydrolytic bacterias
2. Fermentative bacterias
3. Hydrogen producing bacterias
4. Acetogenic bacterias
5. Methanogenic bacterias (Eghezzo, 2004)

The process represents a complex system of transformations of organic matter in which a (mixture of gasses) biogas is being produced. The composition of biogas produced can vary depending on type of substrate (organic matter), digestion system, temperature and other operating parameters. The anaerobic processes of degradation of organic matter can be dived in two main groups depending on the microbial community involved in these reactions as:

- Biochemical processes carried by the involvement of microbial community
- Physico-chemical processes without involvement of microbial community.

Figure. 2.2 represents the phases involved in anaerobic digestion process as well as the products of biochemical transformations that take place within each different process step. Methane formation in anaerobic digestion comprises of four different steps

1. Hydrolysis
2. Acidogenesis
3. Acetogenesis
4. Methanogenesis

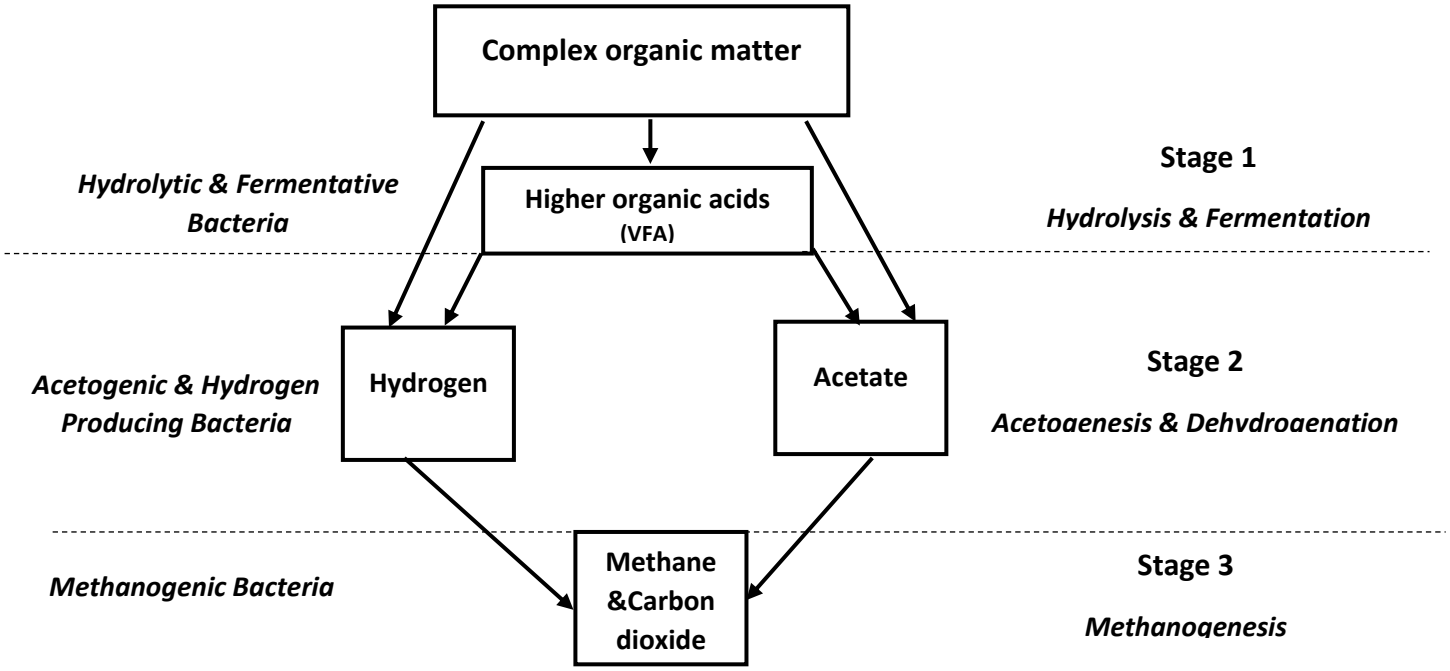

Figure 2.1 Anaerobic treatment process and microbiology

2.2.1.1 Hydrolysis

Generally, in an anaerobic digestion process, the step that causes process failure under imposed kinetic stress is defined as a rate limiting step. Most researchers have reported that the rate-limiting step for complex organic substrate is the hydrolysis step mainly due to the formation of toxic byproducts such as volatile fatty acids (Aslanzadeh, 2014; Fernandes et al., 2009; Heo et al., 2003; Izumi et al., 2010; Maj Duong et al., 2011; Johansen & Bakke, 2006).

Hydrolysis is the first step in anaerobic digestion process during which complex and biopolymer organic matter is broken down to simple and soluble organic matter such as amino acids, monosaccharides and other simple organic matter (Batstone et al., 2002). This first step is very much important for the growth of microorganisms, as they cannot absorb or use complex organic matter as a food source. Therefore, for cell growth of microorganism, organic matter must be soluble and have a low molecular weight for micro-organisms to take up as a food (Gujer & Zehnder, 1983; Gallert & Winter, 2005). Thus, during this step complex proteins, carbohydrates

and lipids are converted to amino acids, fatty acids, alcohols and simple sugars (Gerardi, 2003; Chernicharo, 2007).

This process of solubilization of complex organic matter is carried by a group of strict anaerobes and facultative bacteria such as clostridia, bacterides and streptococci etc. (Gerardi, 2003; Mara and Horan 2003). Genera of these microbes are: Vibrio, Peptococus, Bacillus, Clostridium and Micococcus, (Mara and Horan 2003). The process of degradation of organic matter is carried out by extracellular enzymes excreted by certain microbes which results into the breakdown of complex matter to smaller solubilized products that can easily be taken in by microbial cell as a source of food and nutrients for their growth.

The factors which may affect the hydrolysis step includes the composition of organic matter, size of particle, pH of substrate, Concentration of volatile fatty acids and ammonia nitrogen (NH^{4+}-N), HRT, operational temperature, composition of substrate, particle size and pH of substrate or medium (Gujer & Zehnder, 1983).

2.2.1.2 Acidogenesis

Acidogenesis is the second stage of anaerobic digestion. During this stage, monomers that were formed during hydrolysis are further degraded to small chain acids by acid formers such as obligatory and facultative anaerobes including clostridium spp, lactobacillus, actinomynes, syntrophobacter wolinii, and sytrophomonos wolfei.

The products of second stage includes acetic acid (CH_3COOH), hydrogen (H_2), carbon dioxide (CO_2), butyric acid ($CH_3CH_2CH_2COOH$), ethanol (C_2H_5OH) and propionic acid (CH_3CH_2COOH) by obligatory and facultative anaerobes, known as acid formers. In the second stage, acetogenic bacteria, also known as acid formers, convert the products of the first phase to simple organic acids (acetogenic acid), carbon dioxide and hydrogen. Type of final product produced during fermentation depends on the concentration of hydrogen formed as an intermediate product in this

stage. For example, number of reduced compounds will be reduced if the partial pressure of the hydrogen is too high it would decrease the number of reduced compounds. In general, during this phase, simple sugars, fatty acids and amino acids are converted into organic acids and alcohols (Gerardi, 2003).

2.2.1.3 Acetogenesis

During third phase known as acidogenic phase, anaerobic oxidation is performed (Aslanzadeh, 2014). Microbes which carry out anaerobic oxidation reactions cooperate with the methane forming bacterias. Both acetogenesis and methanogenesis stage of anaerobic digestion works in collaboration and this collaboration depends on partial pressure of the hydrogen present in the system. During acetogenesis, all the products that cannot be directly utilized by methanogenic bacterias to generate methane are converted to methanogenic substrate for example VFAs are oxidized to hydrogen, carbon dioxide and acetate. Hence during this symbiotic relationship inter-species hydrogen transfer occurs (Chandra et al., 2012).

2.2.1.4 Methanogenesis

For easily biodegradable substrates methanogenesis is a rate limiting step. Being a slowest biochemical reaction of the whole digestion process, methanogenesis is a critical step in the whole anaerobic digestion process. During this phase, strict anaerobic methanogens such as methnococcus, methanobacterium, methanosacina and methanobacillus results into production of methane and carbon dioxide from intermediate products. Methanogens are growing microbes and are sensitive to change in temperature and pH. Methane production in this step is carried in two ways: either by break down of acetic acid molecules to produce carbon dioxide and methane, or by reduction of carbon dioxide with hydrogen. Generally, methane production is higher from

reduction of carbon dioxide (Omstead et al, 1980). The methanogenesis reactions can be expressed as follows:

$CH_3COOH \rightarrow CH_4 + CO_2$ ……. Acetoclastic methanogens

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ ……..... Hydrogenotrophic Methanogens

2.2.2 Factors affecting anaerobic treatment process

Anaerobic treatment of wastewater is a very complex process due to close syntrophic relations among several groups of microorganisms separately having different requirements of environmental and physiological conditions. Therefore, the process is highly influenced by the growth and performance of anaerobic microbes that is highly dependent on environmental and physiological conditions. Following parameters including temperature, pH, alkalinity, VFA, nutrient supply, presence of toxic and inhibitory substances is of critical importance for the optimal working conditions of anaerobic microbes (Al Saidi, 2008).

2.2.2.1 Temperature

One of the key parameter influencing the efficiency of anaerobic treatment and selection of microbial species is temperature. Generally, microorganisms are not able to control their inner temperature, therefore, the internal temperature of microbial cell is determined by the external ambient temperature of environment. The effect of external temperature on bacterial cells is very important. For example, the degree of disintegration of numerous compounds are highly related to the temperature, such as ammonia. In most of the biological processes three temperature ranges can be related with microbial growth (Batstone et al., 2002):

- Psychrophilic range: 4 to 15^oC
- Mesophilic range: 20 to 40^oC

- Thermophilic range: 45 to 70◦C, and above

The temperature disturbs the biological processes in two ways:

(i) Affecting the enzymatic reaction rates.

(ii) Affecting the substrate diffusion rates.

Generally, the two most common operated ranges for anaerobic treatment are mesophilic and thermophilic as mostly performance of methanogens species is maximum in these ranges. For temperature, in between 40 and 50°C the methanogens activity is inhibited and the temperature of 42°C is a point of transition from mesophilic temperature to thermophilic range (Gerardi, 2003). As compared to thermophilic range, methanogens have high tolerance to change in environmental conditions at mesophilic range (Buekens, 2005).

As compared to acetogens and methanogens, hydrolysis and acidogenesis phases are not affected by temperature (Parawira, 2004). Temperature fluctuations should be less than 2 - 3°C per day for a stable anaerobic process (Gerardi, 2003). The temperature should be kept constant in anaerobic reactors through insulation and by mixing the contents (Buekens, 2005). According to Massé & Masse (2001) anaerobic treatments should bound to the explicit temperature ranges, or else inhibition of the anaerobic wastewater treatment process may arise. The methanogenic activity is extremely sensitive to temperature variations as compared to the constant temperature.

Although methanogenic activity is increased by 25 to 50 % resulting in subsequent increase in methane production within the thermophilic temperature range but there are certain risks of higher process instability in terms of ammonia inhibition and VFAs production. Ammonia inhibition due the reason that the fraction of unionized ammonia (NH_3) increases with increase in temperature, which is reported to be more toxic than the ionized (NH^{4+}) one, as it is able to penetrate through

the cell membrane of microbes (Chernicaro, 2007). VFAs production increases with increase in temperature to thermophilic temperature (Gallert & Winter, 2008).

2.2.2.2 pH

pH is the concentration of hydrogen ions in aqueous system. The four types of micro-organisms involved in anaerobic digestion have different optimal pH ranges. Optimum range of pH for hydrolysis and acidogenesis is between 5.5 to 6.5 (Ward et al., 2008). Enzymes of acid formers involved in acidogenic stages are active at a pH between 4.5 and 5.0 (Zoetenmeyer et al., 1982). The acid formers are less sensitive to a pH change as compared to methanogenic bacteria (Hwang et al., 2004; Tiwari et al., 2006; Amani, 2010).

The retention time of wastewater affects the pH value and in batch reactor acetogenesis occurs quickly and can result into accumulation of substantial amounts of VFAs resulting into drop in pH below 5.0. Enzymatic activity of methanogenic microbes occurs only at pH above 6.2 (Zoetenmeyer et al., 1982; Amani, 2010). Methanogens are sensitive to acid concentration in reactor that results into decline in pH and therefore, causes decline in their growth rate as the optimum pH range for these microbes is between 6.8 and 7.2 (Ward et al., 2008). When pH drops below 6.0 and increases above 8.0 complete inhibition of methanogenic activity appears (Chernicharo, 2007).

pH in a one vessel reactor such as anaerobic sequencing batch reactor can be adjusted to requirement of methanogens (Parawira, 2004). As methanogenic stage is achieved in anaerobic digestion, the concentration of ammonia may increase which may cause increase in pH value and can increase to above 8.0. But once methane production is stabilized, the pH level remains in between 7.2 and 8.2.

2.2.2.3 Alkalinity

Alkalinity concentration known as buffering capacity of a system have direct impact on pH values inside the reactor. Ability of system to resist change and to maintain constant pH is known as buffering capacity. Enough alkalinity is required in anaerobic reactors to maintain optimum pH for methanogenic activity and to avoid its failure, as it provides buffer capacity to the system (Moosbrugger et al., 1993; Mockaitis et al., 2006). In anaerobic process alkalinity is mainly in the form of bicarbonate and volatile fatty acids alkalinity (Malandra, 2003). CO_2 is generated whenever, organic matter is degraded resulting in the production of bicarbonate, carbonic acid and carbonate alkalinity. Interaction between alkalinity and VFA results in production of fatty acids alkalinity. Thus, from the interactions between the volatile fatty acids and alkalinity the alkalinity of fatty acids is created. Buffering range of VFA alkalinity is in between 3.75 and 5.75 and therefore, it has no significant importance for the anaerobic digestion process (Chernicharo, 2007). The alkalinity within the digester is produced due to the degradation of nitrogen enriched organic matter to form amino groups ($-NH_2$) and ammonia (NH_3). However, when nitrogen concentration is high then ammonia concentration inhibits growth of methanogenic microbes.

During the conversion of organic matter volatile fatty acids are generated and alkalinity in system is consumed to maintain pH and therefore, with time its concentration starts on decreasing resulting in decline in systems pH. When volatile fatty acids are consumed by methanogens then carbon dioxide is produced because of which alkalinity in terms of bicarbonate is generated and the pH is maintained and balanced. This trend is normal and is usually observed when operational conditions are stable. In case of imbalance in the system both methanogenic activity and alkalinity production will also decrease which will lead to acids accumulation in the reactor resulting into further lowering of pH. This trend of pH lowering will ultimately leads to complete inhibition of both

methanogenic activity and biogas production. By measuring alkalinity concentration in system this negative trend can easily be detected. Another way to maintain the pH within optimum range is the addition of chemicals to the reactor when the pH drops due to VFAs build up (Shizas & Bagley, 2002; Buekens, 2005). Therefore, alkalinity is considered as one of the crucial process parameters for assessment of stability of the process.

2.2.2.4 VFA

VFA is an intermediate product anaerobic digestion which greatly affects the pH of reactor when methanogenic bacterias starts utilizing them (Wang et al., 2009; Shao et al., 2008). VFAs act as the stability parameter of an anaerobic process. It should be noted that VFA act as an indicator (Shao et al., 2008). In protonated form VFAs are toxic as they penetrate the cell membrane of the bacteria and get ionized inside these bacterial cells and causes decrease in pH of the cell (Parawira, 2004). Therefore, the syntrophic relationship between the acid formers (produce VFAs) and methanogens (utilize the VFAs) are significant to maintain the pH in the system by overall keeping the VFA concentration low in the system (Parawira, 2004; Shao et al., 2008).

