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Optimization and performance improvement of Anaerobic Membrane Bioreactor (AnMBR) for volatile fatty acid and biohydrogen production

By

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Mohd Atiqueuzzaman Khan, declare that this thesis is submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Civil and Environmental Engineering, Faculty of Engineering and IT at the University of Technology Sydney. This thesis is wholly my own work unless otherwise reference or acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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Abbreviations

Symbol	Description
AD	Anaerobic Digestion
ADBA	Anaerobic Digestion and Bioresource Association
AeMBR	Aerobic Membrane Bioreactor
AnMBR	Anaerobic Membrane Bioreactors
APBR	Anaerobic Packed Bed Reactors
BES	Bio Electrochemical Systems
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substance
ERR	External Rate of Return
ES	Excess Sludge
EV	Electric Vehicle
FAME	Fatty Acid Methyl Esters
FCV	Fuel Cell Vehicles
GC-MS	Gas Chromatogram Mass Spectrometry
GWP	Global Warming Potential
HHPB	Halophilic Hydrogen Producing Bacterium
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
IBR	Induced Bed Reactor
ICE	Internal Combustion Engine
IRR	Internal Rate of Return
LCA	Life Cycle Assessment
MBR	Membrane Bioreactor
MLVSS	Mixed Liquor Volatile Suspended Solids
MS	Mass Spectrometer
MTBE	Methyl Tert-Butyl Ether

OEB	Overall Energy Balance
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
PHA	Polyhydroxyalkanoate
PMC	Photosynthetic Mixed Culture
PVDF	Polyvinylidene Difluoride
SAnMBR	Submerged Anaerobic Membrane Bioreactors
SCFA	Short-Chain Fatty Acid
SDBS	Sodium Dodecylbenzenesulfonate
SDS	Sodium Dodecyl Sulfate
SHPR	Specific Hydrogen Production Rate
SMP	Soluble Microbial Products
SMR	Steam Methane Reforming
SRT	Solid Retention Time
TAN	Total Ammonia Nitrogen
TMP	Trans Membrane Pressure
UASB	Upflow Anaerobic Sludge Blanket reactor
VFA	Volatile Fatty Acid
VOC	Volatile Organic Components
VSS	Volatile Suspended solids

Ph.D. DISSERTATION ABSTRACT

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Thesis title: Optimization and performance improvement of Anaerobic Membrane Bioreactor (AnMBR) for volatile fatty acid and biohydrogen production
Faculty: Engineering and Information Technology
School: Civil and Environmental Engineering
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Abstract

Anaerobic Membrane Bioreactors (AnMBRs) have been widely used for source recovery from municipal and industrial wastewater treatment. Most of the research initiatives are inclined to optimize the production of methane-containing biogas from the anaerobic process. Volatile Fatty Acids (VFAs) and biohydrogen are two major intermediate products of AnMBR that can be recovered to improve the energy efficiency and product revenue from AnMBR.Research studies have investigated the technical feasibility of the production of VFA and biohydrogen using anaerobic digestion. The optimisation of VFA and biohydrogen production has been carried out through reducing their consumption by methanogens. This research study aims the optimisation of VFA and biohydrogen production through process optimisation so that the findings can be applied in a generic AnMBR model producing multiple products. Production of VFA has been investigated by reducing the Hydraulic Retention Time (HRT) and increase Organic Loading Rate (OLR) of the AnMBR. The solvent extraction method was used for VFA extraction and individual concentrations were measured using Gas Chromatogram-Mass Spectrometry. At 8 hrs HRT the concentration of major VFA components were maximum whereas at 550 mg/L COD_{feed} showed the optimum nutrient and COD removal efficiency of AnMBR. Selective production of major VFA components has been investigated by altering the pH of the bioreactor. At pH 7.0 the percentage of acetic acid was highest indicating acetate type fermentation was predominant at that condition. However, a major alteration in the percentage of VFA components were observed at pH 12.0 indicating isobutyric acid as the major VFA components. The result implies that butyrate type fermentation was predominant at pH 12.0. Production of VFA and biohydrogen both were investigated during a stepwise reduction of HRT. Without inhibiting methanogenic activity, the highest VFA and hydrogen yields were 37.08g VFA / 100 g $COD_{feed,}$ and 24.6 mL H_2/ g $COD_{feed,}$ observed at 8 and 6 hr HRT respectively. Optimization AnMBR operating pH was carried out to maximize the production of biohydrogen. The highest yield and production rate were observed to be 122.21 ± 39.05 mL H_2 / L. d and 65.38 ± 3.2 mL H_2 /g COD_{added} respectively and at pH 5.0 at an HRT of 6 hrs.

Keywords

Anaerobic Membrane Bioreactor (AnMBR), biohydrogen, membrane fouling, nutrient removal, inhibition, wastewater, energy recovery, volatile fatty acid, solvent extraction, optimization

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Chapter 1

Introduction

1.1 Research background

1.1.1 Anaerobic membrane bioreactors

Anaerobic digestion process has been widely applied for energy recovery from different waste streams. Anaerobic Membrane Bioreactors (AnMBRs) have been useful to recover energy and at the same time it offers the efficient removal of soluble organic compounds, nutrients, and micropollutants from wastewater. Low energy density, high cost in operation, and membrane fouling are the current drawbacks of the AnMBR technology that limits its performance in the full-scale operation (Khan et al., 2016b).

AnMBR operation also involves environmental impacts such as increasing global warming, aquatic ecotoxicity, human toxicity and abiotic depletion (Pretel et al., 2016a). Therefore, economic and environmental sustainability is yet to be achieved for the operation of AnMBR.

1.1.2 Different AnMBR products

Anaerobic digestion process in an AnMBR consists of four different stages: bacterial hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Adekunle & Okolie, 2015). The initial stage converts the carbohydrates, proteins, and fats into soluble amino acids, fatty acids and sugars. Volatile Fatty Acids (VFAs), biohydrogen and CO2 are the products at the second and third stage of acidogenesis and acetogenesis. Produced biohydrogen, VFA, and CO_2 are finally converted to produce methane and carbon dioxide at the final stage of methanogenesis (Khan et al., 2018; Kim et al., 2003; Lei et al., 2018).

Most of the current research initiatives are focused to produce the biogas as the final product from AnMBR. The produced biogas contains methane that can be readily combustible and does not require any post-treatment before consumption. Research studies have been carried out to increase the production rate and yield of methane-containing biogas through optimizing process conditions like temperature, pH, hydraulic retention time (HRT), solid retention time (SRT), organic loading rate (OLR) etc. (Khan et al., 2016a; Mao et al., 2015). Additionally, different pre and post-treatment processes have been integrated with conventional AnMBR technology to maximize biogas production.

The produced biogas from AnMBR only contains up to 70% methane which is low compared to the gas supplied in household and industrial purposes (~96% methane). Additionally, production of methane has technical issues like a low rate of conversion and process inhibition. Methanogenesis has been identified to be the slowest among all four stages of anaerobic digestion. As a result, a high rate in the initial three anaerobic stages can not necessarily increase the production rate of methane during anaerobic digestion (Ngo et al., 2019).

Additionally, process optimization for the anaerobic process still remains a challenge as the optimum microbial activity at different stages are different from each other. For example, the optimum pH for methanogenic activity is 6.5–8.2 whereas acidogenesis has the maximum efficiency in pH values between 5.5 and 6.5. Apart from different conditions for process optimization, high speed in the initial hydrolysis involves the production of VFA at a faster rate. As the methanogens cannot consume the produced VFA at the same rate, it accumulates inside the anaerobic bioreactor and eventually reduces the pH (Jankowska et al., 2017; Jie et al., 2014; Khan et al., 2016a; Mao et al., 2015). A combination of these factors has made the production of methane challenging from Anaerobic membrane bioreactors.

Most recent research studies have shown that VFA and biohydrogen produced in the intermediate stages of anaerobic digestion can be considered as major products of AnMBR over methane. So far, the available results from different research show that anaerobic digestion can be optimized to produce VFA and biohydrogen through the inhibition of methanogenesis. The alternative approach in product selection eliminates the negative environmental impacts and improves energy density (Intanoo et al., 2014; Intanoo et al., 2012; Kleerebezem et al., 2015; Kougias et al., 2014).

Selecting AnMBR products other than methane involves a major issue in industrial application. A system specifically designed to produce VFA or biohydrogen cannot be utilized to produce methane as the final anaerobic stage is inhibited during the production of VFA and biohydrogen. Heat pre-treatment of the inoculum, heat shock and load shock are generally applied for the inhibition of methanogenesis process.

1.2 Research objectives

Although different studies have proved the technical feasibility of production of VFA and biohydrogen, the currently available research has been applied for one particular type of substrate only. Therefore, a process designed to produce VFA or biohydrogen cannot be configured to produce methane. Moreover, there has not been any single research to extract VFA and biohydrogen from the treatment of municipal wastewater.

The main focus of this research is to develop an AnMBR for VFA and biohydrogen production only by altering the operating conditions. As the approach does not include any selective inhibition of microbial activity, the results are expected to be particularly useful to design a generic AnMBR model where the product spectrum can be changed by altering the operating conditions only. The major objectives of this research are:

1) To compare the value of bioproducts that are produced at different stages of AnMBR in terms of economic, environment and technical viewpoint;
2) To determine the optimum operating conditions for maximizing the production of VFA from AnMBR using low-strength municipal wastewater;
3) To demonstrate the performance of AnMBR during VFA and biohydrogen production using low-strength synthetic wastewater; and
4) To evaluate the optimum hydraulic retention time (HRT), organic loading rate (OLR), and pH for production of biohydrogen of a single-stage AnMBR treating low strength synthetic wastewater.

1.3 Research Significance

The findings from this research can contribute to improve the energy density and reduce environmental impacts from AnMBR. Recovery of VFA and biohydrogen can actually increase the product revenue earned form AnMBR and improve the net profit gain of an AnMBR system. As no design modification has been applied in the bioreactors during the shift of product spectrum from biogas to VFA and biohydrogen, the findings can be applied for industrial purposes. Above all, most biological VFA and biohydrogen production involves anaerobic digestion. Therefore, the current research can be applied to evaluate the AnMBR performance in terms of membrane fouling characterization, COD and nutrient removal.

1.4 Organization and major contents of the thesis

The thesis contains eights chapters, the contributing chapters are displayed in Figure 1.1

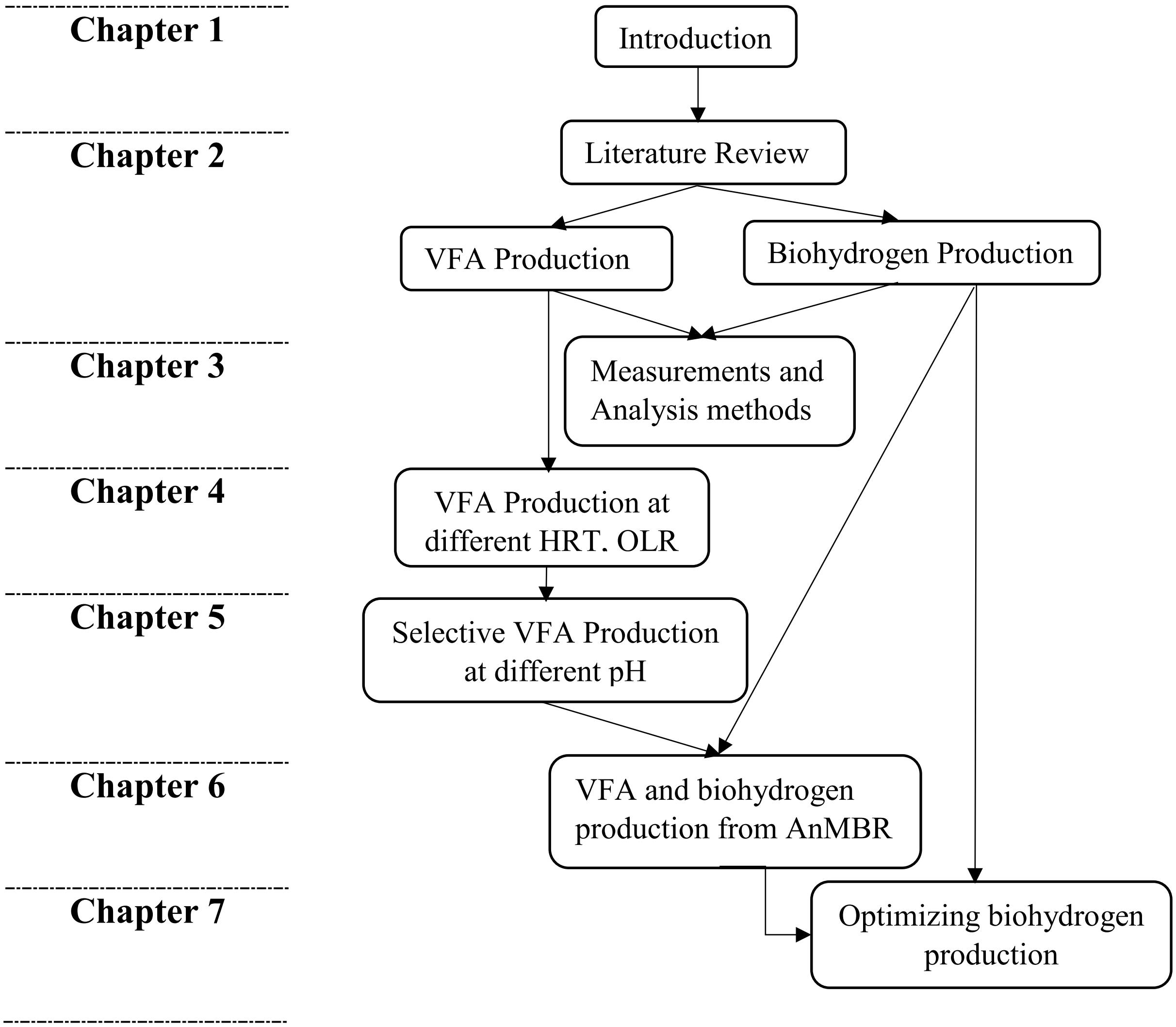

Figure 1.1: Organization and major contents of this thesis

Chapter 1 mainly includes general information about AnMBRs. It includes a brief overview of the products from the different anaerobic stages and the issues involved in biogas production from AnMBR. The research objectives and significance are then mentioned. The final part of this chapter includes the detailed framework of this thesis.

Chapter 2 reviews the fundamentals of anaerobic digestion. Products from different anaerobic stages were compared based on their economic, technical and environmental impact. The chapter includes the discussion about the reason why VFA and biohydrogen were favored over methane as AnMBR products. Finally, it highlights the research findings from the process optimization of VFA and biohydrogen.