2.2.2.5 Nutrients

Nutrients are classified in two groups: macro and micro nutrients regarding the amount needed for biomass growth. Carbon, nitrogen, phosphorous and sulfur are the macro nutrients. Microbes uses nitrogen and phosphorus in soluble form and they come from ammonia nitrogen and orthophosphate, respectively. Macronutrients are required by microbes for their growth, functioning and maintenance of their cells (Singh et al., 1999). Iron, cobalt, nickel and molybdenum are the micronutrients required for the enzymatic activity of anaerobic microbes especially methanogens (Gerardy,2003; Gerardi, 2003). Low dosages of micronutrients and trace

elements are important for the anaerobic reactors to avoid deficiencies and toxicities (Singh et al., 1999; Gerardi, 2003).

Different organic loading rates have different nutrient requirements. COD: N: P for high strength wastewater is 100:7:1 and 350:7:1 for low strength wastewater (Gerardi, 2003; Amani et al., 2010). C: N of 25:1 is optimum ratio of methane gas production (Amani et al., 2010). Too high C: N results in lower gas production as nitrogen will be rapidly utilized by the methanogens. A too low C: N ratio will result in the accumulation of ammonia having an inhibitory effect on the performance of methanogens (Buekens, 2005).

2.2.2.6 Presence of toxic and inhibitory substances

Wastewater often comprises of waste which is noxious to the microbes. Methanogens are can withstand high concentrations of toxic material in wastewater when acclimatized to the wastewater having toxicity (Parkin & Miller, 1983). If not acclimatized then methanogens are highly sensitive and prone to environmental changes and results in reduction in methane gas production (Rajagopal et al., 2013). Toxicity indicators comprises: reduction in methane gas production, pH; increased concentration of volatile fatty acids and a decreased concentration of bicarbonate alkalinity (Gallert & Winter, 2008).

Toxicity from cations

A group of heavy metals such as arsenic, zinc, copper, cyanide, cobalt and chromium are highly toxic for anaerobic microbes. These metals cause inactivation of enzymatic activity of microbes. However, when these metals are present in trace amount they are beneficial to microbial population (Zaher et al., 2007; Amani et al., 2010). Cationic part of metallic salts such as (NH^4, Na^+, K^+,

Mg^{2+}, Ca^{2+}) may also cause toxicity at high concentration while they promote and accelerate microbial growth at low concentration (Chernicharo, 2007; Chen et al., 2008).

Toxicity from ammonia

Ammonia is a source of nutrients for anaerobic microbes that's why it is one of the most important compound for anaerobic digestion. Ammonia is a supplement to buffering capacity of system to maintain stale pH in system. Ammonia is present in system in two forms; saline (NH^{4+}) and free (NH_3). Saline form of ammonia is used as nutrient for microbial population and as a pH buffer for system as it is formed from proteins and ammonia during anaerobic digestion (Angelidaki & Ahring, 1993). Free form is toxic and have inhibitory impact on methanogens when present at higher concentration (Mata-Alvarez et al., 2000; Chen et al., 2008).

Both forms are pH dependent and pH value of a system decides that either free or saline form of ammonia is dominant in system (Chernicharo, 2007; Chen et al., 2008). Saline form of ammonia is dominant pH less than 7.2 and at higher values free ammonia is dominant in system (Chernicharo, 2007). Both forms of ammonia must be in equilibrium for a balanced system as at higher concentration both are toxic for system. At a concentration, greater than 50 mg/L free ammonia can cause toxicity (Rajagopal, 2013). Reduction in glucose degradation rate occurs when concentration of saline ammonia is within the range of 740 to 3500 mg/L (Mata-Alvarez et al., 2000). Therefore, an optimum pH of 6.8 to 7.2 must be kept in anaerobic reactor to avoid ammonia toxicity (Amani et al., 2010).

Toxicity through sulfur

Anaerobic microorganism requires soluble form of sulphur i.e. HS^-. Higher concentration of hydrogen sulphide gas (H_2S) and sulphide (S^{2-}) can cause toxicity of anaerobic reactors (Hilton & Oleszkiewicz, 1988). Sulphur reducing bacterias such as desulfuricans and desulfovibrio are

activated in anaerobic reactor when substrate contains high concentration of sulphates (SO_4^{2-}) (Tian-wei et al., 2014). After activation, they compete with methanogens and results in production of biogas having high concentration of hydrogen sulphide gas and lower concentration of methane gas (Arnaldo & Marcelo, 2011).

Hydrogen sulphide can dissociate into hydrogen sulphide (HS^-) and sulphide (S^{2-}) in water (Hilton & Oleszkiewicz, 1998). At pH, less than 7, H_2S remains un-ionized, while ionized HS^- occurs at pH values within the range of 7.0 to 14.0 (Chernicharo, 2007). Sulphide (S^{2-}) inhibition of system is dependent on presence of non-dissociated hydrogen sulphide (H_2S) in substrate and both are pH dependent (Hilton & Oleszkiewicz, 1998).

90% of un-ionized H_2S is present at a pH of 6.0 and 50 at a pH of 7.0, while only 10% is available at a pH of 8.0 (Hilton & Oleszkiewicz, 1998). When H_2S concentration is greater than 200 mg/L then it could be toxic to anaerobic treatment (Gerardi, 2003; Chernicharo, 2007).

Higher COD concentration of wastewater will result in increased production of methane and therefore, H_2S toxicity will not occur. If COD/SO_4^{2-} ratio is greater than 10 then it will eliminate H_2S toxicity and if it is lower than 10 then it will result in increased production of H_2S (Karvina et al., 2007).

Therefore, to prevent sulphide toxicity, COD/SO_4^{2-} ratio must be increased above 10 to ensure release of sulphide in gaseous form, to increase pH to 8.0 which will favor non-dissociated HS^- formation; reduction in sulphide concentration in substrate and to increase the usage of iron salts will precipitate out in system sulphides (Mohanty et al., 2000).

Toxicity from long chain fatty acids Presence of long chain fatty acids in anaerobic system have toxic effect on anaerobic microbial population because their structure and composition is like lipid

component of methanogens and acteoclastic bacteria. Therefore, VFAs dissolve in their cell walls and starts inhibiting their activities even at a concentration less than 500 g/L (Gerardy,2003).

2.2.2.7 Biogas Production

The product of anaerobic treatment is a biogas production which is a mixture of different gases with most dominant gases are carbon dioxide and methane. Amount of biogas produced represents the health of anaerobic system. Stable anaerobic systems generate more biogas as compared to unstable one.

In biogas, methane is the only one which can be used for electricity and heat production and have some economical value as well. High concentration of carbon dioxide in biogas have decreased heating values and if its concentration is above 30 % then acid concentration in system increases, resulting in pH drop below 7.0 no matter how much alkalinity is present in it (Parkin & Miller, 1983). Among all the gases produced, hydrogen sulfide (H_2S) is the most unwanted because significant amount of this gas can cause damage to equipment's of anaerobic reactor due to formation of sulphuric acid.

2.2.3 Anaerobic treatment systems

Anaerobic wastewater treatment is carried out in different anaerobic reactors having different operational modes and configurations depending on the type of organic matter present in wastewater, temperature and way of microbial growth.

Anaerobic reactors are divided in to two categories depending on the way biomass is retained inside the reactor.

1. **Suspended growth reactors**: In these kinds of reactors microbial biomass is homogenously distributed in the reactor

2. **Fixed film reactors**: Microbial biomass is attached to carriers like rocks, gravels or other type of carriers.

2.2.3.1 Suspended growth reactors

The continuous stirred tank reactor (CSTR) is the simplest reactor design for wastewater treatment and it does not separate HRT from SRT. It is a low-rate reactor having no method to enhance conversion rate of organic matter in reactor except mixing. Mixing devices can either be gas sparging devices or mechanical paddles or propellers. In anaerobic CSTR, SRT can be separated from HRT by providing settling tank for sludge settlement, known as anaerobic contact process.

The upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) processes are also suspended growth processes. The expanded bed reactor is very much alike fluidized bed reactor, except that bed expansion is just 50%. The inert media is done way with in the expanded granular sludge bed reactor, with only granular biomass forming the bed. It is essential to provide granular sludge for the startup of the EGSB reactor.

In UASB high density anaerobic sludge granules are developed under proper anaerobic conditions. This granular sludge then forms a sludge blanket inside the reactor through which wastewater is passed in upward direction. Upward movement of wastewater which provides contact wastewater with biomass providing treatment of wastewater.

2.2.3.2 Fixed film/ attached growth reactors

Microbes in a reactor grow as biofilm on an inert carrier media such as rocks, gravels stones or carrier media. There exists high concentration of biomass inside reactor as physical attachment of biomass on carrier media prevents washing out of biomass. These reactors need to be operated at safely at a velocity to prevent washing out of biomass. The reactors can be operated safely at high

flow velocities without washout. Following are the types of fixed film reactors that are used for industrial wastewater treatment.

For packed bed reactors carrier media in reactor is either stone or plastic for biofilm growth. Packed bed reactor has an inert packing media either of plastic or stone with random packing for biofilm growth. Usually carrier media are of larger size having reduced surface area available for biomass growth which prevents excessive growth of biofilm which may cause clogging of filter media, but provides larger flow channels. Direction of water flow through these media can either be upwards or downwards.

Fluidized bed reactor uses small size inert carrier media typically sand. To achieve 100% expansion of bed, wastewater upflow velocity of 10 to 15 m/h is applied. As smaller size carrier media is used it has larger surface area, therefore, a high biomass concentration is achieved in these reactors. Fluidized bed reactors contain very active biomass in biofilms due to continuous rubbing of carrier media particles and maintains a small thickness of biofilm.

2.2.4 Monitoring Parameters

A successful operation of anaerobic reactor requires a continuous monitoring of certain parameters to check its stability. These parameters include pH, alkalinity, VFA concentration, temperature, total phosphorous, TKN, ammonia, biogas production, biogas composition, SRT, HRT, OLR (Gerardi, 2003). Table 2.1 represents the reported optimal conditions for methanogenic activity.

Table 2.2 represents the anaerobic process instability parameters. Maintenance of physical parameters of anaerobic reactor such as SRT, OLR and HRT prevents adverse changes in above mentioned monitoring parameters.

Table 2.1 Operational conditions for methanogens optimal activity

Condition	Optimum	Marginal
Alkalinity (mg/l, as CaCO3)	1500-3000	1000-1500
pH	6.8-7.2	6.6-6.8,7.2-7.6
Temperature, mesophilic	30-35ºC	20-30º,35-40oº
Temperature, thermophilic	50-56ºC	45-50º,57-60º
Volatile acids	50-500	500-2000

(Gerardi, 2003; Chan et at., 2009)

Table 2.2: Indicators of process instability (Gerardi, 2003; Chan et al., 2009)

Indicator	Decrease	Increase
Biogas Production	X	
Methane Production	X	
Alkalinity	X	
pH	X	
Volatile solids destruction	X	
Volatile acid concentration		X
Percent CO_2 in biogas		X

2.3 Anaerobic vs aerobic treatment

One of the main differences between aerobic and anaerobic treatment processes is that the end products of aerobic treatment are principally solid, while gases are generated after providing anaerobic treatment. (Ditchfield, 1986; Speece, 1983). The various merits of both treatments are highlighted in Table 2.3, and both systems can achieve high organic removal efficiency. Generally aerobic treatment is suitable for low strength waste water having COD value less than 1000 mg/L

and anaerobic treatment is suitable for high strength wastewater. Though worth of methane produced from anaerobic treatment of industrial wastewaters is significant but it is not sufficient to be the only reason for the selection of anaerobic treatment technology. A comparison between anaerobic and aerobic processes is illustrated in Figure 2.3.

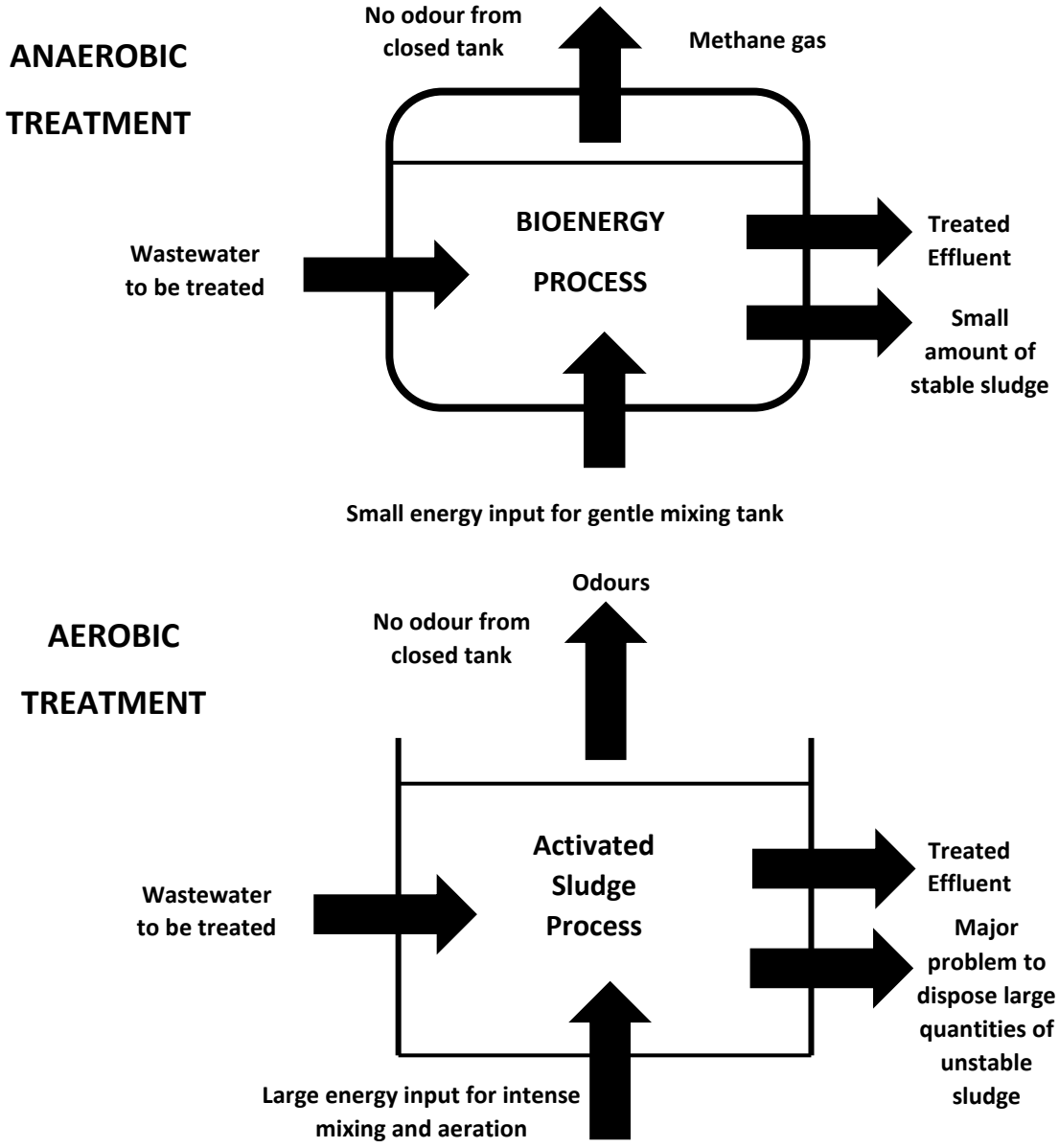

Figure 2.2 A comparison between anaerobic and aerobic treatment process

The advantages of anaerobic treatment compensate the advantages of aerobic treatment when treating high strength wastewater, and requires less energy and have potential for bioenergy and

nutrient recovery. Aerobic treatment achieves higher organics removal as compared to anaerobic treatment. Aerobic effluent has lower concentration of suspended solids as biomass generated in aerobic system is well flocculated and settles very quickly. Thus, the aerobic effluent is of high quality. A comparison between distinctive features of both treatment processes is presented in Table 2.3.

Table 2.3 Differences between aerobic and anaerobic wastewater treatments

PARAMETERS	ANAEROBIC	AEROBIC
REQUIREMENTS		
Operational cost	Low	High
Energy	Low	High
Reactor volume	Small	Large
Mechanical equipment	Little	Much
Maintenance	Not frequent	Frequent
Nutrients	Low	High
Alkalinity	High	Low
DESIGN PARAMETERS		
Organic loading rate (kg COD/m^3.d)	2-30	0.8-2
Hydraulic retention time	Days	Hours
Sludge retention time	> 20 days	5-10 days
Sludge stabilization	Not necessary	Necessary
PERFORMANCE		
Startup	Slow and complex	Fast and simple
Startup time	2 to 4 months	2 to 4 weeks
Degree of treatment	Moderate	High
Sludge production	Low	High
Energy production	Biogas	None
Process stability to change in loading rates	Low to moderate	Moderate to high
Nutrients recovery	Possible	Not possible
COD removal	90%	Above 95%
Post treatment	Required	Not required

The advantages of aerobic treatment over anaerobic treatment include (Chan et al., 2009)

1. It can treat variety of wastewaters having variable composition.
2. Better process stability and control.
3. More BOD/COD, nitrogen and phosphorous removal.

Aerobic microbial communities can trigger a variety of biochemical mechanisms because of having large free energy potentials. Therefore, they can deal with a wide range of chemicals, low substrate concentrations and variable environmental conditions.

Though, aerobic treatment can manage a wide range of wastewaters and may produce but food to microorganism ratio and sludge age are two main parameters that can limit and adversely affect its treatment performance if it is not properly designed and monitored. Main disadvantages of aerobic treatment also include low volumetric loading rates, large sludge production and high-power input. In difference, the respective opposite of these disadvantages is known to be the advantages of anaerobic treatment. Furthermore, anaerobic microbial communities are well suited for both soluble and insoluble organic matter (Vochten et al, 1988).

The main disadvantages associated with anaerobic treatment include the following:

1. Methanogens are the slow growing microorganisms
2. If influent is high in sulfur content, and methanogens are not healthy then it can produce odors.
3. pH must be controlled along with monitoring of volatile fatty acids
4. Does not remove ammonia-nitrogen and phosphorous
5. Methanogens require some micronutrients such as Fe, Co, Ni, Mo for their healthy performance.

Additional major drawbacks of anaerobic treatment are its inability to produce a final quality effluent having high organics concentration, suspended solids, nitrogen and phosphorous, that cannot be discharged directly in to environment. Since anaerobic treatment process lacks several advantages of aerobic treatment process, and vice versa. Therefore, both should be used as a complementary to one another rather than as competitors and anaerobic process must be used as a

pretreatment step and aerobic treatment as a post treatment to produce effluent that can be discharged directly in environment.

2.4 Anaerobic-aerobic treatment

Anaerobic treatment system has a potential for energy generation and low sludge production. Therefore, high strength industrial wastewater is treated anaerobically. Though, it suffers from the slow growth rate of microbes, poor sludge settling and process instabilities. Complete stabilization of organic matter in high strength wastewater is anaerobically impossible and treated wastewater contains high concentration of ammonium and hydrogen sulfide ions. Therefore, noxious anaerobic effluent requires post treatment. The final effluent produced by anaerobic treatment is suitable for aerobic treatment as it contains solubilized organic matter, representing the potential of using anaerobic- aerobic systems (Chan et al., 2009; Chernicharo, 2005).

Following are the benefits of anaerobic–aerobic process identified by Chan et al., (2006) are listed below:

1. Anaerobic pretreatment has great potential of resource recovery through conversion of organic matter to biogas.
2. Aerobic post treatment provides high organics removal and polishes the anaerobic effluent therefore, reduces out fluctuations in anaerobic effluent quality.
3. Anaerobic pretreatment of wastewater reduces variations in oxygen demand for aerobic treatment and therefore, results in to reduced aeration cost.
4. Volatile organics in wastewater can be treated in anaerobic treatment. Therefore, reduces the possibility of volatilization in aerobic treatment.

Therefore, it will be advantageous both operationally and economically to implement anaerobic–aerobic processes for the treatment of high strength industrial wastewater as it couples the benefit of anaerobic treatment (i.e. biogas production) with the benefits of aerobic treatment i.e. enhanced or complete COD and volatile suspended solid (VSS) removal (Ros et al., 2004).

These advantages of anaerobic- aerobic treatment has encouraged the rapid development in these systems for both industrial wastewater and municipal wastewater (Chan et al., 2009).

Generally, there are four types of integrated anaerobic–aerobic bioreactor including;

1. Integrated anaerobic- aerobic system with physical separation of both zones.
2. Integrated anaerobic-aerobic systems without physical separation.
3. Sequencing batch reactors (SBR) having temporal separation of the anaerobic and the aerobic phase.
4. Combined anaerobic–aerobic culture system based on the principle of limited oxygen diffusion in microbial biofilms.

2.5 Anaerobic/Aerobic sequencing batch reactor

Sequencing batch reactors are a variation in activated sludge. SBR combines all step of activated sludge process in a single basin. A batch reactor is neither a continuous flow of wastewater entering nor leaving the reactor. Biomass and wastewater is completely mixed in reactor (Metcalf and Eddy, 2003; Vigneswaran et al., 2007).

An anaerobic sequencing batch reactor is a high rate anaerobic process and is just like aerobic SBR, but the only difference is that it is not aerated during reaction phase. The anaerobic microbes in sludge degrades the substrate and produces methane and carbon dioxide. Anaerobic SBR

operates according to the operational cycle of aerobic SBR such as feed, reaction, settling and discharge.

The main advantages of using anaerobic SBR is its simplicity in operation, biogas production and flexibility of use. Operation of AnSBR requires agitation to enhance substrate transfer to microorganisms for its degradation. transfer of the substrate to the micro-organisms in the granulated biomass for anaerobic degradation. Agitation can be carried out either mechanical stirring or by recirculating the liquid and gas phases (Singh and Srivastava, 2010). Reason for agitation requirement is to maintain homogeneity inside the reactor to enhance biogas production. Major operations of SBR cycle either it is anaerobic or aerobic are shown in Figure 2.4.

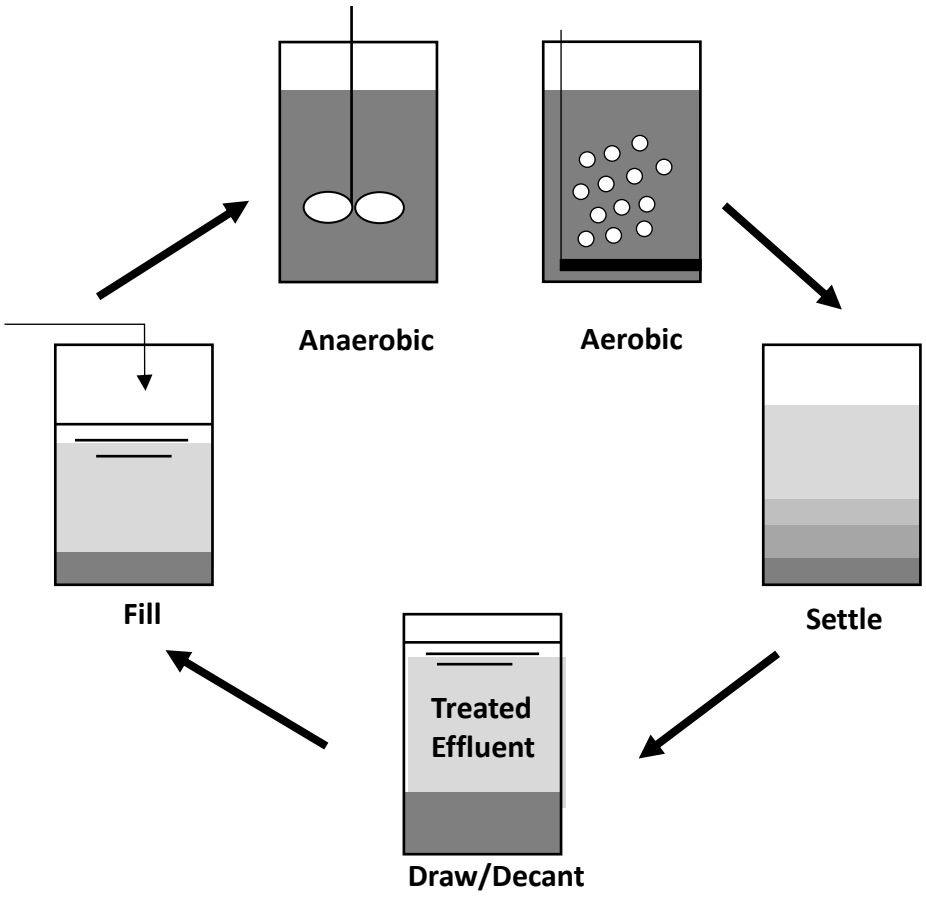

Figure 2.3 Major phases of SBR cycle

2.5.1 Anaerobic/aerobic SBR treatment process

SBR operation is based on a fill-and-draw principle. Usually it consists of five steps i.e. fill, react, settle, decant, and idle and sometimes idle step is not included (Mahvi et al., 2008).

Fill

During this phase reactor receives influent wastewater. Influent wastewater brings food for microorganisms in sludge and therefore, creates environment for biochemical reactions to occur. Mixing and aeration in reactor can be varied during this phase.

React

During this phase, no wastewater enters the system and reduction of organic matter and other nutrients such as nitrogen and phosphorous is carried out. Mechanical mixing or aeration is on throughout this phase. Removal rate of organic matter is drastically increased and most of the carbonaceous mater is removed during this phase as there is no additional organic and volume loadings. Further nitrification and denitrification occurs during the react phase.

Settle

No wastewater enters or leaves the reactor during this phase and sludge settles under non-aeration and mixing conditions. As a flocculent mass sludge settles at the bottom and with a clear supernatant is formed. This is a critical phase of SBR cycle, as if solids do not settle down rapidly, it will cause washout of biomass from reactor during decanting phase

Decant

During this last phase of SBR operation, clear supernatant from reactor is removed through a signal sent to decanter to open the effluent valve after settling phase (Reyad & Arpita, 2016).

2.5.2 Anaerobic/aerobic SBR nitrogen removal

Anaerobic digestion certainly has several benefits both from environmental and economical points of view. However, nitrogen contained in organic matter gets released when organic compounds are degraded thus, effluent from anaerobic reactor contains high ammonia concentration. Ammonia in anaerobic effluent can cause eutrophication and unwanted changes in aquatic population. Therefore, it should be adequately treated before disposal. Removal of nitrogen from wastewater is a collection of complicated chemical processes in a sequence of anaerobic- aerobic systems.

The nitrogen removal basically involves nitrification and denitrification. Nitrification is carried obligate nitrifying autotrophic bacteria. Whereas, denitrification is carried by heterotrophic bacteria which may use nitrate in under anaerobic/anoxic conditions. pH increases during aerobic phase due to CO_2 stripping, whereas alkalinity is produced and the end of denitrification (Lee et al., 1997; Zanetti et al., 2012).

The process of nitrogen removal in anaerobic- aerobic SBR system is controlled by COD/N ratio. TN removal of 95.5% was reported by adjusting the ratio. A low ratio can result in a rapid C deficit, causing an unbalanced nitrification and denitrification (Ho and Choi, 2000). Complete removal of COD and ammonia nitrogen was reported at COD/N ratio of 11.1 (Singh and Srivastava, 2011). Similarly, a low biodegradable COD/Total Kjeldhal Nitrogen (TKN) ratio showed promising results for the process optimization and nitrogen removal (Singh and Srivastava, 2011). Obaja et al. (2003) used SBR system for the treatment of wastewater containing high nutrients concentrations of 1500 mg/L NH^{+4} –N and 144 mg/l PO_3^{-4}–P. A removal efficiency of 99.7% for nitrogen and 97.3% for phosphate was attained at concluded that ratio COD/N must be higher than 1.7 to obtain complete denitrification. (Obaja et al., 2003)

For nitrification, denitrification, Obaja et al. (2003) studied piggery wastewater with high concentration of organic matter, nitrogen and phosphorus content in a single sequencing batch reactor (SBR) with anaerobic, aerobic and anoxic stages. Results showed that SBR proved to be a promising technology for the treatment of wastewater containing high nutrient content.

Organic loading rate and ammonium concentration affects simultaneous nitrification and denitrification in SBR system. With increasing organic loading rate and ammonium concentration, nitrogen removal efficiency decreases. So, by controlling carbon source concentration, total nitrogen removal can be enhanced (Chiu et al., 2007).

2.5.3 Design and operational parameters

Major parameters that are considered important for the operation of anaerobic/aerobic are Hydraulic retention time (HRT), Solids retention time (SRT), Organic loading rate (OLR), gas production rate (m^3CH_4/day), influent COD, C/N ratio

Hydraulic retention time is known as the time spent by wastewater for degradation of organic matter in reactor (Zaher et al., 2007). It is the most important parameter in anaerobic SBR as it affects the production of methane (Gerardi, 2003). HRT for the treatment of various kinds of wastewater in anaerobic SBR is within the range of 0.5 to 3 (Dague, 1993; Timur & Özturk, 1999; Massé & Masse, 2001; Ruiz et al., 2002; Shizas & Bagley, 2002; Ammary, 2005). For the treatment of yeast industry wastewater Krapvinia et al. (2007) varied HRT from 2.5 – 10 days for the treatment of yeast industry wastewater. For the treatment of wastewater containing complex organic matter Upendrakumar et al. (2006) applied HRT of 20 d.

Solids retention time, SRT (days), represents the time that solids (microorganisms) stays in the reactor. This is more important parameter then HRT for several reasons. First of all, it is directly

connected to the time needed for bacteria to multiply in the digester. If the time that microorganism spend in the reactor is shorter than the time they need to multiply (generation time) a situation of "washout" occurs i.e the bacteria is been washed out of the system. The second important point is that SRT directly influence the efficiency or competition of the digestion process. The longer the SRT the more complete the digestion process will be and the higher will be the amount of biogas produced and the less residual sludge is left for further handling. According to Rittmann & McCarty (2001), the minimum SRT for an anaerobic CSTR at 35ºC is 10 days

Organic loading rate (OLR) is a measure of the biological conversion capacity of the system. Feeding the system above its sustainable OLR results in low biogas yield due to accumulation of inhibiting substances such as fatty acids in the digester slurry (Vandevivere, 1999

Mixing is required for the distribution of wastewater constituents and biomass evenly throughout the reactor, for efficient mass transfer from the bulk liquid to the activated bioflocs, and for preventing flocs from coagulating and keeping them in suspension. Depending on the configuration and size of SBR, varying water levels, the aeration strategy etc., one or more types of mixer will be applied (Wilderer, 2001).

Mixing of the digester content has significant impact over the process efficiency in several ways. Mixing provides better biomass distribution in the reactor making it homogenous, and equalize the temperature inside the reactor. The metabolic activities of acetate forming bacteria and methane-forming bacteria requires that they should be in close spatial contact. Slow, gentle mixing ensures that contact. Also, mixing provides for efficient hydrolysis of wastes and production of organic acids and alcohols by acid-forming bacteria (Gerardi, 2003).