Chapter 3 provides detailed information about the materials and methods used in this research. It includes the composition of synthetic wastewater of the reactor feed, sludge characteristics, experimental setup, operating conditions, VFA extraction and quantification process, biogas composition, and volume measurement process.

Chapter 4 demonstrates the optimum HRT and OLR for the production of VFA without any selective inhibition of methanogenesis. It includes the membrane fouling behavior, COD and nutrient the removal performance of the AnMBR along with the composition of VFA moisture at different HRT and OLR.

Chapter 5 shows how the production of individual VFA components can be maximized by altering the pH of the reactor. It includes the change in the composition of VFA mixture at different operating pH. It also highlights the membrane fouling behavior and AnMBR performance at different pH.

Chapter 6 includes experimental results regarding simultaneous VFA and biohydrogen from AnMBR. Corresponding membrane fouling behavior and COD, nutrient removal efficiencies were presented in this chapter.

Chapter 7 focuses on optimizing the production of biohydrogen by varying the pH of AnMBR. HRT and OLR were kept fixed based on the findings from chapter 6. The membrane fouling and COD, nutrient removal efficiency were also studied during this investigation.

Finally, chapter 8 summarizes the major findings from different experiments and lists the recommendations for future research in AnMBR.

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Chapter 2
Literature Review

2.1 Introduction

Recovering resources and energy from wastes and wastewater is deemed to be of primary interest for environmental engineers and researchers. Both aerobic and anaerobic processes have been utilized to design membrane bioreactors for industrial wastewater treatment (Falahti-Marvast & Karimi-Jashni, 2015; Ma et al., 2016). Of these two, the anaerobic membrane bioreactors (AnMBRs) are considered to be a good, low cost alternative that has the advantage of less energy requirement (Pretel et al., 2016b), high organic loading rate (OLR), bioenergy and nutrient recovery (Lei et al., 2018; Chan et al., 2009).

AnMBR is an integrated system where a low pressure microfiltration/ultrafiltration membrane module is coupled with an anaerobic bioreactor. The membrane module separates liquid from biomass and increases biomass concentration. Biogas is generated through anaerobic digestion process in the bioreactor and the filtered liquid from membrane module is collected as permeate (Chang, 2014). Figure 2.1 shows a simplified schematic diagram of anaerobic bioreactor with two major configurations.

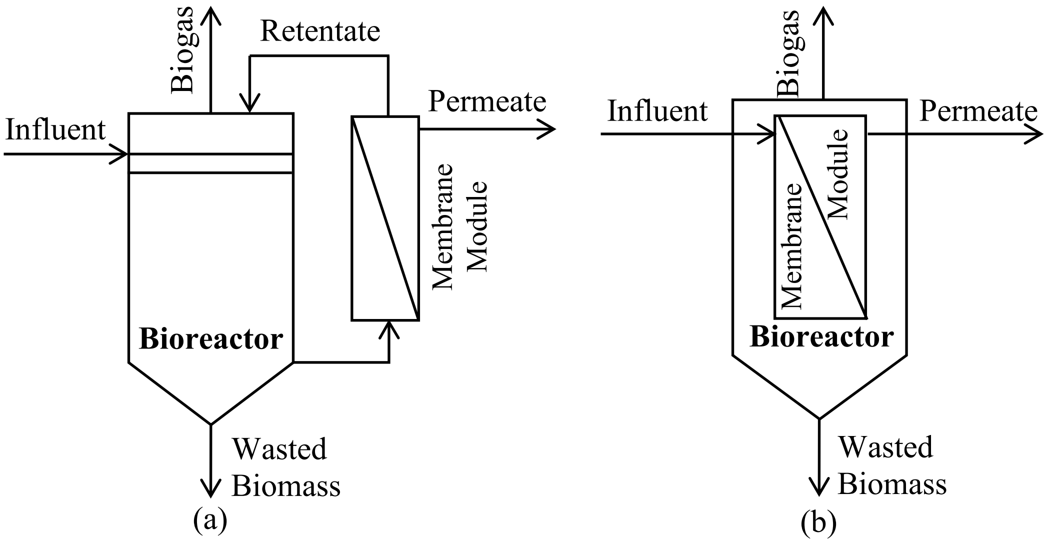

Figure 2.1: Schematic Diagram (a) Side stream (external) (b) submerged of AnMBR configurations

Till now, the industrial application of AnMBRs is limited as it requires a larger membrane area and intensive biogas recycling that contribute to the operation and maintenance costs (Do & Stuckey, 2019; Ozgun et al., 2013; Shin et al., 2014). Since the process offers the prospect of energy recovery, studies have focused on an optimization protocol for maximum methane production from the final stage (Mei et al., 2016). Although it is a much needed initiative to mitigate the growing energy crisis, the environmental impact of the product is one that contributes to greenhouse gas emissions. Experiments have already proven the technical feasibility to extract intermediate products like biohydrogen and volatile fatty acids (VFAs) from the individual anaerobic digestion process (Abdelsalam et al., 2016; Guwy et al., 2011b; Yuan & Zhu, 2016). The current AnMBR models designed to produce methane have a number of limitations in terms of economic feasibility and sustainable energy production (Pretel et al., 2015; Pretel et al., 2014).

The purpose of extracting VFAs and biohydrogen over methane production is governed by two main reasons. Firstly, VFA has already been identified as a suitable precursor for biopolymers and reduced chemicals of high value, such as alcohols, aldehydes, ketones, esters and biofuels (Scoma et al., 2016). Secondly, as a fuel, biohydrogen has a high energy density (Higher Heating Value of 142MJ/Kg compared to 55 MJ/kg of methane) and the combustion product (H_2O) is environmentally friendly (Guwy et al., 2011a; Kim et al., 2011). Therefore, the technical and economic feasibility study for AnMBRs designed to extract these intermediate products can be a promising aspect to improve the economic feasibility of AnMBR.

This chapter provides detailed literature review regarding the value of the bioproducts from AnMBRs, i.e. VFAs, biohydrogen, and methane. Optimum process conditions have been listed for VFA, biohydrogen and methane. Additionally, different operating conditions, technical feasibility has been studied during simultaneous and individual production of different AnMBRs products. The technical overview is followed by an economic assessment that includes the potential for each product and the costs involved in different AnMBRs' operating conditions and arrangements. Finally, to support the aim of the comparison, each component's environmental and societal impact have been discussed.

Major part of this chapter has been published as three different review articles in ERA A-rated journals:

1. **Khan, M.A**., Ngo, H.H., Guo, W.S., Liu, Y.W., Zhou, J.L., Zhang, J., Liang, S., Ni, B.J., Zhang, X.B., Wang, J. 2016b. Comparing the value of bioproducts from different stages of anaerobic membrane bioreactors. *Bioresource Technology*, **214**, 816-825.
2. **Khan, M.A**., Ngo, H.H., Guo, W.S., Liu, Y., Nghiem, L.D., Hai, F.I., Deng, L.J., Wang, J., Wu, Y. 2016a. Optimization of process parameters for production of volatile fatty acid, biohydrogen and methane from anaerobic digestion. *Bioresource Technology*, **219**, 738-748.
3. **Khan, M.A**., Ngo, H.H., Guo, W., Liu, Y., Zhang, X., Guo, J., Chang, S.W., Nguyen, D.D., Wang, J. 2018. Biohydrogen production from anaerobic digestion and its potential as renewable energy. *Renewable Energy*, **129**, 754-768.

2.2 Technical Overview

Anaerobic digestion is considered to be a complex process with a number of biochemical reactions where the reduction process is conducted by the microorganisms in anoxic conditions (Adekunle & Okolie, 2015). The process involves four major stages: bacterial hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The initial hydrolysis stage involves the enzyme-mediated conversion from suspended carbohydrates, proteins and fats into soluble amino acids, sugars and fatty acids. A number of hydrolytic microorganisms such as *Bacterides, Clostridia, Micrococci, Selenomonas*, and *Streptococcus* are the major drivers of the hydrolysis process (Cheng et al., 2018; Adekunle & Okolie, 2015).

During the stage of acidogenesis, the acidogenic bacteria converts the products from the initial hydrolysis stage into hydrogen, CO_2, acetates and VFAs (Adekunle & Okolie, 2015; Liu et al., 2012). The concentration of hydrogen formed as an intermediate product in this stage influences the type of final product produced during the fermentation process. Among the products from acidogenesis, the produced VFAs cannot be converted directly by the methanogens. Hence, the third stage involves the conversion of VFAs (acetic, propionic, and butyric acid) and alcohol into acetate, hydrogen gas and carbon dioxide (Wu et al., 2016).

It should be mentioned that butyric and acetic acids have been reported to be the main precursors for methane production. From 65 to 95% methane is directly produced from acetic acid. The remaining major component, propionic acid remains unconverted as the degradation is thermodynamically less favourable compared to butyrate (Yu et al., 2016b). The final stage of methanogenesis mainly includes the function from acetotrophic and hydrogenotrophic methanogens. The acetotrophic group transform the acetate produced in acetogenesis into methane and carbon dioxide while the hydrogenotrophic methanogens convert hydrogen and carbon dioxide into methane (Andre et al., 2016).

Experiments have shown that the AD process is recognized as a useful mean of producing VFAs (Cysneiros et al., 2012), biohydrogen (Anzola-Rojas Mdel et al., 2016; Jariyaboon et al., 2015) and methane (Andre et al., 2016; Yang et al., 2015; Mao et al., 2015). Each of the production processes involves specific bioreactor arrangements and an optimum set point of process parameters. Figure 2.2 summarizes the major phases of the anaerobic digestion process.

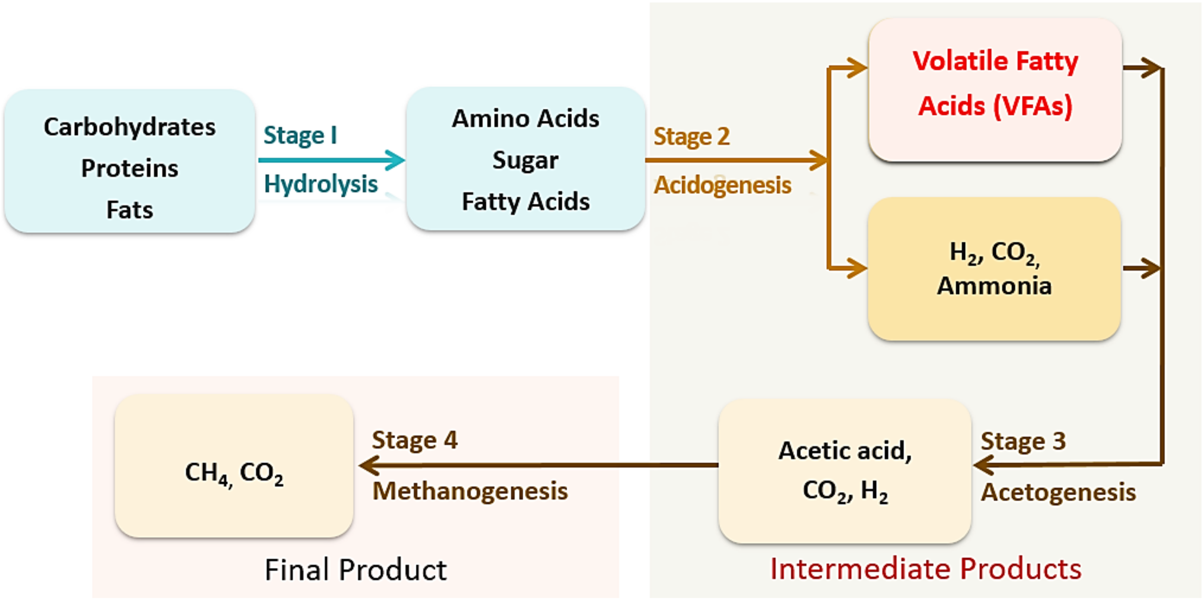

Figure 2.2: Major phases of anaerobic digestion

2.3 Optimizing AnMBR operating conditions

The growth rate of microorganisms in different stages varies widely according to their physiology, nutritional needs, temperature and pH sensitivity. The greatest challenge is to maintain a delicate balance between two major groups: the acid and the methane forming microorganisms. Reactor instability and low methane yield are two predominant issues observed in modern anaerobic model (Adekunle & Okolie, 2015).

An efficient anaerobic digestion process requires the rate optimization for both initial hydrolysis and final methanogenesis processes. When the rate of hydrolysis is higher compared to the final methanogenesis stage, the produced VFA can accumulate in the system and result in a decrease of pH in the reactor, which in turn can lead to the inhibition of the methanogenesis and induce system failure of the digester. Hence, controlling the rate of hydrolysis is important to prevent methanogenesis inhibition due to pH reduction in the system (Chang et al., 2019; Fezzani & Ben Cheikh, 2010; Xu et al., 2014).

Besides being the slowest among the phases, methanogenesis is also sensitive to operating conditions like pH, VFAs/SCOD ratio, OLR, C/N ratio, retention time and the accumulation of ammonia and sulfide (Mao et al., 2015; Yuan & Zhu, 2016). As a result, methanogenesis is deemed to be the most vulnerable and performance limiting part of the anaerobic digestion. Since the current process optimization is based on maximum biogas production, all process operating conditions are tuned to increasing the performance of methanogenic archaea (Mao et al., 2015).

Several different parameters like pH, temperature, mixing, substrate, C/N ratio, and hydraulic retention time (HRT) are important for an optimum performance in the anaerobic process. Although specific substrate properties and expected quality of the digestate define the operating conditions, parameters like values of temperature, pH and C/N ratio could be specified for generic anaerobic digestion models. Table 2.1 summarizes the most common operating ranges applied to create optimum AD performance.

Table 2.1 Optimal operating conditions of Anaerobic Digestion

Parameter	Operating condition	Positive and Negative effects	Recommendation
Temperature	Thermophilic	Rate advantage, high yield of methane. Acidification, low quality effluent, temperature sensitive, high energy requirement (Mao et al., 2015)	Thermophilic hydrolysis/ acidogenesis and mesophilic methanogenesis
	Mesophilic	More stable, higher richness in bacteria Less methane production, nutrient imbalance (Bowen et al., 2014)	
	Hyper-Thermophilic	Resilience in treating high concentrations of proteins, lipids. High energy requirement, More sensitive to temperature change (Lee (M.) et al., 2009)	
pH	6.5 – 8.2	High rate of Methanogenesis Low VFA production (Lee (D.H.) et al., 2009)	pH 5.5-6.5 could be applied to hydrolysis and 7.0 for the methanogenesis (Mao et al., 2015)
	5.5 - 6.5	Maximum VFA production Inhibition of methanogenic bacteria (Kim et al., 2003)	
C/N Ratio	25:1 – 30:1	Optimum overall biogas (Methane) production(Wu et al., 2010)	-

Both OLR and retention time depends on composition and type of waste that needs to be processed along with the model and arrangement of the bioreactors. From Table 2.1, it is evident that the process of methanogenesis and hydrolysis requires different production conditions and both phases have narrowed down the operating ranges that could be applied in AnMBR. Hence, wide and flexible operating ranges could be applied to AnMBR when the optimization of hydrolysis or acetogenesis is considered other than methanogenesis. So far, the current research on anaerobic processes provides only an incomplete picture because studies have been conducted under specific conditions. Only a few studies have provided a generic approach to optimize the AD process on AnMBR (Mei et al., 2016).