Mixing regime can have intermittent or continuous character and can be done by mechanical applications or gas recirculation. The purpose of mixing in a digester is to blend the fresh material

with digestate containing microbes. Furthermore, mixing prevents scum formation and avoids temperature gradients within the digester. However excessive mixing can disrupt the microbes so slow mixing is preferred. The kind of mixing equipment and amount of mixing varies with the type of reactor and the solids content in the digester.

Different types of mixing methods exist for anaerobic digestion (Karim et al., 2005). In general, the mixing of the digester content improves the degradation process as it guarantees standardised conditions within the reactor (Gerardi, 2003). The substrate and nutrients are evenly circulated whereas the pH and temperature are evenly controlled (Sung & Dague, 1995; Zaiat et al., 2001; Gerardi, 2003; Kaparaju et al., 2008). The main reason for mixing is to improve the contact between the liquid phase (substrates) and the biomass which increases the mass transfer fluxes (Zaiat et al., 2001; Rodrigues et al., 2003; Michelan et al., 2009). The hydrolysis rate is enhanced with mixing as it provides a larger surface:volume ratio for the hydrolytic bacteria to work effectively, therefore enhancing the overall reaction rate (Gerardi, 2003; Michelan et al., 2009)

Mixing within the AnSBR is provided by biogas recirculation, liquid recirculation or mechanical mixing (Zaiat et al., 2001; Michelan et al., 2009). Different mixing regimes are reported for AnSBR and can be equipped either by intermittent of continuous mixing regimes (Michelan et al., 2009). According to the mini-review by Zaiat et al. (2001) the mixing conditions in AnSBRs have not been established, although some studies are indicative of conditions. Results related to mixing methods are inconsistent (Kaparaju et al., 2008). According to Michelan et al. (2009) most of the AnSBR mixing regimes are carried out by means of mechanical mixing or biogas recirculation. Literature varies in terms of the type of mixing methods (gas recirculation; liquid recirculation or mechanical mixing), the mode of operation (continuous or intermittent mixing) and the intensities of the mixing modes.

2.5.4 Anaerobic-aerobic SBR modes of operation

2.5.4.1 Single reactor configuration sequential anaerobic-suspended growth aerobic systems

In a single reactor configuration, anaerobic- aerobic SBR system, microbial population enrichment is carried by alternating anaerobic and aerobic phase through controlling mixing and aeration in reactor. The HRT of each phase, oxygen concentration, and mixing can be varied as per treatment requirements. During the operation of anaerobic–aerobic SBR, during anaerobic phase nitrogen gas is purged and air is supplied during the aerobic phase. Different investigations have been done to observe the effects of anaerobic–aerobic residence time on the performance of SBR in terms of COD and nutrients removal. Biogas production and anaerobic phase stability was not considered during the operation of those integrated systems.

The experimental results of various studies have indicated that HRT of anaerobic and aerobic phases in anaerobic-aerobic SBR system significantly affected the system's performance. Mostly anaerobic- aerobic SBR of single reactor configuration were used for the treatment of industrial wastewater such as slaughter house wastewater and textile wastewater for efficient COD removal with no objective of biogas production (Supaka et al., 2004; Gon et al., 2005; Kapdan et al., 2006).

COD removal of 90% for textile wastewater was achieved at HRT of anaerobic/aerobic phase of 17.5/2.5 h was achieved 87% with an anaerobic/aerobic cycle of 14/6 h. It was found that the HRT of anaerobic phase should be long enough to obtain better COD removal (Gon et al., 2005).

For the treatment of synthetic textile wastewater Kapdan et al., (2006) showed similar results. Optimum HRT for maximum COD removal of more than 85% were determined as 12 h for anaerobic and 11 h for aerobic phase with combined HRT of 23 h. Results showed that for HRT

of 19- 20 h in anaerobic phase, COD removal in aerobic phase declined due to the production of toxic end products of anaerobic phase with overall COD removal of less than 80%.

On the other hand, for HRT of 8 and 12 h during anaerobic and aerobic phase, respectively, COD and BOD_5 removal efficiencies of 85 ± 6% and 95 ± 4% were achieved respectively for the treatment of wool dyeing effluents. The optimum HRT for anaerobic and aerobic phase is 8 and 12 h, respectively with total cycle of 24 h. Therefore, results indicated that a longer aeration time results in to better performance, due to effective cell growth. But, from economical point of view this strategy is not feasible (Gon etal., 2005).

For the treatment of slaughter house wastewater anaerobic aerobic SBR system was also used. Three aerobic-anoxic operating conditions were adopted, namely (4+4), (5+3) and (3+5). Through this study, it was found that maximum 86 to 96 % COD and 74.75% of NH_4-N were achieved at (4+4) aerobic-anoxic operating strategy and was found to be the best operating strategy (Kundu et al., 2014).

2.5.3.2 Anaerobic SBR followed by aerobic SBR configuration

For the treatment of piggery wastewater two lab scale SBTR reactors (anaerobic + aerobic) were used. Both reactors had a maximum volume of 1.5L. It was filled up to 0.75L by anaerobic sludge to see the change in efficiency. The overall removal performance was 81-90% for COD and 85-90 % for TKN. Recycling ratio was between 1-3 and was concluded that higher recycle ratio will promote lower concentrations of nitrogen oxides in the effluent (Bernet et al., 2000).

Therefore anaerobic–aerobic SBR has been proved to be a suitable technology for organic removal from textile, slaughter house and piggery wastewater. However, no investigations were carried on control on methanogenic activity, anaerobic reactor stability, biogas production and recovery from

organic shock loads. My research will fulfil these loop holes in anaerobic- aerobic SBR by keeping in view the stability of both reactors to maximize biogas production, organics and nutrients removal as well.

Chapter 3

MATERIALS AND METHODS

3.1 Feed composition

The wastewater, employed in this study, was prepared synthetically having a COD concentration of 1000, 2000 & 3000 mg COD L^{-1} and C: N: P of 100:10:1. The composition of synthetic wastewater is given in Table 3.1. The synthetic wastewater consisted of glucose as a sole carbon source. Ammonium chloride was added as a source of nitrogen whereas potassium dihydrogen phosphate was added as a source of phosphorous. Calcium chloride ($CaCl_2$), magnesium sulphates ($MgSO_4.7H_2O$), iron chloride ($FeCl_3$), cobalt chloride ($CoCl_2$) and zinc chloride ($ZnCl_2$) were added as micro nutrients. Sodium bicarbonate was added to maintain pH of feed within the range of 6.8 to 7.2.

Table 3.1 Composition of feed

Chemical	Units	COD = 1000 mg/L	COD = 2000mg/L	COD = 3000mg/L
$C_6H_{12}O_6$	mg/L	1000	2000	3000
NH_4Cl	mg/L	382	764	1146
KH_2PO_4	mg/L	47.7	95.4	143.1
$CaCl_2$	mg/L	9.73	19.46	29.1
$MgSO_4.7H_2O$	mg/L	9.73	9.73	9.73
$FeCl_3$	mg/L	5	5	5
$NaHCO_3$	mg/L	200	400	600
$CoCl_2$	mg/L	0.1	0.1	0.1
$ZnCl_2$	mg/L	0.1	0.1	0.1

During industrial application of decoupled system, anaerobic reactor was fed with synthetic textile wastewater having a COD concentration of 3000 mg/L and mixture of reactive and basic dyes including cibacron blue, cibacron yellow and methylene blue was added each having a concentration of 2 mg/L.

3.2 System set- up

The lab scale anaerobic-aerobic system consisted of 50 L feed tank, 1.5 L wastewater level controller in anaerobic tank, 6 L anaerobic SBR, 6 L aerobic SBR, 2 L gas collector and effluent collection tank. A photo of the setup is shown in Figure 3.1.

Figure 3.1 Anaerobic- Aerobic SBR system

50 L plastic drum as shown in Figure. 3.2 (a) was provided to store feed wastewater. Wastewater level controller was provided before anaerobic SBR to control water level in anaerobic SBR to It was made of 5 mm thick acrylic sheet (Figure 3.2 (b)).

Figure 3.2 (a) Feed tank **(b) Water level controller**

The AnSBR reactor (Figure 3.3) was prepared using clear acrylic sheets assembled using chloroform. In addition to the holes drilled for the bolting of lid to the cylinder, there were five other holes for the stirrer, immersion heater, thermocouple, NaHCO3 dosing line and the biogas line respectively. The stirrer was connected to an external motor for mixing of the reactor contents during the react phase.

Figure 3.3 Anaerobic SBR

The fill point was at the top of the reactor. The decant point of the reactor was located at the bottom of the reactor. The top lid was secured to the cylinder of the reactor using 12 sets of bolts and nuts arranged symmetrically and rubber gasket was placed in between lid and circumference of cylinder. The bolts and nuts were carefully tightened to make sure the reactor was air-tight but not too tight as to cause cracks to the acrylic material. The dimensions and details of the reactor construction are illustrated in Figure 3.4 and Table 3.2.

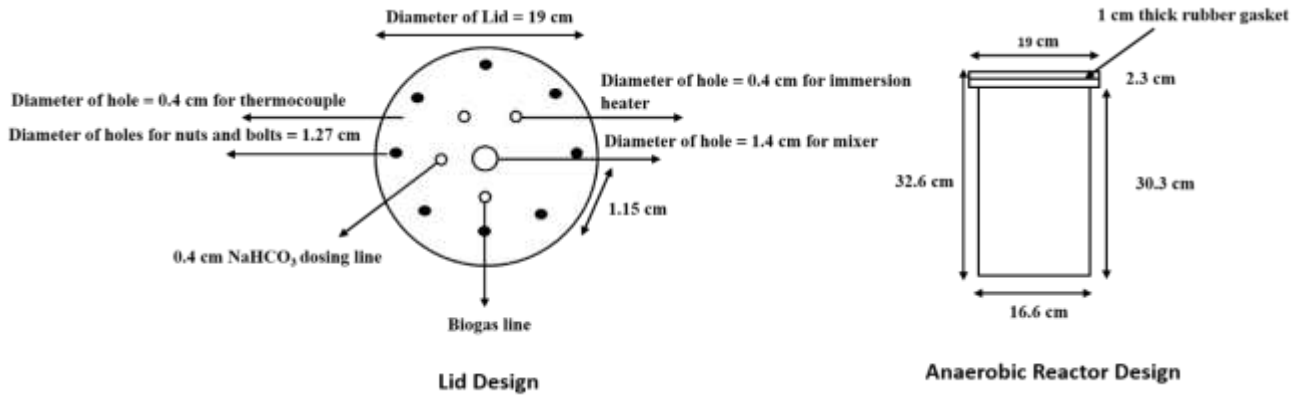

Figure 3.4 Construction drawing of anaerobic SBR

Five ports were provided having length of 2.5 cm and internal diameter of 10 mm. All the five ports were distributed at equal intervals along the effective height of the column. The first port was used as an inlet port for reactor feeding, while the bottom most was used as an outlet port for reactor decanting. The second, third and the fourth port from top were used as sampling ports. The anaerobic reactor was equipped with Cole Parmer, Stir- Pak, model no 50002-20, USA mixer for mixing of sludge and wastewater at a speed of 180-270 rpm, immersion heater and K-Type thermocouple to maintain temperature of 30ºC inside the reactor.

Table 3.2 Design details of anaerobic SBR

Total volume of reactor	6.5 L
Working volume	6.0 L
Inner diameter of cylinder	16.6 cm
Outer diameter of cylinder	17.6 cm
Diameter of Lid	19.0 cm
Number of holes on lid	5
Number of ports on cylinder	5
Diameter of each port	10 mm

The gas tank was of a cylindrical shape made up of 2 mm thick acrylic sheet having an internal diameter of 8 cm and height of 30 cm (Figure 3.5). The generated gas was measured daily for each HRT. The gas tank was initially filled with water saturated with NaCl. The biogas measuring pipe connected to anaerobic reactor was attached to a valve which was operated manually to measure biogas. The volume of liberated gas was measured by the displacement of water in the gas tank.

Figure 3.5 Biogas measuring system

Aerobic reactor was constructed from 5mm thick acrylic sheet (Figure 3.6). Total volume of reactor was 6.5L with a working volume of 6L initially and was then reduced to $1/12^{th}$ to enhance biogas production, organics removal and to reduce aeration cost. Outer and inner diameters were 17.5 and 16.5 cm respectively. Aerobic reactor was equipped with a circular diffuser connected to air pump. Three ports were provided for feeding, decanting and sampling.

Figure 3.6 Aerobic SBR

3.3 Process flow diagram of anaerobic- aerobic SBR system

The feed wastewater was stored in the feed tank. Wastewater was initially fed to anaerobic SBR under gravity. Wastewater level in anaerobic SBR was maintained to 6 L with the help of relay and solenoid valve operated by digital timers. Continuous mixing was provided with the mechanical mixer during the reaction phase anaerobic reactor. During reaction phase, temperature was maintained to $30^{o}C$ with the help of immersion heater, k- type thermocouple, magnetic contractor and thermostat operated via digital timers. During settling phase mixing was stopped via digital timers. Decanting was carried out by the opening of ball valve provided at the bottom of reactor to remove treated wastewater. Opening of ball valve was carried out by the solenoid

valve operated via timers. During decanting, immersion heater was stopped. Biogas generated in anaerobic SBR was measured through water displacement method.

Effluent of anaerobic SBR was fed to aerobic SBR through inlet valve provided at the top of reactor, connected to solenoid valve. Continuous aeration was provided with air pump during reaction phase for mixing of sludge and wastewater. Aeration was stopped during settling phase with the help of digital timers to allow the sludge to settle down. Decanting of aerobic SBR was carried out by the valve connected to solenoid valve at the bottom of reactor operated by digital timers to remove treated wastewater.

The schematic diagram of anaerobic- aerobic SBR treatment units are shown in Figure 3.7.

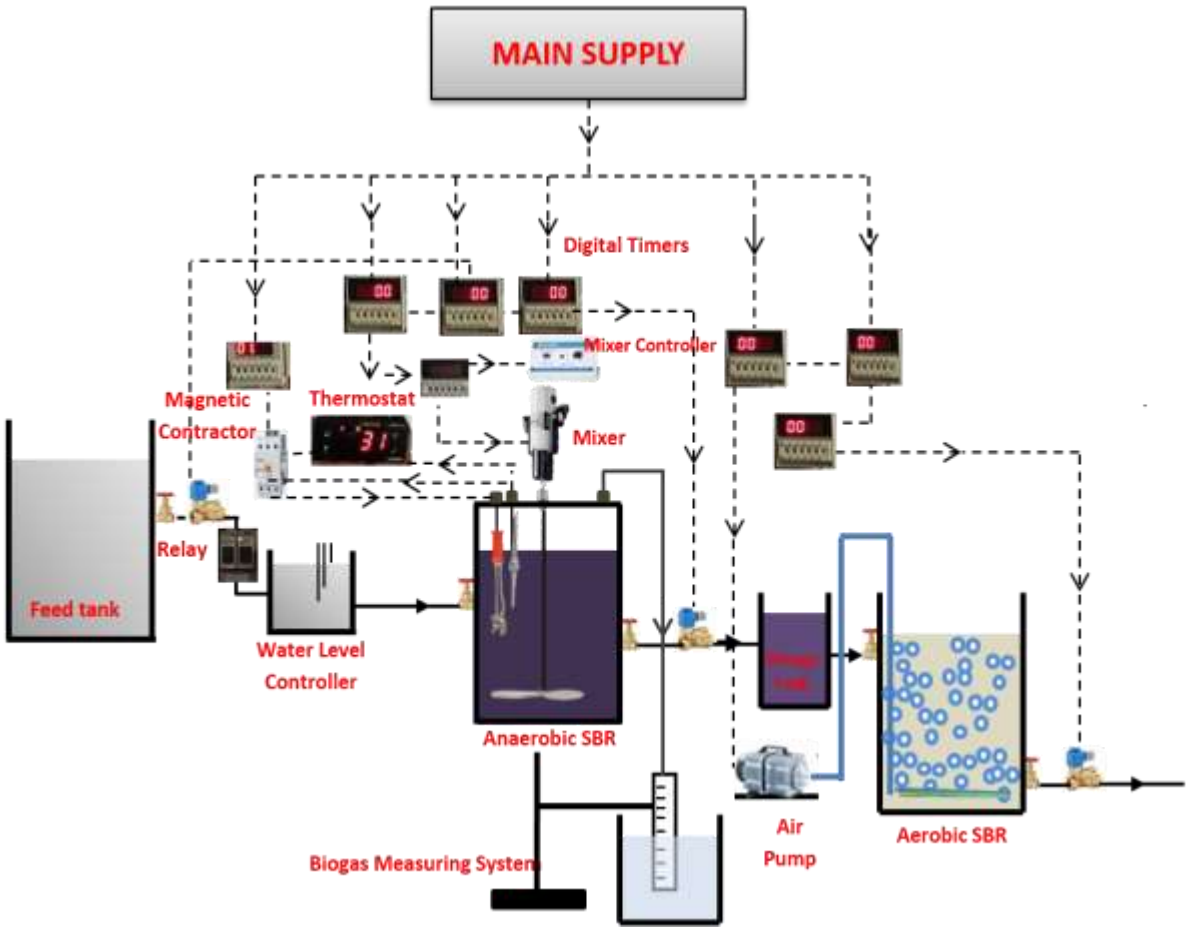

Figure 3.7 Schematic diagram of anaerobic – aerobic SBR system

3.4 Operational stages and conditions in the SBR cycle modes

HRT cycle in each reactor for both coupled and decoupled systems was divided into four discrete phases or periods, i.e., fill, react, settle and draw. Equipment that were activated or deactivated during each phase are shown in Table 3.3.

Table 3.3 Equipment activated/ deactivated during each phase of anaerobic/aerobic cycle

Phase	Inlet valve	Decant valve	Mixer/aerator
Fill	●	○	○
React	○	○	●
Settle	○	○	○
Decant	○	●	○

● Activated

○ Deactivated

3.4.1 Operational stages and conditions in coupled system

For coupled anaerobic – aerobic system, operation time for each anaerobic and aerobic reactor was 12 hours. Table 3.4 represents operating time for different operational stages.

Table 3.4 Operating time for operational stages of coupled anaerobic – aerobic system

STAGE	TIME
Fill	7.5 min
Reaction	11 hours
Settle	45 minutes
Decant	7.5 minutes

The coupled anaerobic-aerobic SBR system was operated for 72 days consecutively at increasing OLR in an anaerobic reactor by increasing COD concentration and keeping HRT of 12h constant in each reactor. Table 3.5 represents the operating conditions under different OLRs.

Table 3.5 Operating conditions for coupled anaerobic – aerobic system

Phase	Run Time (days)	COD (mg/l)	Anaerobic SBR HRT (hours)	Aerobic SBR HRT (hours)	OLR in Anaerobic SBR (kg COD/m^3.d)	SRT in Aerobic SBR (days)
1	1-31	1000	12	12	2	10, 20 & 30
2	31-55	2000	12	12	4	30
3	55-72	3000	12	12	6	30

3.4.2 Operational stages and conditions in Decoupled system

For decoupled system, operation time for anaerobic SBR was 48 and 6 hours for aerobic SBR. Table 3.6 represents operating time for different operational stages.

Table 3. 6 Operating time for operational stages of decoupled anaerobic – aerobic system

STAGE	TIME
Fill	7.5 min
Reaction	47/5 hours
Settle	45 minutes
Decant	7.5 minutes

The decoupled anaerobic-aerobic SBR system was operated for 30 days consecutively. After 20 days, decoupled system was operated to treat textile wastewater having COD concentration of 3000 mg/L and dyes concentration of 6 mg/l. Table 3.7 represents the operating conditions for decoupled anaerobic – aerobic system.

Table 3.7 Operating conditions for decoupled anaerobic – aerobic system

Phase	Operating Period (days)	COD (mg/L)	HRT (h)	OLR in Anaerobic SBR (kg COD/m^3.d)	SRT in Aerobic SBR (days)
4	72-92	3000	48 + 6	1.5	30
5	92-102	3000+Dyes addition (6 mg/L)	48 + 6	1.5	30

During phase 4, after 12, 18, 24, 36, 42 and 48 hours sampling was done from anaerobic reactor and after 1, 2, 3, 4, 5 and 6 hours from aerobic reactor. During phase 5, sample was collected from anaerobic reactor after 48 and 6h from aerobic reactor.

3.5 Tests and analysis

COD, MLSS, MLVSS, VFA, Alkalinity & TKN were measured following the standards APHA methods (APHA, 2012). COD was measured by pH in the anaerobic reactor was measured by pH meter (Cyberscan 500). UV spectrometry of dye mixture was performed within the visible range of light, i.e. 615 nm by using UV visible spectrophotometer (PC Model T60U) to measure the color removal.

COD

COD determination was carried out with closed reflux titrimetric method. A sample of 2.5 mL, 1.5 mL of digestion reagent ($K_2Cr_2O_7$) and 3.5 mL of sulfuric acid reagent were added in COD vials and were capped. And were inverted several times to mix completely. The vials were then placed in a COD digester (Hach 2) which was preheated to 150 ^oC for 2h and the mixture was refluxed. Vials were then cooled at room temperature and the mixture was titrated with ferrous ammonium sulfate (FAS) using ferroin indicator. Following is the equation that was used for COD calculations.

$$\mathbf{COD}\ (\frac{\mathbf{mg}}{\mathbf{L}}) = \frac{(\mathbf{A}-\mathbf{B})\times\mathbf{1000}\times\mathbf{8}}{\mathbf{Sample\ volume\ (mL)}}$$

Where,

A = Volume of FAS used to titrate sample (mL)

B = Volume of FAS used to titrate blank (mL)

TKN

Firstly, digestion of sample was carried out by placing 100 mL sample in volumetric flask and was diluted to 300 mL, 50 mL digestion reagent was added and few glass beads were added. After mixing the flask was placed on heating plate at 360- 400^{o}C under a fume hood until the solution turned clear leaving no charred particles. It was heated for additional 30 minutes. After that flask was allowed to cool down to room temperature. After that pH of mixture was adjusted to 10 by adding NaOH- $Na_2S_2O_3$ solution. After adjusting pH, mixture was diluted to 300 ml by adding distilled water. After that mixture in flask was immediately added to kjeldhal flask on distillation stand. Flask on distillation stand was then connected to condenser and water was then allowed to flow in the condenser. Lower tip of condenser was placed in 50 mL indicating boric acid solution. Contents in kjeldhal flask were distilled in 50 mL boric solution. 200 ml distillate below the surface of boric acid was collected. With time colour of boric acid changed from purple to green that indicated absorbance of ammonia in the indicating boric acid solution and was then titrated against 0.02N H_2SO_4 to quantify the ammonia absorbed by boric acid. End point of titration was conversion of green colour to its original purple colour. Following is the equation that was used for TKN calculations.

$$\mathbf{TKN}\ (\frac{\mathbf{mg}}{\mathbf{L}}) = \frac{(\mathbf{A}-\mathbf{B})\times\mathbf{280}}{\mathbf{Sample\ volume\ (mL)}}$$

Where,

A = Volume of H_2SO_4 to titrate the sample (mL)

B = Volume of H_2SO_4 to titrate the sample (mL)

VFA and Alkalinity

50 ml sample from anaerobic SBR effluent was collected and its pH was measured by pH meter (Cyberscan 500). If pH was above 6.5 then it was titrated with 0.1N H_2SO_4 to bring its pH value to 6.5. After that it was titrated with H_2SO_4 and pH was adjusted to 3.0 and volume of acid consumed was measured for alkalinity calculations.

Following is the equation that was used for alkalinity calculations.

$$\textbf{Alkalinity } \left(\frac{\textbf{mg}}{\textbf{L}}\right) = \frac{\textbf{Volume of acid consumed} \times \textbf{Normality of acid used} \times \textbf{5000}}{\textbf{Sample volume (mL)}}$$

For VFA measurement, titrated sample was the used. Beaker was then placed on heating plate and temperature was allowed to reach up to 70 to 80^{o}C. Sample was then allowed to cool down at room temperature. After that sample was titrated against 0.1 N NaOH until the pH of sample reached to 6.5 and the volume of NaOH consumed was measured for VFA calculations.

Following is the equation that was used for VFA calculations.

$$\textbf{VFA } \left(\frac{\textbf{mg}}{\textbf{L}}\right) = \frac{\textbf{Volume of alkali consumed} \times \textbf{Normality of alkali used} \times \textbf{5000}}{\textbf{Sample volume (mL)}}$$

MLSS/MLVSS

Whattman filter paper was firstly dried in oven at 105^{o}C for 30 minutes to remove moisture from filter paper if any. Filter paper was then placed in desiccator till it cool down to room temperature and initial weight of filter paper was weighed. Then it was placed inside the filtration assembly.

10 mL sludge sample was collected from anaerobic SBR during mixing phase and during aeration phase from aerobic SBR. Sludge sample was then poured on filter paper placed inside the assembly and filtration assembly was stated to suck water from filter paper. When all the water was removed from filter paper and a sludge cake was developed on filter paper, filtration assembly was switched off. Filter paper was then placed in china dish ad was put in oven at 105^{o}C for 1 hour. After 1 hour china dish was placed in desiccator to allow the filter paper to cool down. After that filter paper was weighed and MLSS in sludge sample was measured using following formula.

$$\textbf{MLSS}\ (\frac{\textbf{mg}}{\textbf{L}}) = \frac{(\textbf{A} - \textbf{B}) \times \textbf{1000}}{\textbf{Sample volume}\ (\textbf{mL})}$$

Where

A = Weight of filter paper + residues on it after drying at 105oC

B = Weight of filter paper

For the measurement of MLVSS oven dried filter paper containing residues was then placed in muffle furnace for ignition at 550^{o}C for 30 minutes. After that china dish was placed in desiccator to allow the filter paper to cool down and was then weighed. MLVSS concentration in sludge was measured using following formula.

$$\textbf{MLVSS}\ (\frac{\textbf{mg}}{\textbf{L}}) = \frac{(\textbf{A} - \textbf{B}) \times \textbf{1000}}{\textbf{Sample volume}\ (\textbf{mL})}$$

Where

A = Weight of filter paper + residues on it after drying at 105^{o}C

B = Weight of filter paper + residues on it after ignition at 550^{o}C

3.6 Startup of reactors

One of the crucial steps for stable operation of anaerobic system is the start-up period. For the startup of anaerobic reactor initially 6 L of aerobic sludge having MLSS of 9 g/L was collected from the full scale MBR plant at NUST, Islamabad, Pakistan. Nitrogen gas was purged into the anaerobic SBR to achieve anaerobic conditions. Initially for 20 days sludge was fed just with 6000 mg of glucose after every two days. During this period, pH of sludge was maintained between 6.8-7.2 and the temperature was maintained to 30^0C inside the anaerobic reactor.

After 20 days, anaerobic reactor was then fed with synthetic wastewater for 60 days under an OLR of 1 kg COD/m^3.d at COD concentration of 1000 mg/L. For the startup of aerobic reactor, 2 liters settled sludge was collected from NUST MBR Plant, Islamabad, Pakistan

Chapter 4

RESULTS AND DISCUSSION

4.1 Performance of anaerobic SBR during its start up

The acclimatization of anaerobic sludge with wastewater during the start-up period was monitored by daily measurement of biogas production and effluent COD until steady state was achieved. Anaerobic reactor was then integrated with aerobic treatment to provide post treatment to its effluent. Biogas was not generated during initial stage of start-up, which shows that sludge activity was initially slow. Feed concentration of 1000 mg/L helped to control VFAs production. pH was maintained within in the range of 6.8-7.2 with addition of sodium bicarbonate.

Initially COD removal rate was high due to the activity of inoculated aerobic activated sludge but with time its performance dropped due to anaerobic conditions. However, the treatment performance stabilized with time and no biogas production was observed during this stage. Biogas production was observed after 25 days with an average of 6 L/day as shown in Figure 4.1 clearly indicating a successful startup of anaerobic reactor.

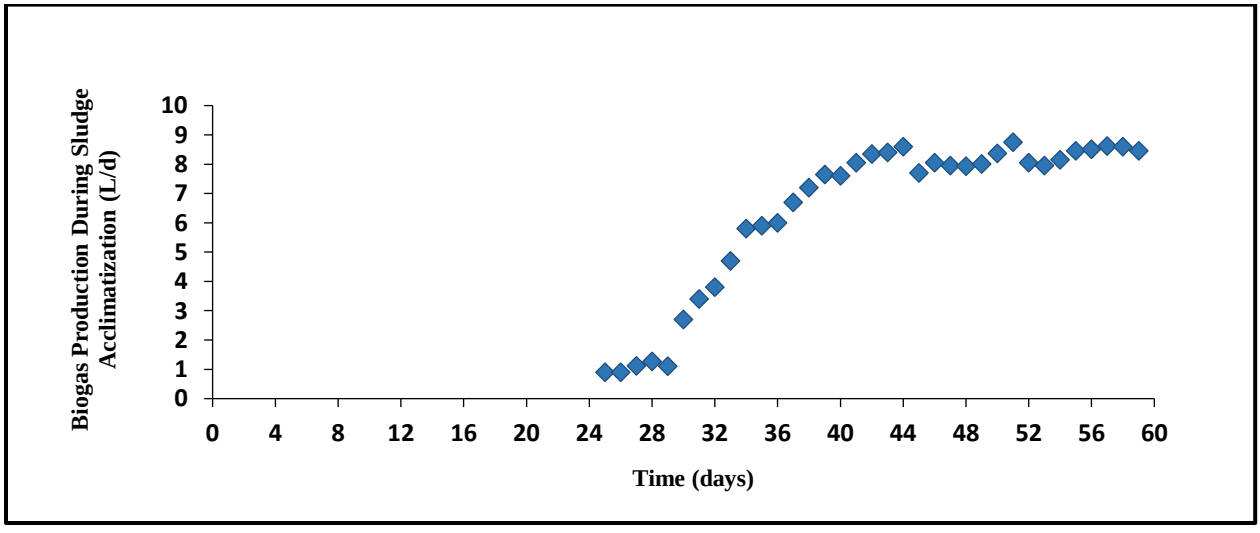

Figure 4.1 Biogas production during startup of anaerobic SBR

4.2 Treatment Performance of Coupled Anaerobic-Aerobic System

4.2.1 Effect of organic loading rate on COD removal

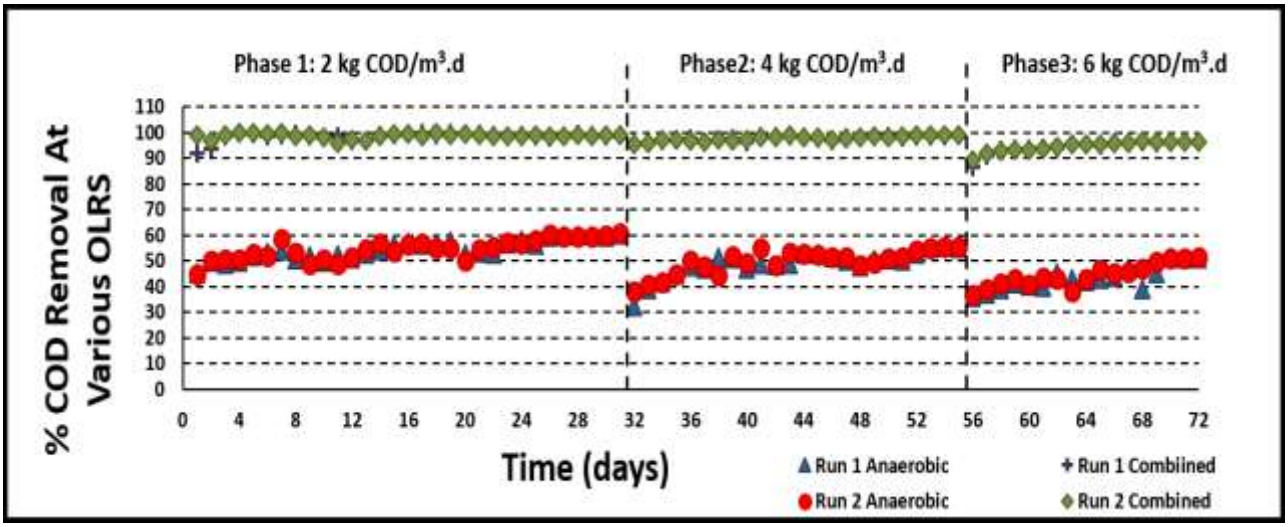

Figure 4.2 % COD removal at various OLRs

Figure. 4.2 illustrates the COD removal trend at three different OLRs. HRT of 12 h was maintained in each reactor under three OLR conditions. Summary of COD removal rates at various OLRs is shown in Table 4.1. At the beginning of each OLR there was a decrease in the COD removal efficiency in both reactors, but the system recovered gradually with time due to adaptation of microbes to the new conditions. The reason for decrease in removal efficiency immediately after increasing OLR is that certain time is required by microbes to get adapted to higher organic load or COD concentrations.