2.3.1 Optimizing volatile fatty acid production

VFAs are produced in the initial hydrolysis on anaerobic digestion. A number of soluble organic acids are included in VFA but the major components are acetic acid, propionic acid, butyric acid, and valeric acid (Khan et al., 2016). So far, the completed research studies on the optimization of VFA production have been performed based on specific types of substrates (Sahinkaya et al., 2019; Scoma et al., 2016; Wang et al., 2014b; Yuan et al., 2011). The literature review below concentrates on the type of bioreactors and optimum process conditions for VFA production.

The two most commonly used technologies for the production of VFAs are attached growth and suspended growth (Eddy, 1991). Both types of growth mechanisms have been implemented in different types of bioreactors. The packed bed bioreactor involves attachment of biomass on the packing material but is compromised by the problem of clogging. In contrast, the fluidized bed bioreactor eliminates the clogging problem where the biomass grows attached to small solid medium such as sand, which remains in suspension by the upward flowing motion of the fluid (Grady et al., 2011). In addition, the continuous stirred tank reactor (CSTR) is ideal to mix waste and microbes thoroughly in the presence of suspended solids and also offers complete mixing of waste and biomass. The most common reactor arrangement involves coupling a gravity settling clarifier coupled with the main bioreactor for separation and recycling the biomass to the bioreactor (Lee et al., 2014).

To produce volatile fatty acids, bioreactors could either be designed to produce VFA as the primary product (Wang et al., 2014b) or as a by-product (Peces et al., 2016). For production of VFA only, several bioreactor designs have provided promising results in terms of VFA production and separation such as: packed bed biofilm column reactor (Scoma et al., 2016), anaerobic leach bed reactors (Cysneiros et al., 2012), two-stage thermophilic anaerobic membrane bioreactor (Wijekoon et al., 2011), continuous stirred tank reactor (Bengtsson et al., 2008) and continuous flow fermentation reactors (Luo et al., 2014b).

The operating conditions for VFA production greatly vary according to bioreactor types, design, substrate composition and product spectrum. A suggestion has been proposed by Lee et al. (2014) between the mode of bioreactor operation and the rate of biomass decomposition. According to their recommendation, the batch or semi-continuous mode of operation is favorable over the continuous mode for UASB, packed and fluidized bed reactors. Apart from the mode of operation, the optimum value of operating temperature, pH, retention time and organic loading rate varies widely for different types of reactor systems and substrate conditions. Some specific actions such as sludge pre-treatment, hydraulic flushing helps the reactor acidification process, and finally helps to maximize VFA production from anaerobic digestion.

A. Temperature

Temperature has a significant effect on VFA production from anaerobic digestion. Yuan et al. (2011) studied the change in VFA concentration produced from waste activated sludge (Kadier et al.) in three different operating temperatures (24.6, 14 and 4 °C). They concluded the highest VFA–COD production of 2154 mg L^{-1} at the operating temperature of 24.6 °C in the shortest time of 6 d, compared to the result of 2149 and 782 mg L^{-1} from 14 and 4 °C, respectively. Additionally, the production rate and yield of VFA produced also improved when the temperature rose within the psychrophilic (4–20 °C) and mesophilic (20–50 °C) ranges (Yuan et al., 2011; Zhuo et al., 2012). This increment could be explained by the solubility of carbohydrates and proteins increasing at a high temperature and the rate of hydrolysis also rose as temperature increased (Liu et al., 2012).

The type of VFA produced has not been altered greatly when the temperature is changed during VFA production. Yuan et al. (2011) also showed that the composition of VFA produced in three different temperatures (24.6, 14 and 4 °C) revealed no significant changes. This outcome included an increase in temperature (from 4 °C to 14 °C) causing a reduction in acetate production from 55% to 43%, yet the production of propionate and butyrate had an increase in percentage from 20% to 29% and 11% to 16%, respectively.

Zhuo et al. (2012) studied the temperature effect on Ultrasonic pre-treated WAS fermentation at four different values: 10, 20, 37, and 55 °C under alkaline conditions. The results included a common trend of change in individual VFA production and no significant alteration in the composition of VFA produced. Increasing the temperature from 45-70° C does not create any positive impact on VFA production (Yu et al., 2013). In contrast, Zhuo et al. (2012) included that at 40° C there was a 40% decrease in total VFA production compared to that that of 37℃. It may be mentioned the microbial species present in different types of waste materials widely differ from each other, their growth rate in different temperature changes will be different. Consequently, identifying the change in growth rate of different types of microbial species could be a future research option for analyzing the impact of temperature in VFA production.

B. pH

The amount of organic content being hydrolysed is the primary factor which is directly responsible for the amount of VFA produced. Along with the substrate composition, pH plays an important role in increasing the production rate and yield of VFA in anaerobic digestion. A comparative study was done to identify the accumulation of VFAs and microbial community structure of excess sludge (ES) at different pH values (Jie et al., 2014). Results found that at a pH level of 10, the accumulation of VFA reached its maximum limit. This finding was supported by another experiment (Wu et al., 2010) where alkaline fermentation of primary sludge for short-chain fatty acids (SCFAs) was studied. Results indicated that a pH range between 8.0–10.0 caused higher SCFAs accumulation when compared to pH 3.0–7.0.

The pH range of extremely acidic (less than 3) or extremely alkaline conditions (above 12) are referred to as inhibitory conditions for the acidogens (Liu et al., 2012). Although the optimal value of pH has been cited as high as 10 for the sludge hydrolysis mentioned above, this value may change to between 5.25 and 11 depending on the type of waste materials (Lee et al., 2014). For example, the anaerobic digestion of kitchen waste requires an optimum pH value equal to 7 (Wang et al., 2016) whereas the optimum pH condition for wastewater treatment ranges between 5.25 and 6.0 (Bengtsson et al., 2008).

In addition to the anaerobic digestion of excess sludge, the highest concentration of VFA is determined by the fermentation with inoculum and the HRT of the reactor. Based on these two additional factors the optimum pH values are changed. For example, Wang et al. (2014b) examined the effect of pH on different types of inoculum in eight different batch reactors over a fermentation period of 20 days. Results from this experiment indicated the maximum concentration and yield (51.3 g-COD/L and a yield of 918 mg/g VSS $_{\text{removal}}$) for VFA at pH level 6.0.

For production of VFA, the ratio of VFA to SCOD refers to the amount of soluble substances converted into VFAs (Jiang et al., 2013). Experiments also show that the pH range of 5.0 to 6.0 produced the highest value of VFA/SCOD ratio (75%), regardless of the type of which inoculum was used while producing VFA from food waste. However, this experiment did not include the results for an extreme alkaline state (pH > 10) (Wang et al., 2014b).

Although the composition of produced VFA primarily depends on the composition of the substrates, any changes in pH values can also control the type of VFA produced from acidogenic fermentation (Lee et al., 2014). Before the selective production of any specific type of volatile fatty acid, the optimum pH level needs to be determined.

C. Retention Time

In anaerobic digestion of waste materials, the retention time of the waste and the microbial culture in bioreactor are important process parameters. Retention time includes hydraulic retention time (HRT) and solid retention time (SRT) which refer to the volume of the reactor and the allocated time for selected predominant microbes respectively. Experimental results have proved that that the production of VFA depends more on the hydraulic retention time compared to the temperature of a reactor (Kim et al., 2013).

A high value of HRT provides enough time for the acidogenic bacteria to reduce the waste into soluble derivatives and consequently it favors the VFA yield (Bengtsson et al., 2008). The hydraulic retention time for a system depends on the type and composition of the substrate. For instance, a HRT of 1.5 day was applied to VFA production and profile in anaerobic leach bed reactors digesting a high solids content substrate (Cysneiros et al., 2012) whereas 1.9-day HRT produced best performance in acidogenic anaerobic digestion of OFMSW (Romero Aguilar et al., 2013).

HRT values are only beneficial for VFA production up to a certain value, while prolonged HRT is responsible for the accumulation of VFA in the reactor. An experiment was performed to

produce VFA from acidogenic fermentation of food (Lim et al., 2008). The results demonstrated that the production of VFA increased as the HRT increased from 96 h to 192 h, but there was no further increase in VFA production once the HRT exceeded to 288h.
It has been identified that the growth rate of methanogens is slower compared to the growth rate of acidogens. As a result, a low SRT does not allow enough time for the methanogens to consume VFA and produce methane and carbon dioxide (Lee et al., 2014). In contrast, the acidogens require a minimum SRT to perform the hydrolysis of the substrates. A long SRT provides sufficient time for the methanogens and enables more biogas production, for instance, wastewater treatment using submerged anaerobic membrane bioreactors (SAnMBR) has a SRT range from 30 to 90 days (Huang et al., 2013).

D. Organic loading rate

The Organic loading rate (OLR) of a process is directly governed by the bioreactor arrangement and type and composition of substrates. So far, no direct relationship has been observed regarding the change in OLR and the yield or production rate of VFA. However, the general trend of VFA production could be predicted with the change in OLR. For example, lactic acid fermentation from food waste with indigenous microbiota shows that the concentration of lactic acid initially increased with increasing the OLR. The lactic acid concentration rose from 29 g/L to 37.6 g/L when the OLR was increased from 14 to 18 g-TS/L d (Tang et al., 2016). Yet, for the same experiment when the OLR was increased from 18 g-TS/L d to 22 g-TS/L d the acid production decreased sharply to 22g-TS/L d. These results could be attributed to the contention that if the organic loading rate reaches beyond the optimum value the rate of hydrolysis is reduced.

A study of fermentation included two-phase olive oil mill solid residue over a range of different OLRs from 3.2 to 15.1 g COD/L/d. The result indicated that the maximum VFA concentration increased up to 12.9 g COD/L/d, and consequently a gradual decline was observed beyond 12.9 g COD/L/d (Rincon et al., 2008). Similar results were observed during the production of VFA from food waste (Lim et al., 2008) using in once-a-day feeding and drawing-off bioreactor. An increase in VFA production was observed from the organic loading rate of 5 g/L/d to 13 g/L/d, but beyond 13 g/L/d the reactor became unstable.

It can be summarized that production of VFA increases with the initial increase in OLR and the rate of production drops when OLR is increased further regardless the type and composition of the substrate. However, more research studies need to be done to characterize the range of optimum values in OLR along with the bioreactor design and type of substrates.

E. Other Parameters

In addition to the optimized process parameters, some specific additional measures can offer positive results for VFA yield and production rate. Actions such as hydraulic flush could increase the VFA production for a particular process. Experiments indicate that the hydraulic flush increased VS degradation and VFA production by 15% and 32% respectively, in buffered leach bed reactors that digested a high solids content substrate (Cysneiros et al., 2012). Furthermore, some chemical additives increase the production of VFA significantly; Table 2.2 summarizes the information concerning some common additives and their respective results in VFA production.

Table 2.2: Effect of adding surfactants and/or enzymes on the production of VFA (Modified from (Lee et al., 2014))

Additive(s)	**Waste**	**Dosage**	**Maximum VFA Concentration (mg COD/L)**		**Reference**
			without additives	**With additives**	
Sodium dodecylbenzenesulfonate (SDBS)	Waste activated sludge + primary sludge	0.02 g/g TSS	118 (mg COD/ g VSS)	174 (mg COD /g VSS)	(Ji et al., 2010)
Sodium dodecyl sulfate (SDS)	Waste activated sludge	0.1 g/g dry sludge	191	1143	(Jiang et al., 2007)

α-Amylase + neuter protease	Waste activated sludge	0.06 g/g dry sludge	-	1281	(Luo et al., 2011)
SDS + α-amylase + neuter protease	Waste activated sludge	SDS = 0.1 g/g dry sludge Enzyme = 0.06 g/g dry sludge	-	1457	(Luo et al., 2011)

2.3.2 Optimizing biohydrogen Production

In recent years the production of biohydrogen has attracted much research interest because it enables using waste materials compared to conventional electrolysis and thermo-catalytic reformation. An anaerobic system could be designed to produce biohydrogen as the major product (Abbasi & Abbasi, 2011) or as a by-product with biodiesel or methane (Intanoo et al., 2016). Dark and photo-fermentation processes are the two major options for producing biohydrogen through the anaerobic method (Rittmann & Herwig, 2012). The dark fermentation process involves the production of biohydrogen and VFA through the stage of acidogenesis by acidogenic bacteria such as *Clostridium spp.* Photo-fermentation process enables the biohydrogen production from VFA with the presence of light, the predominant microbial community is photosynthetic bacteria such as *Rhodobacter* or *Rhodopseudomonas spp.* (Lee et al., 2012).

Unfortunately, the yield of biohydrogen from experiments has been significantly less than the expected theoretical yield; the difference is being that some of the raw materials are converted into by-products. During acidogenesis, butyrate and ethanol are produced that are termed as fermentation barriers to limit the hydrogen production. In connection, during anaerobic digestion, only one third of the electron potential is transferred to produce hydrogen, leaving the remaining two thirds being transferred to fermentation by-products (Abdallah et al., 2016). Different types of bioreactors have been employed for biohydrogen production including anaerobic down-flow structured bed reactor (Anzola-Rojas Mdel et al., 2016), upflow anaerobic sludge blanket reactor (UASBR) (Intanoo et al., 2014), continuous stirred tank reactor (Luo et al., 2010),continuously external circulating bioreactor (Liu et al., 2014) etc. Reactor models including a separate hydrogen fermenter using the conventional bioreactor design have shown promising results indicating a maximum yield and production rate of hydrogen; 1.13 mol H_2/mol glucose and 0.24 mol H_2/L-d, respectively (Bakonyi et al., 2015). The configuration of the hydrogen fermenter along with subsequent downstream processing (biohydrogen recovery and purification) are two key factors that define the efficiency of a bioreactor producing biohydrogen (Kumar et al., 2015).