At OLR of 2 kg COD/m^3.d, an average of 59.0% of COD was removed in anaerobic reactor and remaining was treated aerobically which gave combined removal of 98.5 % after achieving steady state this removal rate was 99.7 % with COD concentration of 3 mg/L in effluent and therefore met the NEQS. Literature has reported that if high amount of COD removal is accomplished in the anaerobic reactor, insufficient COD or other nutrients will be left in the effluent, which may not support the performance of aerobes in the following aerobic reactor. Hence, in the optimization of

the COD removal in anaerobic reactor, it was crucial to make sure that adequate, but not too much COD is left in the effluent for successful functioning of the aerobic bioreactor (Chan et al., 2009) therefore, OLR in anaerobic reactor was increased from 2 to 4 kg COD/m^3.d .

During OLR of 4 kg COD/m^3.d in anaerobic reactor, initially decline in COD removal was observed and then it increased gradually with time with an average of 52.13% and remaining COD was treated aerobically which gave an average combined removal of 97.65 % after achieving steady state this removal rate was 99% with COD concentration of 20 mg/l in effluent .

During OLR 6 kg COD/m^3.d in anaerobic reactor, initially decline in COD removal was 44.0% and combined average removal of 95.0% after achieving steady state this removal rate was 96.3% with COD concentration of 111 mg/L in effluent and met the NEQS level i.e <150 mg/L NEQS). Reason for decreased removal efficiency was the COD concentration of anaerobic effluent that was 1686 mg/L reactor and aerobic treatment is suitable mostly for wastewater having concentration lower than 1000 mg/L concentration of anaerobic effluent was greater than 1000 mg/L (Chan et al., 2009). Another reason for decline in percentage organics removal is due to the shorter reaction time for increased organics concentration, which caused the reactor to work as a hydrolytic-acidogenic system (Donos et al., 2009).

Various studies have shown that higher OLRs exhibited reduce COD removal efficiencies in wastewater treatment systems (Torkian et al., 2003; Sanchez et al., 2005). Mohan et al., (2007) treated dairy wastewater in anaerobic SBR and also reported decline in COD removal efficiency with increase in OLR. Overall, COD concentration in anaerobic effluent during OLR 2, 4 and 6 kg COD/m^3.d was 410, 958 and 1686 mg/L. COD concentrations after providing aerobic treatment were 15, 48 and 165 mg/L.

Table 4.1 Summary of COD removal at various OLRs

OLR (kg COD/m^3.d)	Anaerobic SBR % COD Removal		Combined Anaerobic-Aerobic SBR % COD Removal	
	Average	Range	Average	Range
2	59	45.2-60.5	98.51	92-99.8
4	52.13	32.2-55.4	97.65	95.2- 99.0
6	43.83	35.63-51.36	94.5	86.7-96.3

4.2.2 TKN removal

An average drop in TKN removal from both anaerobic and aerobic SBR was observed by increasing OLR in anaerobic SBR. During OLR of 2, 4 and 6 kg COD/m^3.d, average % TKN removal of 30, 23 and 20, respectively in anaerobic SBR and 81, 55 and 47 % from combined treatment, respectively. Overall combined performance of both SBR systems decreased from 81.0 to 47.0 % having concentration of 19 and 159 mg/L, respectively and therefore it didn't met the NEQS at higher organic loading rate i.e. ammonia concentration should be less than 40 mg/L.

Decline in TKN removal of aerobic SBR might be due to loss of nitrifying activity at higher organic loads (Mohan et al., 2007). Higher organic load has been reported to inhibit nitrification decreasing the ammonia and nitrogen removal rates. This view was also supported by Tawfik et al., (2002) and Lyssenko and Wheaton (2006). According to He et al. (2007), high loading of organic material results in lower nitrification efficiency because of loss of ammonium through assimilation by heterotrophs. The overall ammonia removal dropped at a higher organic loading rate, which can be attributed to the domination of heterotrophic bacteria at a high organic loading rate which exerted a negative effect on the rate of nitrification (Tawfik et al., 2002).

4.2.3 Effect of organic loading rate on stability of anaerobic reactor and biogas production

Stability indicators of any anaerobic reactor are pH, VFAs concentration and total alkalinity. These indicators help to determine the balance between methanogenesis and acidogenesis (Murto et al.,

2004; Boe, 2006). In digestion process, acidogenic element generates acid, but under normal condition it is buffered by reactor's alkalinity. During the anaerobic degradation of organic matter, CO_2 is generated which is a major source of alkalinity (Akuzawa et al., 2011).

Results showed that, several factors like COD removal, biogas production, pH, volatile fatty acids production and alkalinity are greatly affected by changing the organic loading rate and wastewater concentration, and this trend is shown in Table 4.2 and Figure 4.3 and 4.4.

Table 4.2 Summary of stability indicators at various OLRs

OLR (kg COD/m³.d)	pH Range	VFA's Range (mg/L)	Alkalinity Range (mg/L)	Biogas Production (L/day)	VFA/Alkalinity ratio
2	6.23- 6.99	475-225	605 -720	9.45-12.35 (11)	0.31-0.79
4	6.07- 6.87	575-470	1020-1200	10.5-11.65 (11.27)	0.39- 0.56
6	5.7- 6.3	730-700	1026-1280	9.85-11.55 (10.77)	0.5-0.73

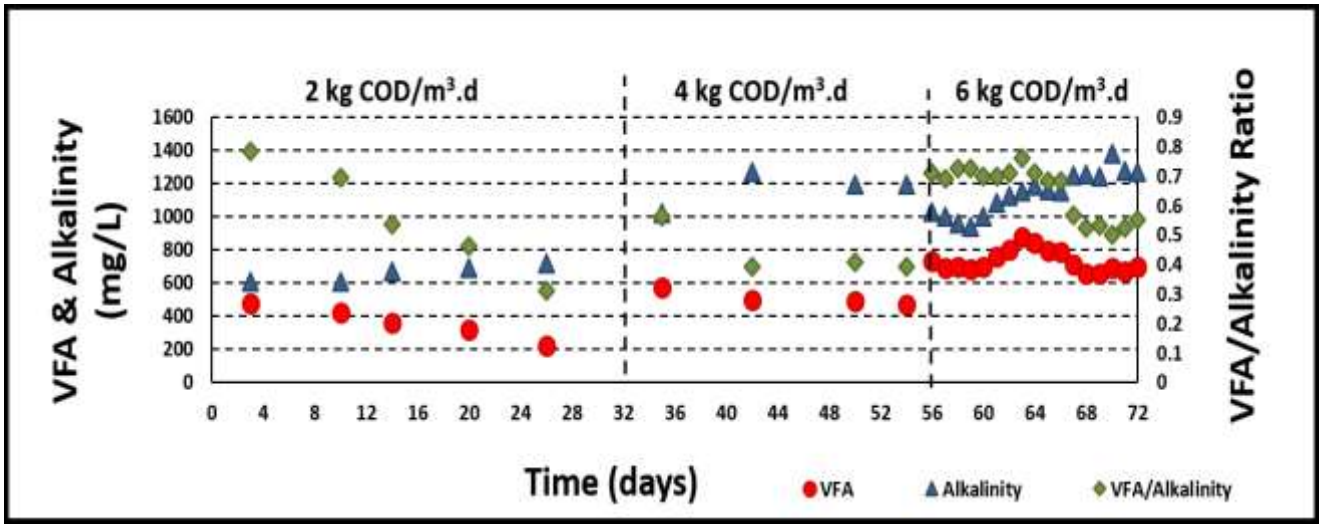

Figure 4.3 VFA, Alkalinity and VFA/Alkalinity ratio at various OLRs

VFAs were measured to determine the adaptability of anaerobic system to high organic loading rate or not. A high concentration of VFA occurred initially in anaerobic reactor effluent during

each phase when OLR was increased, indicating prevalence of acid fermentation and overloading of anaerobic reactor (Fuchs et al., 2003; Akuzawa et al., 2011) but once microbes were adapted to change in loading rate, VFA concentration returned to lower values with increase in alkalinity .The VFA concentration in the effluent during first phase decreased from 475 to 225 mg/L, from 575 to 470 mg/L and from 730 to 700 mg/L for OLRs 2, 4 and 6 Kg COD/m^3.d, respectively. For the treatment of dairy wastewater, Mohan et al., (2007) reported similar trend of increased VFA's production with increase in OLR.

These results confirm that when the load applied to the system increases, the methanogenic bacteria can not completely degrade the VFA produced; therefore, the efficiency and stability of the reactor are affected negatively (Wang et al., 2009).

The alkalinity represents the buffering capacity of the system and how it can neutralize the acidification of the VFAs. This stability indicator is interesting because it is more sensitive to process changes than the pH. During OLR 2, 4 and 6 kg COD/m^3.d alkalinity increased from 605 to 720 mg/L, 1000 to 1200 mg/L and from 1000 to 1200 mg/L, respectively.

Stability of an anaerobic system can be evaluated by the Volatile Fatty Acids/Alkalinity ratio. (Barampouti et al., 2005) suggest that the ideal ratio of VFA/Alkalinity is in the range of 0.1 to 0.3 to avoid the acidification of the process. Increase in OLR resulted into increase in VFA/Alkalinity causing instability of anaerobic reactor, this can be attributed to insufficient alkalinity generated in reactor. Anaerobic reactor became stable during OLR 2 and 4 kg COD/m^3.d after achieving VFA/Alkalinity ratio of 0.3-0.4 of effluent in the range of 6.7-7.0. When the OLR increased to 6 kg COD/m^3.d the VFA to alkalinity ratio reached a value of 0.7 indicating, system instability (Zhao and Viraraghavan, 2004) ; During this period, complete instability in reactor performance was observed in terms of pH of effluent which was in the range of 5.3 to 5.7 whereas

VFA/Alkalinity ratio was fluctuating between 0.5-0.7 resulting in the decline in biogas production and COD removal efficiency. As reported by Zhao and Viraraghavan (2004) if the ratio of VFA to alkalinity increase above 0.3 to 0.4 indicate system instability and a proper ratio is between 0.1 to 0.2. On contrary, Sánchez et al., (2005) and Malpei et al., (1998) have stated that, optimum ratio of VFA to alkalinity should be less than 0.3 or 0.4.

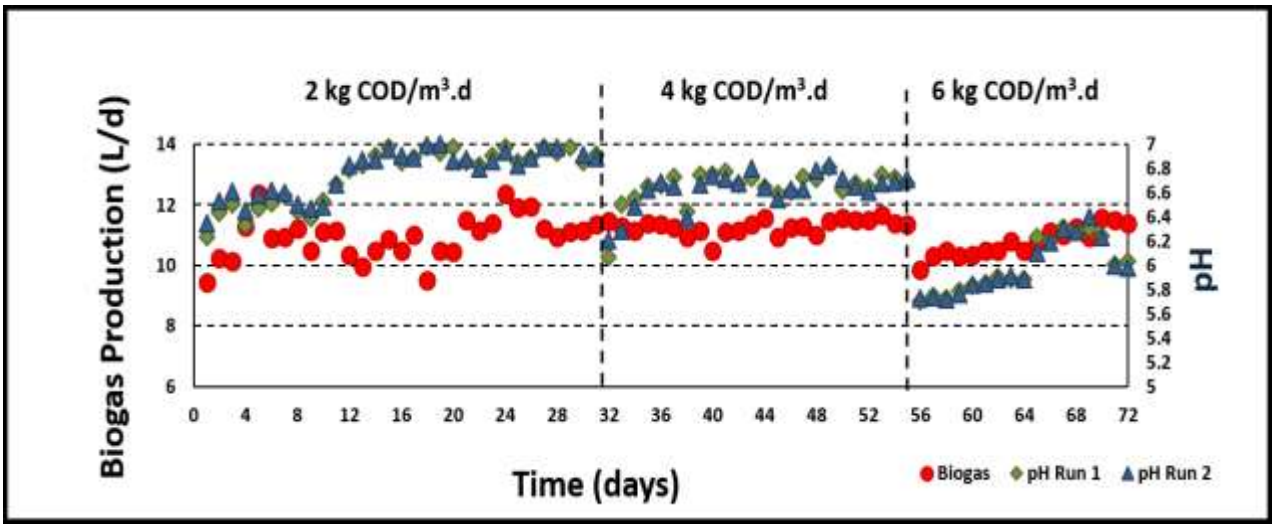

Figure 4.4 Biogas production and pH of anaerobic SBR at various OLRs

pH of effluent was within the range of 6.23 to 6.99, 6.07 to 6.87 and 5.7 to 6.3 during OLR of 2, 4 and 6 kg COD/m^3.d, respectively as shown in Figure. 4.3. Decline in pH of effluent was observed with increase in OLR due to the reason that the conversion of organic substrate leads to fatty acids production in the process that consumes the alkalinity and as a consequence pH starts to decrease. Decline in pH of effluent with increase in OLR of anaerobic reactor, indicated anaerobic process failure.

Average biogas production was 11.0, 11.3 and 10.7 L/day during OLR 2, 4 and 6 kg COD/m^3. d, respectively as shown in Figure. 4.3. Biogas production was in the range of 9.45 to 12.35, 10.5 to 11.65 and 9.85 to 11.55 during OLR of 2, 4 and 6 kg COD/m^3.d, respectively.

The effect of organic loading rate on biogas composition may be used as a direct indicator of the vitality of the anaerobic digester. The end product of anaerobic digestion process is production of biogas and the amount of biogas represents the "health" status of the process itself. Stable anaerobic digestion results in higher amount of biogas produced compared to unstable one. No significant difference in biogas production during OLR 2 and 4 kg COD/m^3.d was observed. Whereas there was a decrease in biogas production during OLR of 6 kg COD/m^3.d due to accumulation of volatile fatty acids (VFAs) in the anaerobic reactor because of high organic loading rate, which results in adversely affecting methanogenic activity, biogas production and having VFA/Alkalinity ratio above 0.3.