Bioreactors with two-stage assembly operations enable the simultaneous production of biohydrogen and methane. The particular advantage here is the ability to separate operating conditions (temperature, pH or retention time) being applied specifically to the microbes on each stage (Intanoo et al., 2016; Intanoo et al., 2014; Jariyaboon et al., 2015). However, the major drawback of two-stage arrangement is initial installation cost for reactor vessel and membrane module exceeds that for the single stage arrangement (Khan et al., 2016). Therefore, the cumulative product revenue is comparable to the additional costs involved in initial installation and operations such as controlling temperature, pH and membrane fouling.

Although the type and organic content in the substrates are the major factors that control the production of biohydrogen, several process parameters are related to the production of biohydrogen. These include temperature, pH, substrate composition, retention time, loading rate etc. (Bakonyi et al., 2015; Bakonyi et al., 2014). The following section details the effects of temperature, pH, retention time and organic loading rate for production rate and yield of biohydrogen.

A. Temperature

Not many studies have compared the productivity of biohydrogen when using thermophilic, mesophilic and psychrophilic processes. Results for research data show that the overall production of biohydrogen did increase during thermophilic operation compared to the mesophilic strategy (Jariyaboon et al., 2015). The findings included a faster acclimatization rate of thermophilic inoculum compared to the mesophilic inoculum. Another analysis considered hydrogen production using two-stage induced bed reactors (IBR) from dairy waste processing (Zhong et al., 2015). The results indicated a value of 131.5 ml H_2/g-COD $_{removed}$ at 60 °C compared to 116.5 ml H_2/g-COD $_{removed}$ at 40 °C.

In the thermophilic scenario (temperature 55 ° C) research was carried out for simultaneous production of biohydrogen and methane using a two-stage upflow anaerobic sludge blanket reactor (UASB) (Intanoo et al., 2014). Results were the maximum hydrogen production rate and highest H_2 yield equal to 2.2 L/d and 80.25 ml H_2/g, respectively, during a COD loading rate of 90 kg/m^{3}d. In contrast, another study (Limwattanalert, 2011) documented the maximum amount of hydrogen produced in terms of maximum yield being 114.5 ml H_2/g COD removed in the mesophilic context (37 ° C).

The results obtained from these experiments confirm the veracity of two concepts. Firstly, in the thermophilic scenario, there is an improved solubility of the polymeric components such as lignocelluloses present in the substrates. Secondly, increasing the temperature, in turn, increases the activities of the enzymes (Zhong et al., 2015). Another important aspect of biohydrogen production is the inhibition of methanogenic activities. To increase the biohydrogen production the population of hydrogen-producing bacteria should be increased and at the same time, repressing hydrogen-consuming bacteria such as methanogens. Two common methods for repressing the methanogens are heat shock and load shock treatment. For heat shock treatment, the sludge is treated at 100 °C for 30 min in an autoclave prior to use in cultivation (Jariyaboon et al., 2015). Research findings indicated that in the thermophilic state, the inhibition of methanogen is higher compared to the mesophilic one (40 ° C) (Zhong et al., 2015).

The research findings do not provide any generalized temperature range that would be particularly beneficial for biohydrogen production. To identify the optimum temperature for any process, faster acclimatization of the inoculum and inhibition of the methanogenic activities should be considered under the optimum loading rate.

B. pH

For biohydrogen production, the growth rate microorganisms and dynamics of fermentation largely depend on the initial pH of the bioreactor. A change in pH triggers a microbial shift that eventually defines the metabolic pathway of the microorganisms. A variation of the hydrogen ion concentration causes a change in pH that eventually leads to the variation of discharges detected by the redox potential. Research has shown that activities of the fermentation products largely rely on the pH and it is an important ecological factor for hydrogen producing bacteria (Ruggeri & Tommasi, 2015).

Although the optimum value of pH in a bioreactor varies according to the substrates' composition, research findings have indicated a favorable range that is common for all biohydrogen production processes through anaerobic digestion. Results from one experiment indicated the initial increase of pH in the acidic range favored biohydrogen production. This particular study concluded a pH value of 6.9 for maximum yield of hydrogen and a value of 7.2 for maximum average production rate for biohydrogen (Wang & Wan, 2011).

Another experiment involved the production of biohydrogen in batch reactor using an initial concentration of 6000 mg/L glucose as a substrate (Liu et al., 2011). Their findings showed a pH value equal to 4 could discourage microbial growth. In addition, they reported that at pH 7.0 the hydrogenase activity was low, which finally resulted in a low biohydrogen yield (ranged from 0.12–0.64 mili-moles/mili-mole glucose). They concluded that pH values from 5.5 to 6.8 are the most favorable for biohydrogen production. Ruggeri & Tommasi et al. (2015) performed a research study aiming to produce biohydrogen from noodle manufacturing wastewater. By analyzing Clostridium butyricum CGS5, the results included a pH value of 5.5 for maximum hydrogen production where a pH of 4.5 could have inhibitory effects.

Controlling the pH in a lab scale experiment may not reflect the real costs when the experiment is conducted in an industry context. However, the type of waste material and bioreactor type should be defined for more precise tuning of pH value in an anaerobic process.

C. Retention time

For biohydrogen production, hydraulic and solid retention time are critical design and operating parameters, since the reaction time between the microbial species and substrate removal efficiency both depend on HRT and SRT. Improving the production of biohydrogen implies the inhibition of bioactivity of hydrogen-consuming bacteria (both homoacetogens and hydrogenotrophic methanogens). Various studies' results contend that low HRT inhibits the activities of methanogens (Romero Aguilar et al., 2013). In addition, if the HRT is too short there is the potential of biomass washout from the system.

According to the experiment undertaken by Kumar et al. (2016), HRT values between 3 to 6 hours are favorable for the maximum biohydrogen production rate (25.9 L H_2/L-d) and yield (2.21 mol H_2/mol galactose), respectively at an OLR of 120 g/L-d with a high rate of continuous stirring in a tank reactor. Furthermore, a reduction of HRT from 2 hours reduced the production of biohydrogen indicating a biomass washout from the system.

Research studies were done to observe the specific hydrogen production (SHP) from a mixed substrate having a mixture ratio of 80:20 from municipal solid waste and food waste in a dry thermophilic anaerobic co-digestion (55 °C and 20% solid content) (Angeriz-Campoy et al., 2015). The applied SRT for the experiment ranged from 6.6 to 1.9 days and results indicated a decrease in SRT actually increased the production of hydrogen. The maximum rate of biohydrogen production in this experiment was 2.51 L H_2/L reactor day, and SHP was 38.1 mL H_2/g VS added at an SRT of 1.9 days.

The findings are supported by another experiment aiming to produce biohydrogen from the fermentation of different galactose–glucose compositions (Kumar et al., 2014). At HRT 6 and 18 hours, the maximum hydrogen production rate and maximum hydrogen yield of 4.49 L/L/d and 1.62 mili-moles/mole glucose were attained. For the galactose, HRTs of 12 and 24 h produced a maximum production rate and yield valued at 2.35 L/L/d and 1.00 mole/mole galactose, respectively. It can be summarized that longer SRT and shorter HRT improve the efficiency of biohydrogen production. This outcome favors the population of active biohydrogen producers and consequently results in a high substrate conversion rate and a high percentage of yield (Jung et al., 2011).

D. Organic loading rate

The nutrient content comprising carbon sources are converted into molecular hydrogen gas during the anaerobic digestion process. For this reason, the organic loading rate needs to be optimized according to bioreactor design giving consideration to the maximum amount of produced biohydrogen. Results from research studies that have been already performed could be utilized to get a general connection between biohydrogen production and organic loading rate.

It has been observed that the initial increase in the loading rate aids the production of biohydrogen (Zhang et al., 2013). The results include an initial increase in the organic loading rate from 4 to 22 g COD/L-d has a positive effect on biohydrogen production. This is in terms of production rate of 0.196 mol d^{-1} L^{-1}, and subsequently, the biohydrogen production rate fell down to 0.160 mol d^{-1} L^{-1} when the organic loading rate increased from 22 to 30 g COD/L-d. The maximum microbiological uptake for a certain bioreactor arrangement depends on whether the solid retention time is enough to enable the microorganisms to degrade the organic content efficiently. An experiment was undertaken in up-flow anaerobic packed bed reactors (APBR) with sugarcane vinasse indicated the optimum value of OLR equal to 84.2 kg-COD m^{-3} d^{-1}. The mentioned OLR was able to produce the results of 1117.2 mL-H_2 d^{-1} $L^{-1}_{reactor}$ and 2.4 mol-H_2 mol^{-1} $_{total\ carbohydrates}$ as biohydrogen production rate and yield, respectively.

HRT and OLR are closely related to each other and defining a specific value for either one actually depends on both. The influence of OLRs and HRTs on hydrogen production was observed using a high salinity substrate by halophilic hydrogen-producing bacterium (HHPB) (Zhang et al., 2013). The maximum biohydrogen yield was 1.1 mol-H_2/mol-glucose with optimum OLR of 20 g-glucose/L/day (range studied 10–60 g-glucose/L-reactor/day) and HRT of 12 h (range studied 24–6 h).

Kim et al (2012) studied the bio-hydrogen production from lactate-type fermentation at different OLRs (10, 15, 20 and 40 g/L/day) and HRTs (6, 12 and 24 h). At an OLR of 40 g/L/day, the optimum HRT was identified as 12 h for continuous biohydrogen production (Kim et al., 2012). The results implied low of yield biohydrogen if the HRT was decreased or increased from 12h indicating the scenario of biomass washout or more biohydrogen consumption by methanogens respectively. Table 2.3 summarizes the effects of OLR and HRT on biohydrogen production using different types of substrates.

Table 2.3. Results of maximum hydrogen production yield and optimal HRT and OLR (Modified from (Zhang et al., 2013))

		Optimum Values			
Inoculum	**Substrate**	**HRT**	**ORL**	**Max. H_2 Yield**	**Reference**
Anaerobic digester sludge	Starch	12 h	40 g-COD/L/day	0.92 mol-H_2/mole-glucose	(Arooj et al., 2008)
Anaerobic digester sludge	Glucose	8 h	48 g-glucose/L/day	2.9 mol-H_2/mole-glucose	(Hafez et al., 2010)
Anaerobic granular sludge	Cheese whey	6 h	138.6 g-lactose/L/day	2.8 mol-H_2/mole-lactose	(Davila-Vazquez et al., 2009)
Anaerobic sludge	Glucose	12 h	40 g-glucose/L/day	1.2 mol-H_2/mole-glucose	(Kim et al., 2012)
Clostridium bifermentans 3AT-ma	Glucose (Containing 2% of NaCl)	12 h	20 g-glucose/L/day	1.1 mol-H_2/mole-glucose	(Zhang et al., 2013)

E. Other Parameters

Very few experiments have investigated the positive effect on adding chemical additives and other relevant unit operations to increase the production of biohydrogen. Some specific treatment processes like recycling the substrates have shown promising results. Heat pre-treatment of inoculum can lead to positive results concerning the biohydrogen production rate. Luo et al., (2010) showed that hydrogen yield increased from about 14 ml H_2/g VS in a mesophilic context to 69.6 ml H_2/g VS under thermophilic conditions.

Addition of 2.8%Tween 80® (T80) and 1.7 g/L polyethylene glycol (PEG 6000®) during the treatment of organic fraction of municipal solid waste (OFMSW) has been proven to be beneficial for production of biohydrogen (Elsamadony et al., 2015). When these two additives were added the hydrogen yield increased to 116.7 ± 5.2 ml_{H2}/g $Carb._{initial.}$

Fe content has also been proved to have positively influence the production of biohydrogen. The characterization of most H_2-evolver enzymes occurs more easily with the presence of iron content in the active core/site. Experiments refer to an H_2 production rate of 41.6 l/day at 10.9 mg $FeSO_4$/l, and this is 1.59 times higher compared to 2.7 mg $FeSO_4$/l (Lee et al., 2009).

2.3.3 Optimizing methane production

Production of methane containing biogas through anaerobic digestion is the most common production method and has led to proven results through a number of experiments. Biogas has already been identified having the potential to replace fossil fuels in the future (Prajapati et al., 2013). Till now, most research approaches regarding process optimization are focused on the production of methane (Andre et al., 2016; Elsgaard et al., 2016; Zhong et al., 2015). During anaerobic digestion, methane is produced from the final stage of methanogenesis; this stage is referred to as the most vulnerable of all the phases and relies on the following: temperature, pH, retention time, total ammonia nitrogen (TAN), and nutrient content of the bioreactor (Khan et al., 2016; Mao et al., 2015).

Differently designed and configured bioreactors significantly affect the process of methane production, particularly in terms of retaining stability and efficiency. Several types of bioreactors have been utilized to study the production rate and yield of methane from different substrates. Among them, dry anaerobic digestion (Andre et al., 2016), field scale plug flow reactors (Arikan et al., 2015), anaerobic sludge blanket reactors (UASB) (Intanoo et al., 2016), continuously stirred tank reactor (CSTR) (Luo et al., 2010), induced bed reactors (IBR) (Zhong et al., 2015) and anaerobic membrane bioreactors (AnMBR) (Pretel et al., 2015) could be mentioned. Another bioreactor arrangement included a degassing membrane unit coupled with a UASB reactor. It improved the methane production rate to about 94% with a liquid recirculation rate equal to 0.63 L/h (Luo et al., 2014a).

A number of research studies have been conducted so far to optimize production of methane from anaerobic digestion. The findings are mainly based on lab-scale operation (Mao et al., 2015; Zhong et al., 2015). The final stage of methanogenesis in anaerobic digestion has been referred to have dependence on a number of process parameters such as temperature, pH, hydraulic and solid retention time, organic loading rate, total ammonia nitrogen (TAN) etc. (Mao et al., 2015; Zhong et al., 2015). For a particular process variable, the optimum value is determined considering the remaining process parameters are fixed at optimum condition. Although an approach for tuning the process conditions simultaneously or dynamic modelling can provide more accurate result, a generic relationship can be established between methane production and change in temperature, pH retention time and OLR from literature review (Andre et al., 2016; Mao et al., 2015).

The following sub-section includes a simplified explanation about effects of temperature, pH, retention time and organic loading rate in methane production. The additional treatment methods and additives for increased biogas production have been mentioned in the next section. Finally, the major challenges in implementing these concepts into industrial scale anaerobic digestion plant have been discussed.

A. Temperature

Temperature has a direct influence on the thermodynamic equilibrium of the biochemical reactions of anaerobic digestion and also controls the activities, growth rate and diversity of the microorganisms (Lin et al., 2016). During the production of methane, the microbial data in thermophilic and mesophilic system refers hydrogenotrophic and acetoclastic methanogenesis respectively. Therefore, the dominant pathway for methane production is defined by operating temperature of the digester (Zamanzadeh et al., 2016).