4.2.4 Biomass concentration and SRT

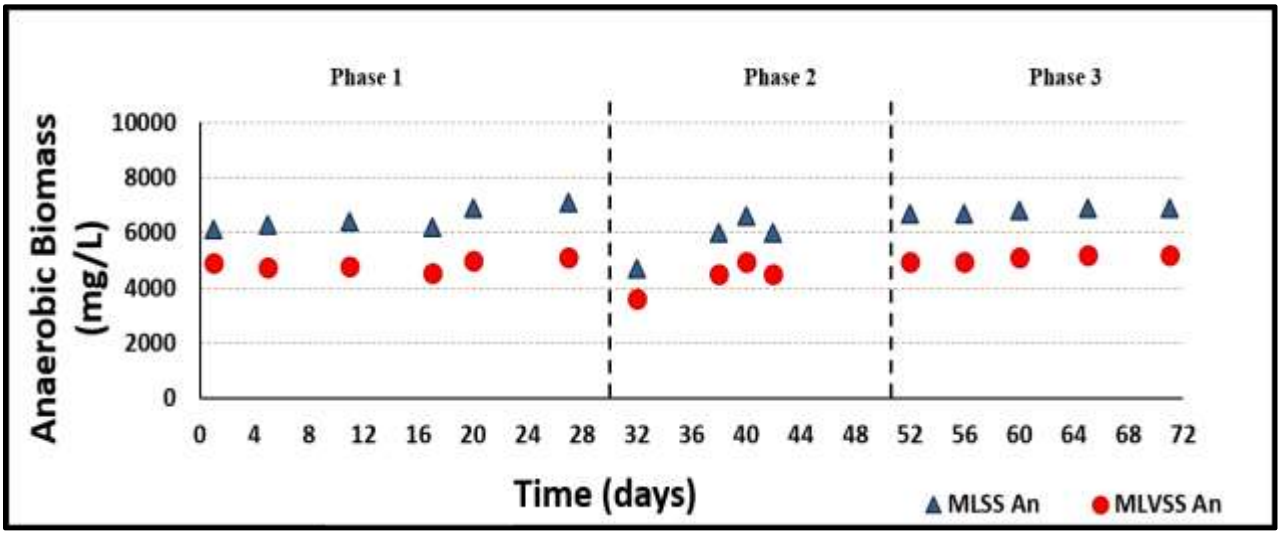

Figure 4.5 Biomass concentration in anaerobic SBR

MLSS in case of anaerobic reactor kept on increasing at a very slow rate from 6000- 7000 mg/L from OLR 2 to 4 kg COD/m^3.d as shown in Figure 4.5. On 31st day, sludge was wasted from anaerobic reactor at SRT of 120 d for 3 days therefore MLSS concentration dropped to 5000 mg/L. MLSS concentration again started to increase at a very slow rate and reached to about 6900 mg/L on day 60. MLVSS/MLSS ratio of 0.78 to 0.82 was maintained throughout the study.

Biomass concentration for aerobic SBR is shown in Figure 4.6. Initially no sludge was wasted for 4 days from aerobic reactor during phase 1 for the adaptation of microorganisms to the new environment. When MLSS concentration reached to 8000 mg/L, sludge wastage was started by increasing SRT to 10, 20 & 30 days gradually due to which MLSS & MLVSS concentration decreased with MLVSS/ MLSS ratio of 0.81.

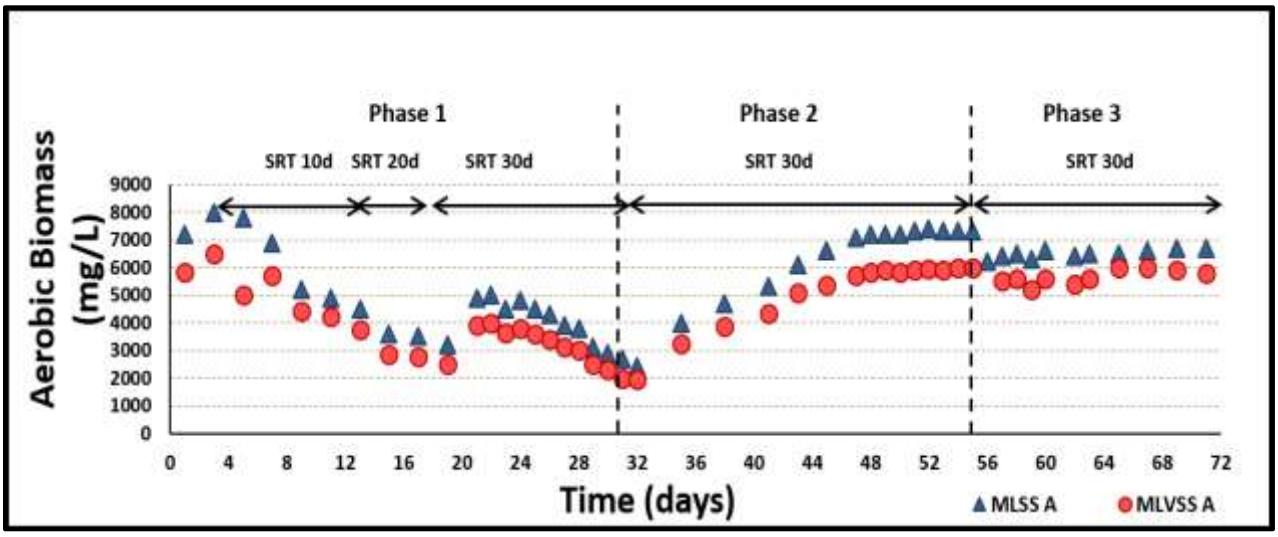

Figure 4.6 Aerobic biomass concentration

Biomass concentration for aerobic SBR is shown in Figure 4.6. Initially no sludge was wasted for 4 days from aerobic reactor during phase 1 for the adaptation of microorganisms to the unfamiliar environment. When MLSS concentration reached to 8000 mg/L, sludge wastage was started by increasing SRT to 10, 20 and 30 days gradually due to which MLSS & MLVSS concentration kept on decreasing with MLVSS/ MLSS ratio of 0.81.

During phase 2, sludge was wasted at SRT of 30 days and MLSS & MLVSS concentration started to increase and maintained to MLSS of 7300 mg/L and MLVSS of 6000 mg/L with MLVSS/MLSS ratio of 0.82.

During phase 3 MLSS and MLVSS concentration increased with time and maintained to MLSS of 6700 and MLVSS of 5800 mg/L with MLVSS/MLSS ratio of 0.86 at SRT of 30d. SRT for aerobic SBR was optimized at 30 days.

With an increase in substrate concentration during phase 2 and 3 when OLR in anaerobic SBR was 4 and 6 kg COD/m^3.d, resulted into increase in the growth of microorganisms, thereby enhancing substrate removal along with the biomass growth. MLVSS/MLSS ratio above 0.80 in both reactors indicated good quality of sludge.

4.3 Treatment Performance of Decoupled Anaerobic – Aerobic SBR

Initially, the focus was set to achieve high organics removal and biogas production. When treatment performance and stability of both reactors declined at OLR of 6kg COD/m^3.d, it was then decided to revisit the strategy and to decouple anaerobic SBR from aerobic SBR by increasing HRT and to use aerobic reactor just for polishing purpose with a small HRT. COD concentration of synthetic wastewater remained unchanged i.e. 3000 mg/L as this was the concentration that caused instability of both reactors and could not fulfilled the criteria for aerobic treatment i.e. COD less than 1000 mg/L. Working volume of aerobic SBR was reduced to 0.5 liters.

Anaerobic reactor performance in terms of COD removal, biogas production and reactor stability and TKN removal in aerobic reactor was observed for 20 days at HRT of 48 and 6 h, respectively.

4.3.1 COD Removal

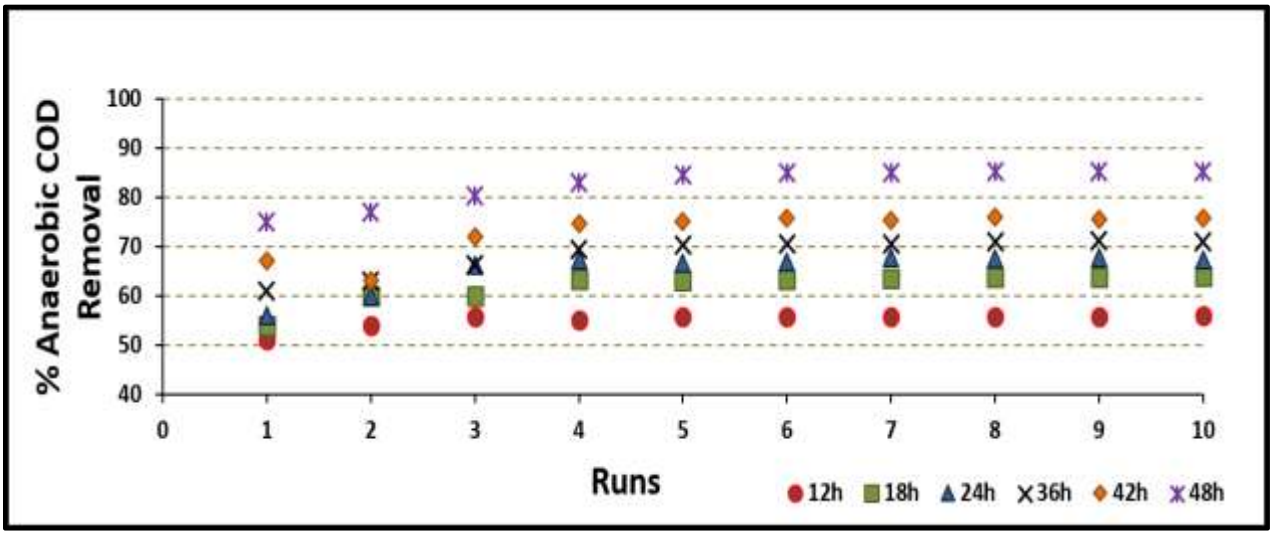

Figure 4.7 Hourly COD removal in anaerobic SBR

Anaerobic reactor performance subsequently increased after OLR reduction. Increasing HRT in anaerobic SBR caused increase in organics removal from 55.0 to 83.0%. A low HRT caused pre-acidification, resulting in accumulation of COD (as VFA), which did not subsequently convert to methane, resulting in an accumulation of VFA. This result agrees with the trend observed by Nachaiyasit and Stuckey (1997). Range of % COD removal for 12, 18, 24, 36, 42 and 48 h was 51.0 to 55.9, 54.0 to 64.0, 56.0 to 67.7, 61.0 to 71.0, 67.0 to 75.7 and 75.0 to 85.2%, respectively. Hourly analysis in Figure 4.7 of anaerobic reactor shows that COD removal increased with time and at 12, 18, 24, 36, 42, 48 h average removal percentages were 55.0, 61.0, 65.0, 69.0, 73.0 and 83.0%, respectively.

Hourly analysis of effluent after providing post treatment as shown in Figure 4.8 showed that total average COD removals at 1, 2, 3, 4, 5, 6 h were 94.5, 95.3, 96.0, 96.5, 97.5 and 98.6%, respectively. Overall COD concentration from decoupled system was 42 mg/L which was less than NEQs i.e. 150 mg/L.

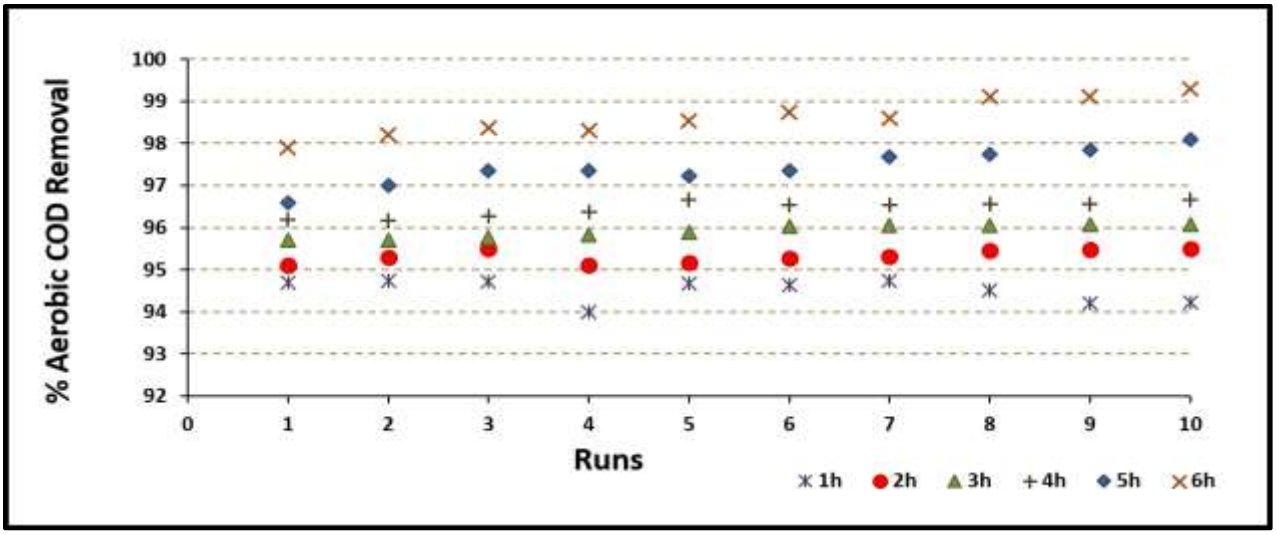

Figure 4.8 COD removal after providing aerobic treatment

4.3.2 TKN Removal

Average TKN removal in anaerobic reactor was 35.0%. After providing aerobic post treatment 6 hourly removal rates were 59.0, 67.5, 77.7, 87.1, 93.2 and 99.5% respectively. Overall TKN concentration was less than 15 mg/L and therefore met the NEQS of ammonia in effluent i.e. less than 40 mg/L.

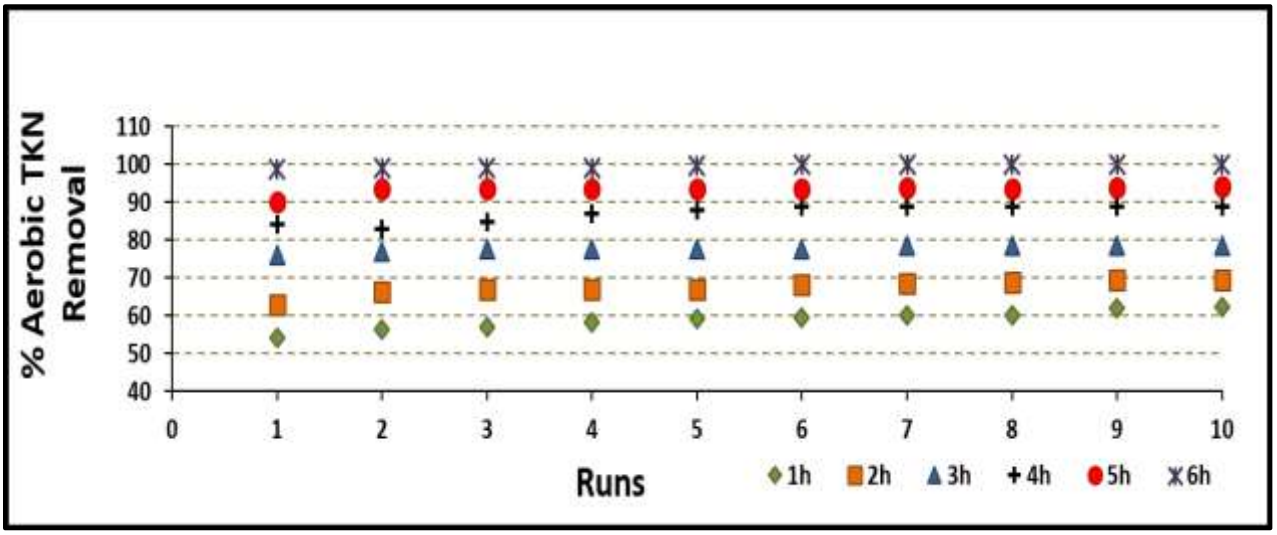

Figure 4.9 TKN removal after providing aerobic treatment

TKN removal in anaerobic SBR was lower as compared to aerobic SBR due to the probable reason that denitrification could be responsible for these extra nitrogen losses in the aerobic reactor (Bernet, 2000). High TKN removal percentage was observed in decoupled system with a reason reported that higher organic load inhibit nitrification decreasing the ammonia and nitrogen removal rates. This view was also supported by Tawfik et al. (2002) and Lyssenko and Wheaton (2006). According to He et al. (2007), high loading of organic material results in lower nitrification efficiency because of loss of ammonium through assimilation by heterotrophs.