In thermophilic conditions (55–70 °C), the growth rates for the methanogens are higher compared to the rate in mesophilic systems (37 °C) (Sun et al., 2015). The high rate of reaction enhances the system’s load bearing capacity and the productivity of the thermophilic system compared to the mesophilic system. In contrast, the high reaction rate of acidogenesis in thermophilic process involves accumulation of propionic acid in the digester. It is not degraded due to the fact that propionate degradation requires five to six times lower hydrogen concentration compared to butyrate (Liu et al., 2012).

The accumulated propionic acid then inhibits the activities by the methanogens. Results from an experiment show that when the propionic acid concentration reached above 1000 mg/L as COD equivalent, it inhibited acetoclastic methanogenesis (Shofie et al., 2015). Furthermore, more energy input is required to maintain the system at a high temperature. Conversely, the mesophilic system offers a high yield of methane, better process stability, and greater richness in bacteria with less additional energy required for the system (Bowen et al., 2014).

Considering the facts mentioned above, a two-stage anaerobic process has been suggested including a thermophilic hydrolysis/acidogenesis and mesophilic methanogenesis process (Mao et al., 2015). Selecting the process operating temperature for methane production largely depends on the type and composition of the substrate. The hyperthermophilic (70-80 °C) anaerobic digestion process performs the best in treating the co-substrates as the decomposition of organic materials is easier at high temperature (Wang et al., 2014a; Wang et al., 2012).

On this theme, a research study has been carried out to find out the optimum temperature for methane production from cattle and pig slurry (Elsgaard et al., 2016). Results here found that most methane was produced from stored digestate at 43–47 °C. The results indicated a sharp increase in the production rate of methane in the 30 to 40 °C temperature range. This is because the mesophilic populations of methanogens were favored by the post-digestion storage system.

B. pH

The pH of a reactor has a direct influence on the yield of methane production as the growth rate and activities of the microorganisms are greatly affected by the change in pH values (Yang et al., 2015). For single stage configuration, the optimum range has been reported to be 6.8–7.4 for methane production (Mao et al., 2015). The narrow optimum range could be explained by the observation that the acidogenic and methanogenic activities reach their peak at pH range 5.5 - 6.5 and 6.5-8.2 respectively (Mao et al., 2015).

As rapid acidification by accumulation of propionic acid (mentioned before) easily reduces the pH of the digester below 6.5, maintaining pH in a single stage digester is particularly challenging during the production of methane (Fezzani & Ben Cheikh, 2010; Mao et al., 2015). The alternative two-stage assembly for anaerobic digestion makes it possible to maximize the different stages of anaerobic digestion separately with optimum pH values for acidogens and methanogens. Intanoo et al. (2014) performed an experiment to produce biohydrogen and methane simultaneously from cassava wastewater using two-stage upflow anaerobic sludge blanket reactor (UASB). The pH of the initial hydrolysis stage was maintained at 5.5 while the pH of the second stage was not controlled. Instead, the experiment documented a low concentration of sodium hydroxide (230–350 mg/L) stimulating the activities of the methanogens in the second stage.

Furthermore, the production of ammonia can have a positive impact on resisting the sharp decrease of pH in a reactor. The experiment conducted by (Yang et al., 2015) revealed an increased yield of CH_4 (7.57 times higher) when the pH was increased up to 8.0 compared to the conditions of pH uncontrolled group.

C. Retention Time

Both the hydraulic and solid retention time control the efficiency of biological methane production from the anaerobic digestion process (Mao et al., 2015). A low value of HRT involves the potential risk of biomass washout from the system, leading to a low methane yield. Results show that for the algal biomass an HRT less those 10 days decreases the methane productivity (Kwietniewska & Tys, 2014).

Unlike the HRT, a low value of SRT favours methane production. Experiment on dewatered-sewage sludge in mesophilic and thermophilic conditions implied that biogas production trebled when the SRT was reduced from 30 to 12 days (Nges & Liu, 2010). However, a SRT shorter than the optimum value can cause VFA accumulation, increased alkalinity and washout of the methanogens. In the same experiment a 9-day SRT created an imbalance in the process and resulted in the problem of foaming. In addition, Lee et al., (2011) mentioned an SRT from 2.5–4 day results in a complete washout of methanogens and the inhibition of methanogenesis.

To study the effect of hydraulic retention time, 24 full-scale biogas plants in Germany were studied for the digestion of cow manure and crops (Linke et al., 2013). From the experiment, the yield of methane was expressed as a function of HRT, proportion of crops in the input and the temperature. It was observed at temperatures less than 20 °C digestate required a long time to reach the expected degradation (100 days for *HRT* = 60d) compared to the scenario where above 35 °C degradation was very fast (<40 days for *HRT* = 40d). As a consequence, the hydraulic retention time should be determined considering the operating temperature and the organic content of the substrate in a particular bioreactor.

D. Organic loading rate

Although the methane yield greatly depends on the percentage of the carbon component in the waste material, an organic loading rate exceeding the rate of decomposition or hydrolysis of the digester can actually cause a process imbalance and decline in methane production (Mao et al., 2015).

Quantification of VFA by High performance liquid chromatography (HPLC) (Zamanzadeh et al., 2016) or pH drop in digester could be utilized to find out the optimum loading rate (Aboudi et al., 2015; Farajzadehha et al., 2012). However, observing pH drop is more feasible for general applicability. A high organic loading rate leads to a high rate of initial acidogenesis that increases the amount of acid production.

As mentioned previously, (i) the low rate of methanogenesis and (ii) accumulation of propionic acid acts to reduce the pH of a digester. Qiao et al., (2013) in this connection studied thermophilic co-digestion coffee ground in a submerged anaerobic membrane reactor. The results showed a high concentration of propionic acid (1.0–3.2 g/L) consumed 60% of the total alkalinity when OLR was increased from 2.2 to 33.7 kg-COD/m^3 d. Table 2.4 lists the optimum values of OLR for different type of substrates and reactor configurations.

Table 2.4: Optimum OLR and pH range for methane production using different type of substrates

Substrate	Reactor type	pH	OLR	Reference
Sugar beet cossettes, pig manure	Semi-continuous stirred tank reactor	7.4-7.8	11.2 gVS/$L_{reactor}$ d	(Aboudi et al., 2015)
High COD wastewater	AnMBR	>7.4	11.81 kgCOD·kgVSS $^{-1}$·d^{-1}	(Yu et al., 2016a)
Dairy waste	Two stage induced bed reactor	6.8–7.5	32.9 g-COD/l-d	(Zhong et al., 2015)
Olive mill solid residue	Continuously stirred tank reactors	7.3-7.5	9.2 g COD/L day	(Rincón et al., 2008)
High-strength municipal wastewater	Upflow anaerobic sludge blanket reactor	7.6 – 8.4	7.2 to 10.8 kg m^{-3} d^{-1}	(Farajzadeh ha et al., 2012)
Food waste	Thermophilic and mesophilic digester with recirculation	7.6-8.1	18.5 gVS/d	(Zamanzad eh et al., 2016)
Olive mill wastewater	Two stage semi-continuous mesophilic digesters	5.0-6.3 (acidogenesis) 7.0 – 7.4 (methanogenesis)	8.17 ± 0.36 g COD/L/ d (acidogenesis) 4.59 ± 0.11 g COD/L/ d (Methanogenesis)	(Fezzani & Ben Cheikh, 2010)
Vegetable waste	Stirred tank reactor (Acidogenesis) fixed-bed biofilm (Methanogenesis)	5.1 ± 0.1 (Acidogenic reactor) 7.6 ± 0.1 (Methanogenic reactor)	3.0 g VS/L/d	(Zuo et al., 2015)

From table 2.4 it is clear that the limitation in organic loading rate could be avoided in the two-stage anaerobic processes as it eliminates the possible inhibition of methanogenesis by acidification (Intanoo et al., 2014; Jariyaboon et al., 2015; Zhong et al., 2015). In this connection, a study aimed for simultaneous production of hydrogen and methane from palm oil mill effluent using two-stage thermophilic and mesophilic fermentation (Krishnan et al., 2016). The total hydrogen and methane yields were 215 L H_2/kgCOD^{-1} and 320 L CH_4/kgCOD^{-1}, respectively, with a concurrent removal of 94% organic content from the substrate.

E. Other Parameters

Different additives and physical and chemical pre-treatment methods have been applied to increase the biogas production. Results confirm that adding Co and Ni increases the amount of methane produced from anaerobic digestion and addition small amount of nanoparticles containing Co, Ni, Fe and Fe3O4could increase biogas production up to 1.7 times (Abdelsalam et al., 2016).

A novel AD process was developed to produce pipeline quality bio-methane (>90%) from biochar-amended digesters through an enhanced CO_2 removal process. The biochar-amended digesters achieved the removal of CO_2 between 54.9–86.3% and the methane production rate rose to 27.6% (Shen et al., 2015).

Anaerobic co-digestion of different substrates also improved the amount of methane created; pig manure with dewatered sewage sludge may increase methane production by 82% (Zhang et al., 2014). Table 2.5 summarizes the effects of different types of additives/ treatment processes on increasing biogas production.

Table 2.5: Additives/ treatment processes for increasing biogas production

Substrate	Additives/ pre-treatment process	Results	References
Cattle dung slurry	1 mg/L Co, 2 mg/L Ni, 20 mg/L Fe and 20 mg/L Fe_3O_4	Biogas production up to 1.7 times	(Abdelsalam et al., 2016)
Rice straw	3% NaOH (35°C and for 48h)	Energy recovery increased by 59.9%	(Zhang et al., 2015)
Maize straw	NaOH (4% and 6%) pretreatment & Fe dosage (50, 200, 1000 and 2000 mg/L)	57% and 56% higher biogas and methane yield, respectively	(Khatri et al., 2015)
Swine manure fibers	Aqueous ammonia soaking (AAS)	98% increase in the methane yield	(Jurado et al., 2016)
Organic solid waste	Ozone dosage (0.16 g O_3/gTS)	37% increase in biogas volume	(Cesaro & Belgiorno, 2013)
a mixture of grass and maize silage	High pressure (9 Bar)	77% increase in methane content in biogas	(Lemmer et al., 2015)
Swine manure	Vegetable wastes (50% dw/dw)	An improvement of 3- and 1.4-fold in methane yield	(Molinuevo-Salces et al., 2012)
Nannochloropsis LEA, *Nannochloropsis* alga (WA)	Thermal pre-treatment (150–170 °C)	40% increase in methane production (to 0.31 L/gVS)	(Bohutskyi et al., 2015)

F. Challenges and recent advances in methane production

The previous discussion on optimization contains simple approach to maximize the production of methane in lab-scale operation. However, full-scale industrial operation involves a number challenges, such as:

- Although in general, high temperature favors production of methane for large-scale industrial operation, ambient condition, type of waste and associated cost to maintain the temperature should be taken into account. For example, a research study on a 400 m^3 BARC digester in Maryland (ambient temperature of 13 °C) showed that the energy requirement decreased to 70% when the temperature was reduced from 35 to 28 °C (Arikan et al., 2015).

- There is always a trade-off between the high organic loading rate and cost associated to maintain the pH at optimum range (6.5 – 8.2) for methanogens (Mao et al., 2015). The extraction of propionic acid can reduce the chance of rapid acidification in the digester. Results from research studies show that, removing propionic acid by solvent extraction can achieve an extraction yield of propionic acid up to 97% (Wang et al., 2009).

- Apart from optimizing one parameter at once; the optimization becomes more challenging when simultaneous changes in temperature, pH, retention time and OLR are taken into account. The type and reactor configuration along with substrate composition defines the appropriate approach in this regard.

- Table 2.4 clearly indicates a high organic loading could be applied to the digester with separate acidogenesis and methanogenesis stage. Implementing this idea in industrial scale involves the challenge of overcoming high capital (Membrane, tank, bioreactor) and operation (Fouling control, temperature and pH maintenance) costs (Khan et al., 2016; Pretel et al., 2015).

Major fraction of research on anaerobic process has a common target, improvement of energy conversion efficiency through optimizing the anaerobic process for methane containing biogas production (Abdelsalam et al., 2016; Huang et al., 2016; Intanoo et al., 2015). To maximize the production of methane, the most recent research works include the tolerance of anaerobic digester under extreme operating conditions, for example high OLR (up to 40.0 kgCOD·m^{-3}·d^{-1}), high salt concentration (up to 15 g/L sodium and 152 mg/L calcium concentration) and a wide range of pH values (from 6.2 to 8.5) (Xing et al., 2015; Yu et al., 2016). Among them, some experiments have already proved that the removal of intermediate products from anaerobic process (VFAs and biohydrogen) can enhance the methane yield from the final stage (Intanoo et al., 2015; Peces et al., 2016).

Currently, for methane production, one of the common performance management options includes the headspace flushing with N_2 and CO_2 where the increased CO_2 solubilization relieves the O_2 stress on methanongenesis and results higher CH_4 yield (Koch et al., 2015). Experimental results from recycling the AD effluent also showed improved productivity of methane (Li (L.) et al., 2015). Additional common performance management options include adding cellulolytic organisms, optimimzing subtrate feeding frequency, and dosing nanoparticles etc. Some of the results include a rise upto 1.8 times methane production by adding 1–20 mg/L Co, Ni, Fe or Fe_3O_4 nanoparticles (Abdelsalam et al., 2016; Manser et al., 2015; Martin-Ryals et al., 2015).

Besides process optimization, recently developed idea such as the two-stage anaerobic digestion model provides the option for rate maximization by applying different operating conditions for hydrolysis/acetogenesis and methanogenesis. Intanoo et al. (2015) developed a two-stage AD process using upflow anaerobic sludge blanket reactor (UASB) that focuses on both producing hydrogen and methane from wastewater. Results from this experiment showed 39.83 l H_2/kg COD removal at a COD loading rate of 25 kg/m^{3}d that refers more than 80% methane production compared to the production rate of 50-75% from a single stage anaerobic bioreactor.

Another option has been adding biogas AD accelerants to provide localized substrate concentration and favorable conditions for microbes (Mao et al., 2015). The research achievements in methane production clearly show a lot of promise. A combination of these factors have been responsible to promote technology of biogas production using anaerobic digestion.

2.4 Economic assessment of AnMBR products

In spite of having great promises, the application of AnMBR is very limited compared to aerobic membrane bioreactor (AeMBR) in wastewater treatment or other waste disposal industries. The primary reason is attributed to the concern that the amount of energy recovered here cannot necessarily exceed the initial installation and high operational cost. However, this limited economic feasibility may be a result of not considering the situation for maximizing intermediate AnMBR products. The following paragraphs include individual and comparative discussions about economic feasibility when AnMBRs with different arrangements are designed to produce VFAs, biohydrogen and methane individually or simultaneously.