4.3.3 Stability of anaerobic reactor and biogas production

VFA/Alkalinity ratios in Figure 4.10 shows that VFA/Alkalinity ratio decreased with time with average ratios of 0.52, 0.45, 0.37, 0.35 and 0.30, respectively showing that reactor stability achieved by increasing HRT in anaerobic reactor.

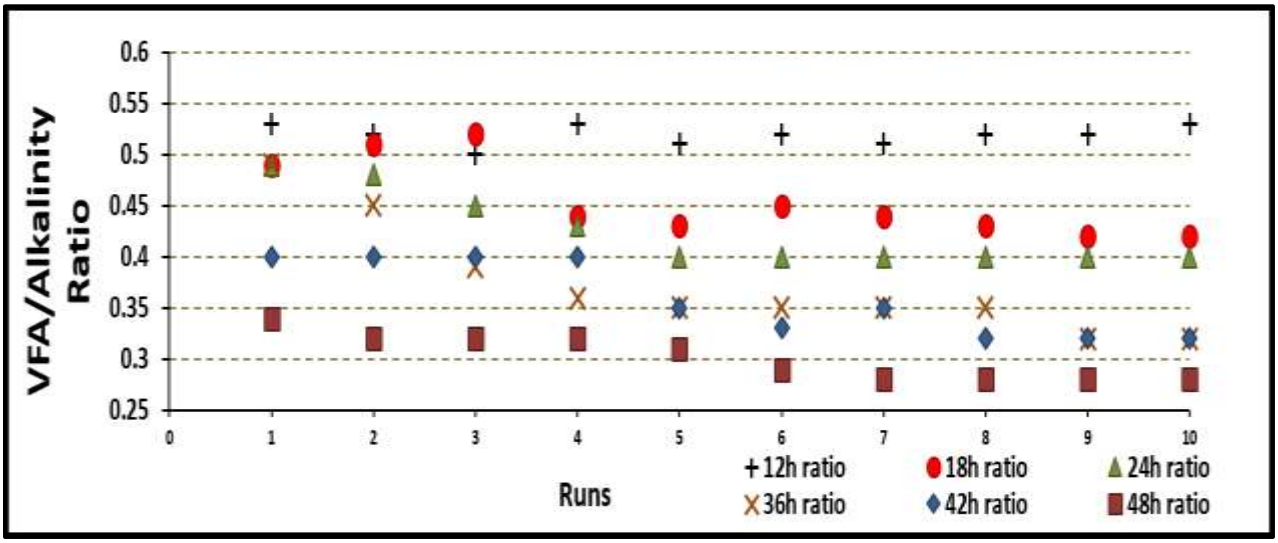

Figure 4.10 VFA/Alkalinity ratio

pH of effluent was within the range of 6.8 to 7.2 being an optimum range for methane producing bacteria (Fuchs et al., 2003). Optimum pH inside the reactor therefore resulted into increase in average daily production of biogas i.e. 22.7 L/d as shown in Figure 4.11.

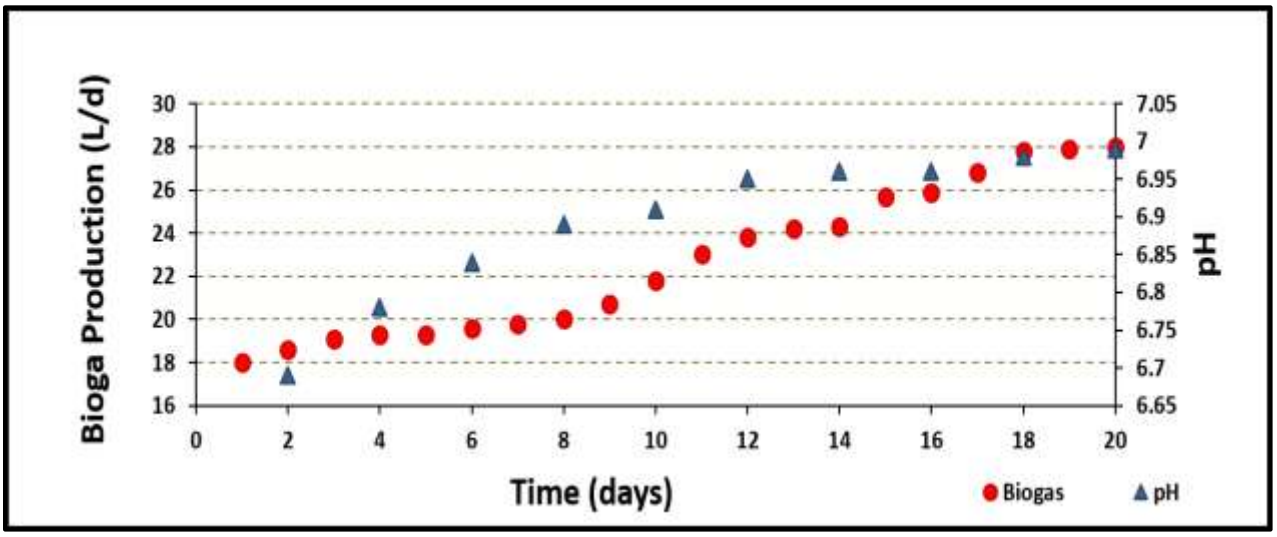

Figure 4.11 Biogas production and pH

4.3.4 Biomass concentration

MLSS concentration in anaerobic reactor was maintained to 7000 mg/l with no sludge wastage and 7000 mg/L in aerobic SBR was maintained at 30 d SRT.

4.4 Industrial application of decoupled anaerobic – aerobic system

Lastly the decoupled anaerobic-aerobic system was operated to treat synthetic textile wastewater having mixture of basic and reactive dyes including cibacron blue, cibacron yellow and methylene blue. The decoupled system process proved to be efficient for the treatment of a synthetic textile wastewater. Treatment performance of decoupled anaerobic – aerobic system is shown in Figure 4.12.

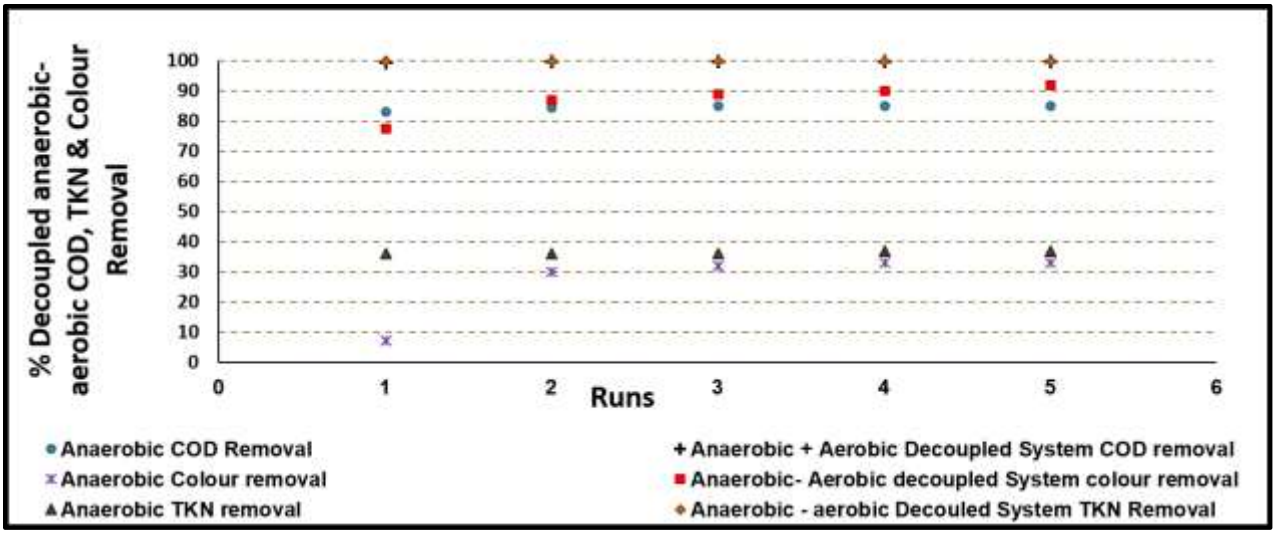

Figure 4.12 Treatment performance of anaerobic – aerobic SBR of synthetic textile wastewater

Synthetic textile wastewater treatment performance by decoupled anaerobic - aerobic system is shown in Figure 4.12. It was found that maximum COD was removed in anaerobic reactor, on the other hand majority of dyes were removed by the subsequent aerobic process. An average COD removal efficiency of 85.0% was obtained in anaerobic SBR and increased to 99.6% after providing aerobic post treatment. Dye removal efficiency was in the range of 7.0 to 33.0% for anaerobic SBR and increased to 77.5- 92.0% after providing aerobic post treatment. Anaerobic SBR effluent was visibly colourless but UV visible spectrophotometry detected concentration of dyes in effluent. This may be explained by the possibility of reduction of azo dyes into colorless aromatic amines during the anaerobic process as reported earlier (Pandey et al. 2007; Vanderzee and Villaverde 2005). Anaerobic conditions are required for azo dye removal, while aerobic process is required for biodeterioration and biodegradation of aromatic amines (Vanderzee and Villaverde 2005). Literature has reported that aromatic amines generated after azo dye reduction are resilient to anaerobic digestion, whereas they may be partially or completely degraded aerobically (Libra et al. 2004; Sponza and Isik 2005; Koupaie et al. 2011; Supaka et al. 2004; Ali 2010). The incomplete removal of dyes by aerobic polishing is due to the aerobic biological

treatment of aromatic amines resulting into either auto-oxidized products, and/or complete mineralization results to generation of CO_2, NH_3, etc. (Popli and Patel 2014).

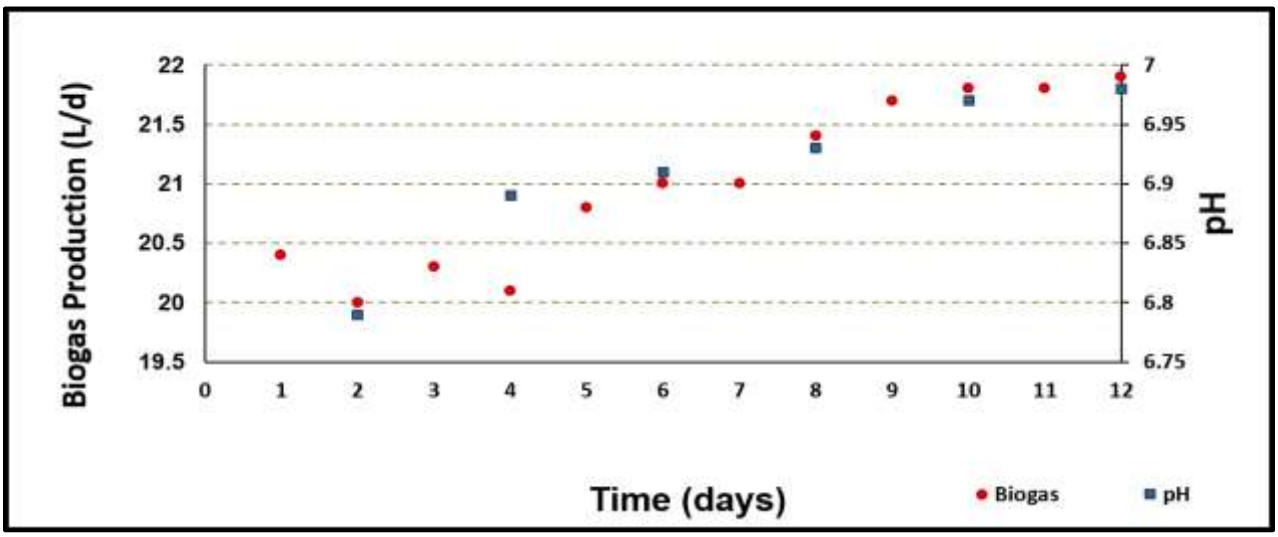

Figure 4.13 Biogas production and pH of anaerobic effluent

Biogas production increase with time and its trend is shown in Figure 4.13. There was no significant difference in biogas production after introduction of synthetic textile wastewater with average production of 22 L/d. Anaerobic SBR performance remained stable having VFA/Alkalinity ratio in the range of 0.27-0.3. MLSS concentration in anaerobic reactor was maintained at 7000 mg/L with no sludge wastage and 6000 mg/L in aerobic SBR at 30d SRT under the synthetic textile wastewater application.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The feasibility of a coupled and decoupled anaerobic-aerobic SBR system for COD and TKN removal from high strength synthetic industrial wastewater was demonstrated in this study. The following conclusion may be drawn from this research study:

- For coupled anaerobic-aerobic system, increasing organic loading rate (OLR) in anaerobic SBR from 2, 4 and 6 kg COD/m^3.d caused instability of both reactors having VFA/ Alkalinity ratio between 0.4 to 0.7.
- Biogas production declined from 11.0 to 10.7 L/d, decline in chemical oxygen demand (COD) removal in anaerobic SBR from 59.0 to 43 % and combined removal from 98.5 to 94.5% and % total kjeldhal nitrogen (TKN) removal from 51 to 27% was observed in coupled anaerobic-aerobic system.
- Decoupling of anaerobic-aerobic system proved to be a cost effective and stable treatment with overall 98.6 % organics removal and 99.5 % of TKN removal. Biogas production of 22.7 L/d was observed in decoupled system. Biogas production increased up to 50%, by increasing HRT in anaerobic SBR from 12 to 48 h.
- Decoupled anaerobic-aerobic system also proved to be an economical and stable treatment for synthetic textile wastewater, containing reactive and basic dyes with overall COD, color and TKN removal of 99.6, 92.0 and 99.9 % respectively.
- Decoupling strategy proved to be economical in terms of biogas production by causing 50% increase in its daily production.

- TKN removal enhanced from 47 to 99.9 % by decoupling of anaerobic- aerobic system and using aerobic SBR for polishing the anaerobic SBR effluent
- Optimized HRT for anaerobic SBR is 48 and 6 h for aerobic SBR with SRT of 30 d for high strength industrial wastewater having COD concentration of 3000 mg/L

5.2 Recommendations

- Performance evaluation of decoupled system for treatment of real industrial wastewater such as textile, paper & pulp, food processing and pharmaceutical may be explored.
- Comparison of decoupled anaerobic-aerobic SBR system with anaerobic SBR & aerobic MBR system may be investigated.
- Investigation of biogas composition in terms of methane and carbon dioxide needs to be carried out.

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