2.4.1 Assessment of methane Production

The current commercialization of AnMBR digestion focuses on maximum biogas production and its main constituent, methane. It is clear to which extent product of methane remains the major driver of the anaerobic digestion process. Firstly, compared to the other AD products methane has the advantage of limited downstream processing, the created biogas can directly be utilized for fuel with or without further purification, and for chemical intermediates. The second advantage is, production of methane involves low energy consumption, and the process uses all biodegradable organic matter and produces a high yield (Kleerebezem et al., 2015). Although Methane is considered as a suitable energy source with low cost, the production rate of methane varies with substrate composition. As a result, stable methane production rate has been a common problem for anaerobic digestion, because the feed with low organic content cannot provide sufficient organic carbons for methane production.

Pretel et al. (2015) evaluated the design parameters for an submerged AnMBR under different solid retention time (13-41 days), organic loading rates (10-15 g/l MLSS) and operating temperatures (15 - 30 °C). In addition, the initial installation (sizing and construction of reactor, pumps and membrane) and operating cost (gas spurging, filtration and pump operating) of 100% biogas or total methane recovery were calculated against the product revenue from methane. According to the results, profit from total methane recovery had negative values represent net profit (ranging from -0.005 to -0.002 euro/m^3) against the total cost range from

0.130 to 0.079 euro/m^3. This indicated that the revenue earned from methane production could not exceed the initial installation and operating cost of an AnMBR.

2.4.2 Assessment for the production of VFA

The large-scale production of VFA is governed by the chemical synthesis that includes the process of methanol carbonylation and catalytical oxidation reaction between ethylene and carbon monoxide (Scoma et al., 2016). However, detailed cost analysis is not yet available to compare the economics between conventional carbonylation and anaerobic digestion processes. From the anaerobic digestion process, the extraction of VFAs could be performed simultaneously with methane or aiming at the complete recovery of VFAs only. Peces et al. (2016) investigated primary sludge pre-fermentation under semi-aerobic conditions. Their experiments demonstrated both VFA recovery (43 g CODVFA kg−1 VS) and improved methane recovery at both 20℃ and 37℃ operating conditions.

VFA produced from the initial hydrolysis stage of the anaerobic digestion process is a source of reduced chemicals such as alkanes, aldehydes, alcohols, and ketones (Huang et al., 2016; Morgan-Sagastume et al., 2011; Peces et al., 2016; Scoma et al., 2016). Polyhydroxyalkanoate (PHA), a biopolymer used for biodegradable plastics production could be produced more economically from VFA enriched photosynthetic mixed culture (PMC) rather than the current pure culture systems by commercial industries (Fradinho et al., 2014).

A comparison between the revenue earned from methane and VFA generation was performed by Kleerebezem et al. (2015) based on a cardboard production facility producing 5000 m3/day wastewater in a closed cycle. The results included a revenue of 3.6 k€ from total methane recovery compared to 20.2 k€ revenue from PHA produced in a single day. However, their cost analysis did not consider the operational cost for methane or PHA production and also the cost involved in downstream processing for product recovery, but the significant economic room encourages more detailed research work on economic feasibility assessment when VFA is produced from AnMBR.

2.4.3 Assessment for the production of biohydrogen

The production of biohydrogen using the anaerobic process has been a great idea for overcoming the problems posed by carbon emissions (Intanoo et al., 2015; Jariyaboon et al., 2015). The current industrial hydrogen production involves coal, natural gas and oil as favorable raw materials but all these processes are energy intensive and require significant quantities of fossil fuel (Hosseini and Wahid, 2016). Biohydrogen production via anaerobic fermentation could reduce production costs which compromise the efficiency of the current industrial process; it is a renewable enterprise and may represent sustainable and efficient energy in the future (Jung et al., 2011; Xia et al., 2015).

Biohydrogen production from municipal waste and wastewater has already proved its sustainability but has the current drawback of low hydrogen yield (Hosseini and Wahid, 2016). The large-scale application of biohydrogen production has been greatly compromised by safety and economic issues involved in hydrogen storage (Lowesmith et al., 2014; Mohammadshahi et al., 2016). The current hydrogen storage system suffers from technical issues that include the corrosion and embrittlement in common materials such as carbon steels (Rezende et al., 2015).

For maximum hydrogen production, recently developed models mostly include simultaneous production of biohydrogen and methane (Intanoo et al., 2015; Jariyaboon et al., 2015) from the two-stage UASB reactor. Results from these experiments have provided improved methane recovery with the produced biohydrogen. Hence the cost recovery from these two stage anaerobic process is higher compared to the conventional anaerobic process.

The cost of hydrogen as fuel still remains on the higher side and production of biohydrogen could be a cost effective option. Not only the simultaneous production with methane but also the individual production could be a feasible option. No research data is yet available regarding the condition when biohydrogen is considered as the only product from the AnMBR. Although multiple stage arrangements have a drawback for additional cost of initial installation (Reactor and membrane installation) and process operation (membrane fouling, temperature, pH control), the cost recovered through the production of hydrogen could be compared with the additional amount for multiple stage assembly.

A typical cost analysis model includes the energy supply system (production cost, production level, available resources, etc.), energy markets (fuel prices, price adjustment), consumer choice behaviour (consumer utility, fuel demand, vehicle adoption) and refuelling infrastructure finally (Shafiei et al., 2017). The following discussion will focus on the production and storage cost of hydrogen as fuel.

Economically, industrial production of hydrogen has not been considered as a feasible option as fossil fuels are as raw materials in common cases such as steam methane reforming and coal gasification. Also, in each of these production system external costs are associated with carbon capture and storage. Furthermore, hydrogen production rate and yiled from anaerobic process is variable as the carbon content is different raw material. Some cost comparisons have been done regarding different hydrogen production processes (Bakenne et al., 2016) but it is particularly challenging to compare hydrogen production costs to continuously changing fuel and oil prices. Table 2.6 contains the summary of overall hydrogen production cost from different raw materials and processes.

Table 2.6: Cost of hydrogen production using different energy sources

Raw material	**Process**	**Production cost ($\$/kgH_2$)**	**References**
Natural gas	Steam Methane Reforming	0.75	(Parthasarathy & Narayanan, 2014)
Natural gas	Steam Methane Reforming (with carbon capture & storage)	2.67	(Dincer, 2012)
Nuclear	Electrolysis	2.4	
Nuclear	High Temperature Electrolysis	3.5	(Acar & Dincer, 2014)
Nuclear	Copper–chlorine	1.7	(Wang et al., 2010)
Nuclear	Sulfur–iodine cycle	1.9	
Coal	Gasification (with carbon capture & storage)	1.8	
Solar	Electrolysis	7.7	(Nowotny & Veziroglu, 2011)

Solar	Photovoltaic electrolysis	9.1	(Bhandari et al., 2014)
Solar	Photoelectrochemical	3.5	(Acar et al., 2015)
Wind	Electrolysis	7.2	(Wang et al., 2010)
Wind	Electrolysis	7.3	(Hwang, 2013)
Biomass	Gasification	1.65	(Koumi Ngoh & Njomo, 2012)
Biomass	Gasification	1.4–2	(Kalamaras & Efstathiou, 2013)
Biomass	Pyrolysis	1.3–2.2	
Biomass	Gasification	4.60–7.86	(Dowaki et al., 2007)
Geothermal	Steam electrolysis	1–2.6	(Yilmaz et al., 2012)

From table 2.6, it is evident that the lowest production cost for steam methane reforming process is 0.75 USD per kg of hydrogen whereas the maximum retail price of gasoline was 3.5 USD per gallon considering the time from 1994 to 2011. However, the costs associated with carbon capture and storage from CO_2 produced from gasoline have not been accounted for in this calculation. Additionally, it is expected that by the year 2030 the supply/demand gap in global oil and gas production will increase when demand for energy will rise by 60% (Bakenne et al., 2016). It will therefore be predicted that oil and gas prices will rise over time in the next few decades.

The costs involved in carbon capture and storage are variable since the type of carbon capture and storage process differ, and the expenses required for building the infrastructure for CCS will also vary. The US National Energy Technology Laboratory has estimated USD 16/t CO_2 for carbon capture and storage (Martínez Arranz, 2016). Given the current trend of fossil fuel usage, global CO_2 emissions could rise to 44 billion tones by 2040 (Theo et al., 2016) and means that approximately $704 billion USD will have to be spent on capture and storage technologies and processes. Such costs can only be reduced if the increased demand for energy is satisfied by the production of biohydrogen from renewable energy sources.

An average of 50 million metric tons of hydrogen is produced worldwide annually where 76–77% of the produced hydrogen is converted from natural gas and oil (Naphtha), 19–20% is produced from coal, and the remaining 3–4% is produced from renewable sources (Bakenne et al., 2016). As the majority of hydrogen production involves the usage of fossil fuel as raw materials, production costs have not been competitive enough compared to the traditional energy sources such as gasoline or petrol. For example, Lee (Lee, 2016) performed cost benefit analysis and evaluation of financial feasibility of full commercialization of biohydrogen. The study was performed on cost-capacity scaling methods for different biohydrogen production plants. Their final result showed 2.20, 3.37, and 3.85 benefit/cost ratios for three different scenarios respectively. Additionally, Internal Rate of Return (IRR) and External Rate of Return (ERR) were calculated to 42.45%, 58.71%, 62.77%, and 14.40%, 16.05%, and 16.53% in payback periods of 11.33, 8.95 and 8.52 years.

Another study involved the cost benefit analysis of carbon footprint from hydrogen fuelled scooter and Internal Combustion Engine (ICE) scooters (Chang et al., 2016). The experiment came up with a result that the hydrogen fuelled scooter from Steam Methane Reforming (SMR) process has the smallest carbon (0.0115 kg CO_2). As a result, the cost involved in carbon capture and storage would be less compared to the biohydrogen production process (Lakshmikandan & Murugesan, 2016). Same study measured the total life cycle and came up with a result that the total life cycle cost (excluding fixed costs i.e. cost of the vehicle, hydrogen production unit etc.) is maximum (USD 6632) for the hydrogen fuelled scooter whereas the ICE scooters have an amount of USD 4233. However, the reason was quite obvious as the hydrogen fuelled scooter was using the SMR process. Hydrogen fuel generation from biomass could be an interesting research option to reduce the total life cycle costs for hydrogen fuelled vehicles.

On the other hand some cost benefit analysis has produced negative results leaving the concept of hydrogen fuelled vehicles not being a feasible option. Ito & Managi (Ito & Managi, 2015) investigated the economic validity of diffusion of hydrogen Fuel Cell Vehicles (FCV) and all Electric Vehicle (EV) in Japan. The differences between net present value between benefit and cost were studied to find out the economically feasible option between these two. The highest net positive value (NPV) was – 19 billion dollar based on 5 million FCV vehicle diffusion scenarios. However, the major limitation of this study lies in the calculation of total cost estimation. The authors estimated the differences in vehicle purchase and operating costs for FCV and Internal Combustion Vehicles (ICV) and added the differences to find out the total cost. Surely, the actual cost was not reflected during the cost benefit analysis of this study.

In terms of energy storage and transport, hydrogen fuel could be considered as an economically favorable option for long term storage. A study compared the cost comparison of pumped-hydro, hydrogen storage and compressed air energy (Klumpp, 2016). This calculation included the average discounted electricity generation cost, termed as "Levelized Electricity cost" (Hao et al.) for three different energy sources. For a long term storage scenario, the findings included a reduction of 70% LEC for hydrogen storage compared to 10% and 20% reductions for pumped hydro and compressed air storage, respectively. This research study suggested that by 2030 hydrogen storage would emerge as the best source of energy for all storage-discharge paths.

2.4.4 Cost comparison considering different product spectrum from AnMBR

High fluctuations of industrial toxicants, different sources of waste result unstable biogas production rate as different amount of organic compounds are available for methanogenesis. This could be the single major problem acting against the widespread industrial application of AnMBR. Studies have been conducted to breakdown the initial installation and operating costs involved in AnMBR treating wastewater from different sources (Lin et al., 2011; Pretel et. al., 2014). Table 2.7 provides a summary based on the results from both experiments and it clearly indicates that major portion of the operating cost is associated with high energy requirement when biogas is recycled into the system.

Table 2.7 Breakdown of total life cycle capital cost, operating cost and energy consumption in different AnMBR process (data adapted from Lin et al., 2011; Pretel et. al., 2014)

Submerged AnMBR treating 20000 m^3 volume municipal wastewater				**AnMBR treating (3.2±0.7 m^3/day) sulphate-rich urban wastewater**	
Total Life cycle capital cost (%)		Operating Cost (%)		Energy consumption (%)	
Tank Installation	11.3	Gas Scouring Energy	46.7	Biogas recycling blower	73.5
Membranes	72.3	Pumping Energy	13.7	Sludge feeding pump	14.6
Screens	5.9	Sludge Disposal	7.2	Stirring power reactor	8.3
Gas Blower	5.5	Chemical Consumption	32.5	Permeate pump	1.8
Other Costs	5.0			Other Consumers	1.8

The heavy burdens of AnMBR economy mainly include low flux, membrane fouling, high capital and operational costs. Over last few years there have been a significant development on the reduction of membrane acquisition or replacement costs because the costs for membrane modules have significantly decreased (Ozgun et al., 2013). Regardless the AnMBR arrangement, during methane production high amount of energy is always required for gas scouring and this energy supplement requires up to 46.7% of total operational cost of AnMBR (Lin et al., 2011; Pretel et. al., 2014).

For maximum methane production, the production of VFA is controlled down to the `level where the reduction of pH does not inhibit the methanogenic activity (Yuan and Zhu, 2016). Simultaneous VFA and methane production could be an option, but the complete inhibition of methanogenic activity could provide the opportunity to reduce the cost of installation, energy consumption and application of wider operating range in AnMBR operation (Kleerebezem et al., 2015).

Unlike VFA, research models have already been developed to produce biohydrogen from AnMBR (Bakonyi et al., 2015; Kim et al., 2011). For biohydrogen production, two stage anaerobic digestion process offers improved process stability through COD elimination in methanogenic stage, eliminates the limitation in organic loading rates and provides an option to treat sewage sludge, dairy wastewater, food waste and agro-industrial wastes (Guwy et al., 2011). In this connection, assessments are required to compare the low cost of operation and added biohydrogen production with the high initial installation cost for multiple stage arrangement.

Energy required for gas recycling, range of applicable organic load, pH and temperature control for methanogens, rate control for hydrolysis/ acidogenesis and unstable methane production are the key factors that stand on the way of the economic feasibility of currently established AnMBR models. The alternate approach to produce biohydrogen and/or VFA only could be a potential solution that can improve the economic feasibility of AnMBR. The technical feasibility achieved from different anaerobic models has been correlated in table 2.8. It summarizes the economic and technical challenges associated with different products spectrum and provides the potential research options based on the theories and limited available results.

Table 2.8: Summary of the proposed AnMBR models for different product band from AnMBRs

Production Band	AnMBR Model	Major Challenges	Recommendation
Biohydrogen, VFA and Methane	Multiple stage	High installation cost, high operating cost, process optimization	Economic feasibility could be assessed whether the cost recovery by producing hydrogen and VFA could exceed the installation cost.
VFA and methane	Single stage	Process optimization, reactor design	Feasibility study for multiple stage AnMBR
VFA	Single/ Multiple stage	Process optimization, utilization of all terminal stage products	A new AnMBR model with the inhibition of methanogenesis step (Kleerebezem et al., 2015) could be implemented through research
Biohydrogen and methane	Multiple stage	High installation cost, High operating cost (biogas recycling, control against membrane fouling)	Developed research models have proven the technical feasibility (Intanoo et al., 2014; Jariyaboon et al., 2015; Zhong et al., 2015). Economic feasibility could be assessed.
VFA and biohydrogen	Single/ Multiple	Process optimization, product spectrum control	The alternate approach (Kleerebezem et al., 2015) could be implemented by research

2.5 Environmental Impact

Although AnMBR does good work by treating the waste materials or wastewater, negative environmental impacts associated with the products and effluents does not make it the best option for anaerobic digestion process. The current major product methane and its combustion product carbon dioxide have been identified as major contributors in greenhouse gas emission.

The world has clearly recognized the devastating effects of climate change and current political agendas do clearly focus on reducing CO_2 emissions from burning of fossil fuels (Cucchiella and D'Adamo, 2013). Many strategies have set out to develop renewable and clean energy sources to mitigate the problem of finite fossil fuel reserves and environmental problems associated with these fuels (Wei et al., 2013).

The carbon dioxide emission rate has been growing exponentially by the continual increase of fossil fuel usage. Optimizing the process parameters in AnMBR for maximum methane production provides a sustainable option for bioenergy production. However, the development of this emerging technology would also contribute to the rising trend of global carbon dioxide emission. Besides contributing into the greenhouse gasses, there are other environmental issues associated with the AnMBR products; the following paragraphs contain the effect of AnMBR products on the environment.

Global warming, acidification, eutrophication, abiotic depletion and maritime aquatic eco-toxicity have been identified as the major environmental impacts from the products of AnMBR (Pretel et al., 2016). In a separate study, Pretel et al. (2013) evaluated the environmental impact of different products and effluents originating from submerged anaerobic MBR (SAnMBR). The assessments were based on three operating temperature conditions - ambient 20°C, 33°C and controlled 33°C. The results obtained from the study are summarized in table 2.9.

Table 2.9: LCA results of submerged AnMBR. Method: CML 2 baseline 2000 V2.05/West Europe, 1995/Normalisation/Excluding infrastructure processes (modified from Pretel et al., 2013)

Impact Category	Ambient 20 °C	Ambient 33 °C	Controlled 33 °C (at ambient 20 °C)
		Total (X 10^{-14})	
Eutrophication	158.8726	159.1307	191.6357
Marine aquatic eco-toxicity	11.6750	10.9076	362.4733
Acidification	7.7487	6.6890	184.0135
Terrestrial eco-toxicity	7.4031	7.0542	31.7411
Fresh water aquatic eco-toxicity	70.7456	76.8873	80.7569
Abiotic depletion	3.2047	2.8501	576.6242
Global warming (GWP100)	2.5455	2.3352	227.7044
Human toxicity	69.7208	76.3144	95.9476
Photochemical oxidation	0.3407	0.3145	24.0949
Ozone layer depletion (ODP)	0.0061	0.0055	1.1397

The content of nitrogen and phosphorus in the digestate is not dependent to the anaerobic digestion process and their percentage mainly depends on the type of substrate that is being processed (Jeong et al., 2018; Puchongkawarin et al., 2015). Negative environmental effects like eutrophication, aquatic eco-toxicity, acidification and human toxicity are directly attributed to the degradation rates of total COD, amount of total nitrogen, phosphorus and finally the production rate of methane (Kaya et al., 2019; Pretel et al., 2013). Thus, tuning AnMBR parameters for improved nutrient recovery could be an option can partially reduce some negative effects but controlling the product spectrum could be an effective option to reduce environmental impacts caused by methane.

The impact category of GWP (Global Warming Potential) is associated with the amount of energy required for AnMBR operation. The model designed for methane production has already been identified as energy intensive for gas scouring and supplying heat energy to increase methane production (Lin et al., 2011; Pretel et. al., 2014). Both factors contribute to the impact category of GWP in AnMBR operation. Apart from the energy requirements, the produced raw biogas from AnMBR constitutes the major component of methane and CO_2.

There is no argument that methane and carbon dioxide directly contributes to the greenhouse gas emissions followed by the environmental GWP on the environment. Direct discharge of methane into the atmosphere is also possible by the fugitive emission from AnMBR. In addition, if not handled properly, dissolved methane could also be present in the AnMBR effluent. Low temperature operating conditions in AnMBR can create an effluent that contains more than 50% of methane (Pretel et al., 2016). Since the GWP of methane is approximately twenty-three times that of carbon dioxide, 5% emission could simply undermine and negate the positive impact of anaerobic digestion (Kleerebezem et al., 2015). To capture dissolved methane from bioreactor effluent, degassing membrane system has been a relatively new concept but the recovery system is yet to achieve the optimization. Impact categories like human toxicity, fresh water aquatic eco-toxicity, terrestrial eco-toxicity and marine aquatic eco-toxicity are directly affected by the presence of dissolved methane in the AnMBR effluent (Pretel et al., 2013).

Fatty acid methyl esters (FAMEs) derived from VFAs can be used as a green solvent due to their low toxicity and high biodegradability (Jung et al., 2016). Another derived product, PHA, could be degraded by the microorganisms that secretes depolymerase enzymes to hydrolyse the bonds of ester polymers (Elain et al., 2016). Besides, recovery of VFA diverts part of available organic carbons in the anaerobic digestion; it eventually reduces the methane production followed by the reduction of environmental impacts associated with greenhouse gas emission (Puchongkawarin et al., 2015). Therefore, anaerobic digestion process designed for maximum VFAs production could be applied in AnMBR design. Practical research work in this connection would contribute to eliminate the negative environmental impacts from AnMBR associated with methane production.

Conservation of the environment and sustainability are now the most prioritized section in aspects of any fossil fuel's evaluation. The primary combustion product of fossil fuels is CO_2 and CO where additional impurities' supply of air also leads to the production of NO_X and oxides of sulfur (Nicoletti et al., 2015). Renewable energy sources like hydrogen, geothermal, wind and solar do not affect the environment by producing greenhouse gasses like NO_x and CO_2(Esen et al., 2007). For hydrogen, the only possible product from the combustion of hydrogen is water vapor which has no significant direct effect on the environment or human health. The statistics related to the emission of pollutants, such as SO_x, NOx, carbon monoxide and carbon dioxide emphasize the superiority of hydrogen over fossil fuels. To provide an idea of the effects of air pollution, Figure 2.3 lists the common industrial pollutants that are now typical in industrialized countries.

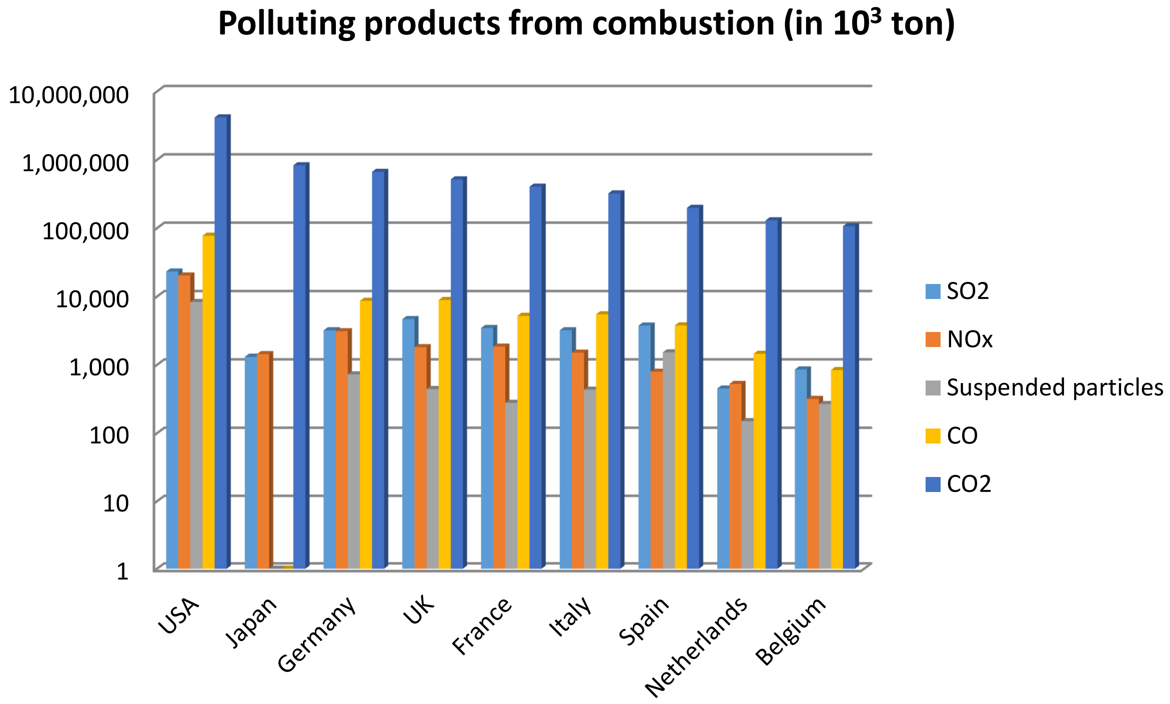

Figure 2.3: Comparison between the main pollution emissions in the industrialized countries (Data adapted from (Nicoletti et al., 2015)).

Nicoletti et al. (2015) compared the weighted percentages of pollutants in combustion flue gas with reference to hydrogen, carbon, methane and octane. From the results listed in Table 2.10, it is clearly evident that the combustion of hydrogen offers zero emissions regarding CO_2 and SO_2 apart from the emission of nitrogen oxides. The formation of NO_x is a function of flame temperature and duration (Geng et al., 2016). Considering the broad flammability range of hydrogen, its combustion can be influenced how an engine has been designed, so the aim should be to reduce NO_x emissions.

Table 2.10: Weighted percentages of pollutants in combustion flue gas for common fuels

Fuel	**kg Pollutants /kg of fuel**				
	CO_2	**SO_2**	**NO_x**	**Un-burnts, particulates**	**H_2O**
H_2	0	0	0.016	0	7
C	1.893	0.012	0.008	0.1	0.633
CH_4	2.75	0.03	0.0075	0	2.154
C_8H_{18}	3.09	0.010	0.0115	0.85	1.254

The table does not include pollutants like Volatile Organic Materials (VOCs), radioactive materials and heavy metals that may be present with fossil fuels during combustion. Impacts on the environment that are additional to greenhouse gas emissions have been mentioned by Khan et al. (Khan et al., 2016b). For example, methane from anaerobic digestion could be present in the liquid effluent from a bioreactor that leads to environmental problems like eutrophication, marine aquatic eco-toxicity, freshwater aquatic eco-toxicity, terrestrial eco-toxicity, human toxicity, etc. Since hydrogen is not soluble in water, the production of hydrogen from anaerobic digestion could eliminate these serious environmental problems.

The advantage of hydrogen as a clean energy source is evident in that it reduces the release of pollutants into the environment. To limit the rise of global average temperature < 2°C, maximum allowable emission limit carbon dioxide should be around 565-886 billion tones until 2050 (Ashnani et al., 2015). Achieving this target would only be possible if more emphasis is given on developing and employing alternative energy sources like hydrogen.

2.6 Impact on scientific society

Recent publications have reported that the scientific community is increasingly interested in producing biofuels from biodegradable wastes (Ozgun et al., 2013; Smith et al., 2014). The scientific community engaged is bioresearch believes that a high level of viability and sustainability of biofuels has been achieved by employing these biodegradable wastes as feedstock. Until now, the community's perception on biofuel generation is in the primary stage as the conventional energy production process still offers cost effectiveness over bioenergy. Considering the contribution in global power generation, only 1.8% of the power is from bioenergy (Sawin et at., 2015).

Although producing energy from anaerobic digestion process is in the early days, statistics show that the number of anaerobic waste management plant has been increasing sharply around the world. For examples, in 2014, the Anaerobic Digestion and Bioresource Association (ADBA) in London, UK reports a cumulative methane production rate of 19000 m^3/h from 32 commissioned anaerobic plants, compared to a production rate of 2,000 m^3/h from 6 new commissioned anaerobic processes in 2013 (More, 2015). In addition, in Europe, with a capacity of 8 million ton of organic waste, 244 anaerobic plants are operated to process about 25% organic wastes (Adekunle and Okolie, 2015).

There is no doubt, industrially AeMBR is still favored over the AnMBR despite the fact that AnMBR requires less energy compared to the aerobic system. The large scale introduction of AnMBRs has been limited for two main reasons. Firstly, people are more interested in the amount of bioenergy produced regardless of the type of waste material being treated. The low energy density compared to fossil fuels is a limitation for some applications and poses challenges to new business models (Richard, 2010).

Current research initiatives only provide an incomplete picture when comparing the drivers of different energy models. Most studies so far have selected single cases or regions to analyze specific situations. In this case, the promising results obtained from different anaerobic digestion models have not been implemented through design modification of existing AnMBR arrangements. Research initiatives on the valorization of the intermediate products are still in its infancy and no large-scale industrial application yet has been occurred.

For the scientific community, developing cost effective synthesis and storage system for hydrogen energy is the primary area of focus as industries have already started making preparations for the application of hydrogen energy. Among other renewable energy sources, hydrogen has already been identified as the main alternative of fossil fuels because of its ability to power fuel cells in zero-emission electric vehicles. Market introduction has just been made for the fuel cell electric vehicles by the car makers. Automobile companies are entering the pre-commercial phase by progressing from prototype vehicles to small-scale production (Ball and Weeda, 2016). By the year 2025, the United States alone aims to put 3.3 million zero-emission vehicles powered by hydrogen fuel cells. Until 2023, the state of California alone aims to invest $20 million annually to reach a goal of 100 hydrogen filling stations throughout the state (O'Malley et al., 2015).

Despite the fact that hydrogen has a high potential as a renewable energy source, it has not yet been considered by the general consumers because of its requirement of high cost, lack of available skills and technical knowhow. Relatively expensive hydrogen production by electrolysis has garnered considerable attention because it offers more flexibility for large-scale integration of intermittent renewable energies. Production of hydrogen by the anaerobic digestion process has been proven technically feasible but lack of investment and operating costs (Ferrer et al., 2015; Pretel et al., 2014; Pretel et al., 2015) are prohibitive, suggesting high values relative to the conventional single stage AnMBR. Before large-scale application can commence, the process demands optimization and comparative economic feasibility assessment of the current technologies (Mei et al., 2016; Miranda et al., 2016).

In the existing wastewater treatment plants using anaerobic digestion, VFA has already been identified to aid the biological nutrient recovery process and increasing the methane production from the final stage of anaerobic digestion. Major challenge lies ahead to reduce the cost of biosynthesis process for PHA, as the production cost in oil-derived plastics is still favorable (Elain et al., 2016; Fradinho et al., 2014; Peces et al., 2016). No research has been performed yet to produce an integrated PHA production process from VFA by assessing the cost for process operation and downstream processing required for product recovery. There have been pilot-scale attempts to maximize VFA production in the anaerobic digestion (Huang et al., 2016; Ma (H.) et al., 2016; Xia et al., 2016; Yin et al., 2016) but the findings are yet to be implemented using different AnMBR arrangements.

The scientific community and some industries have already adapted methane as the final product from the AnMBR but currently it is no better than fossil fuels. Since methane has only been considered as the end valued product, the appetence of AnMBR has not been made for limited economic feasibility. Research developments to produce alternate products have showed promise, but only for fragmented pictures or specific substrate conditions. Most of the achievements involve anaerobic digestion with different bioreactors, only a limited number of experiments have been performed on AnMBRs. Compared to different AnMBR products, it is evident that the community's perception of VFA and hydrogen is yet to be ascertained. The employment of both products requires more research in terms of economic feasibility and large scale application. Before industrial application, it is required to develop generic research models of AnMBR where the product spectrum could be controlled by altering the operating conditions or bioreactor arrangements. The feedstock composition would be the challenging factor when concentration is given for a particular product.

2.7 Concluding remarks

[1] Production of methane from anaerobic digestion involves technical issues like reactor acidification, low biogas recovery due to dissolved methane and low energy density. In addition to the technical issues, the environmental impact of AnMBR includes negative effects like high GWP, aquatic ecotoxicity, human toxicity, etc. Production of methane can be deemed vulnerable as the process depends on a narrow range of strict operating conditions. Therefore, from the technical and environmental viewpoint, methane-containing biogas is not the best option for energy recovery from AnMBR.

[2] Intermediate AnMBR products like VFA and biohydrogen can eliminate the technical difficulty in AnMBR through the inhibition of methane production stage. Economically, production of VFA can be considered as a more feasible option compared to methane and biohydrogen. In contrast, the production of biohydrogen can be more favorable as an AnMBR product because of its fuel properties and clean combustion product.

[3] For full-scale industrial production, extracting VFA from AnMBR can be a particular challenge as the extraction and purification process can be cost intensive. Cost analysis based on direct comparison of the product revenue earned from VFA and methane indicates VFA as a better alternative compared to methane.

[4] The current hydrogen production processes based on fossil fuel still involves negative environmental impacts as the combustion of fossil fuels contributes to the CO_2 Rand CO production. Although the production cost of biohydrogen still remain on the higher side compared to conventional processes, more research initiatives are needed to optimize the production of biohydrogen from AnMBR.

[5] Temperature, pH, OLR, and HRT can be critical for VFA, biohydrogen and methane production from AnMBR. Each AnMBR product requires a very specific set of operating conditions and temperature and pH both can be generalized regardless of the bioreactor design and arrangement. Optimum values of OLR and HRT are dependant on the bioreactor type and design. Therefore, OLR and HRT needs to be defined for a particular type and arrangement of a bioreactor.

[6] Currently, available research studies have employed selective microbial inhibition such as inhibition of methanogenesis for VFA and biohydrogen production. It can be a potential issue in full-scale industrial operation as the composition of the feed solution can vary within a very wide range. A system tuned to produce VFA or biohydrogen only does not have the flexibility to produce methane. A generic AnMBR model can provide with the opportunity to select single/ multiple products from AnMBR. It can eventually improve the overall energy recovery from AnMBR and at the same time reduce negative environmental impacts caused by methane production from AnMBR.

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Chapter 3

Experimental Investigations

3.1 Introduction

This chapter includes the sludge characteristics, experimental setup, operating conditions of AnMBR, TMP measurement, biogas analysis and VFA extraction and quantification (for chapter 4-7). The solvent extraction method was applied for the extraction of VFA while Gas Chromatogram – Mass Spectroscopy was sued to analyze the quantity of individual components of VFA mixture. During AnMBR operation at a certain operating condition, the sludge samples have been collected from the bioreactor periodically and the bioreactor effluents have been collected regularly for the analysis of COD and nutrient removal. TMP was measured and recorded for each set of operating conditions.

3.2 Materials and methods

3.2.1 Wastewater Characteristics

The synthetic wastewater solution contained glucose as the organic carbon source which is easily biodegradable, and $NaNO_3$ and KH_2PO_4 as sources of nitrogen and phosphorus, respectively. The following table summarizes the specific concentrations of major and trace nutrients present in the feed solution.

Table 3.1: Composition of different components in feed solution

Compounds	Concentration (mg/L)
Organics and nutrients	
Glucose ($C_6H_{12}O_6$)	560
Sodium Nitrate ($NaNO_3$)	147.6
Ammonium sulphate ($\underline{NH}_4Cl$)	23.22
Potassium phosphate ((KH_2PO_4))	26.4
Trace nutrients	
Calcium chloride ($CaCl_2{\cdot}2H_2O$)	0.736
Magnesium sulphate ($MgSO_4{\cdot}7H_2O$)	10.14
Manganese chloride ($MnCl_2{\cdot}7H_2O$)	0.55
Zinc sulphate ($ZnSO_4{\cdot}7H_2O$)	0.88
Ferric chloride anhydrous ($FeCl_3$)	2.9
Cupric sulphate ($CuSO_4{\cdot}5H_2O$)	0.782

Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	0.84
Sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$)	2.52
Yeast extract	60

3.2.2 Sludge acclimatization

The AnMBR used for this experiment had mixed liquor seed sludge from two different water treatment plants in Sydney, Australia (Cronulla water treatment plant and Central Park water treatment plant). At the beginning of each experiment, the sludge in the reactor had a mixed liquor suspended solid concentration of 10 g/l. The system was purged with nitrogen to get any unexpected air and oxygen out with diffused aeration tubes. The sludge mixture (30:70 ratios from Cronulla and Central Park respectively) was acclimatized in the reactor for 90 days until a constant COD and nutrient removal was obtained. Characteristics of the seed sludge have been listed in table 3.2.

Table 3.2: Characteristics of seed sludge

Parameters	Units	Cronulla WW treatment plant	Central Park WW Treatment plant
pH	–	7.1 ± 0.2	7.3 ± 0.2
TSS	% w/w	14.5 ± 0.5	11.3 ± 0.5
VSS/TSS	% w/w	74.12 ± 1.9	68.30 ± 0.5
COD	mg/L	1102 ± 10	890 ± 2
TN	mg/L	117.2 ± 2.5	142.91 ± 3.1
TP	mg/L	27.24 ± 1.2	21.36 ± 1.5

3.2.3 Experimental Setup

An anaerobic submerged membrane bioreactor was used in this experiment with a working volume of 3.5 L. A submerged hollow fibre membrane module was used in this experiment (PVDF, 0.04 m^2, 0.07–0.1 µm pore size, 1.0 and 2.2 mm of the inner and outer diameter respectively) (Figure 3.1). Continuous mode of operation was applied in the membrane with 8 minutes of suction and 2 minutes of relaxation time.

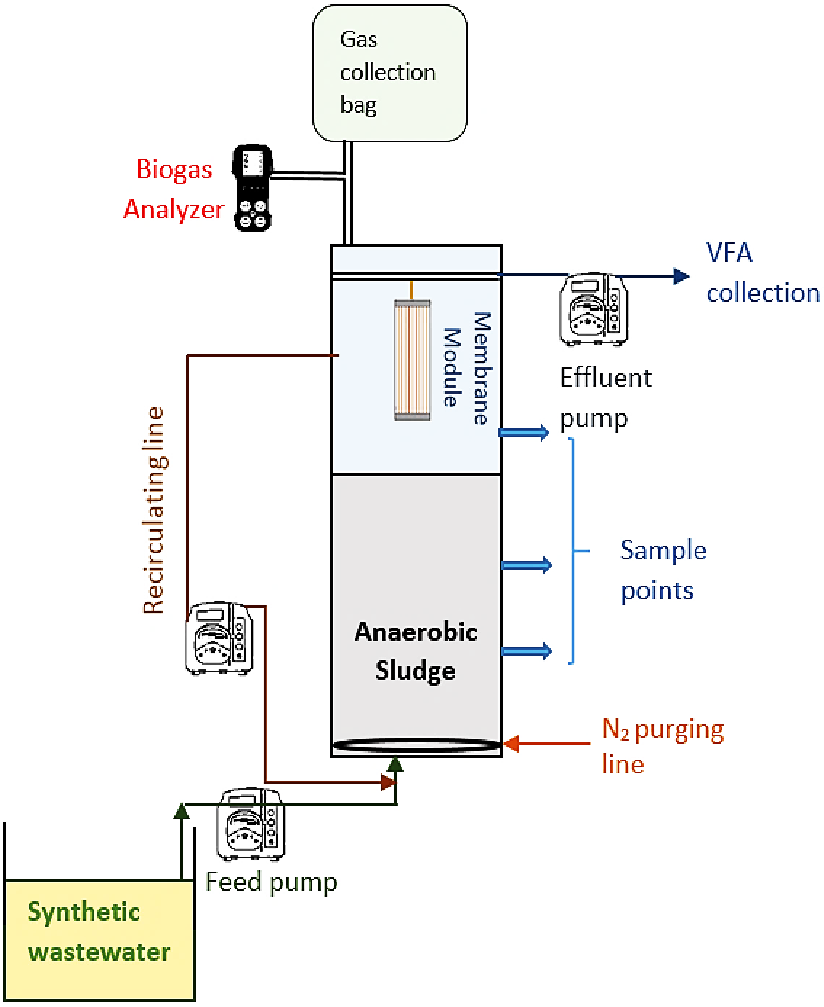

Figure 3.1: Schematic of experimental setup for VFA and biohydrogen production

At the beginning of each trial, the concentration of Mixed Liquor Volatile Suspended Solids (MLVSS) was maintained at 10 g L^{-1}. The temperature of AnMBR was controlled at 22 ± 2 °C. The pH level in AnMBR was maintained by adding Na_2CO_3 in the feed solution. During each stage of the AnMBR operation, the maximum level of Dissolved Oxygen (DO) was maintained at 0.01 ppm. Nitrogen was purged to reduce DO through e aeration tubes installed at the bottom of the reactor.

3.2.4 AnMBR operation

For different experimental investigations, AnMBR was operated in the continuous mode. Each set of operating condition was maintained for about for 3 weeks followed by a one-week period to recover and stabilize the reactor to the new pH level. VFA samples were collected and analyzed at 4-day intervals and each time two different samples were analyzed simultaneously to obtain the most accurate results. At the end of each trial, Mixed Liquor Volatile Suspended Solids (MLVSS) were measured and the value was maintained at 10000 ± 500 mg/l. The COD and nutrient removal performance was recorded at every two operating days. SRT was maintained at 60 days, excess sludge was collected from the reactor at the end of every week.

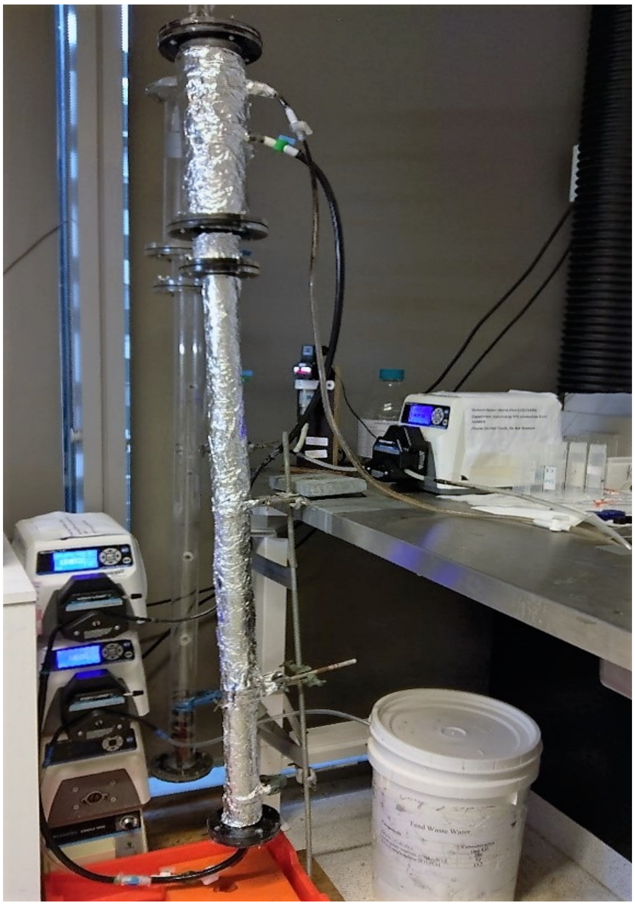

Figure 3.2: AnMBR setup for the production of VFA

The COD and nutrient removal performance was recorded at every two days. Excess sludge was collected from the reactor at the end of every week to maintain the SRT at desired value.

Minimum dissolved oxygen in the reactor was 0.01 ppm and the temperature was kept constant at 22 ± 1 °C.

3.2.5 Organics and nutrients removal

For each set of investigation, nutrients were measured as phosphate (PO_4^{3-}—P) and nitrate (NO_3^-—N) using the cell test method (Spectroquant, Merck) and a photometer (NOVA 60, Merck). The COD influent and effluent was measured using reagents from Hanna Instruments in a photometer by the EPA 410.4 method.

3.2.6 Biomass concentration and growth rate

The MLSS and MLVSS were measured by filtering the mixed liquor through 1.2µm filter paper followed by drying for 2h in an oven at 105 ° C. The filter paper was then put in a desiccator for 24 h to measure the MLSS and then put into an oven at 550 ° C for 30 min followed by another 24 h desiccation to measure the MLVSS.

3.2.7 Mass balance

Mass balance for carbon, nitrogen, and phosphorus were carried out by multiplying the fractions of individual components with the mas of each individual compound. Glucose was the single major source of carbon in the feed solution which is later converted to different VFA components, CO_2 and CH_4. Through the optimization of VFA and biohydrogen, the production of CH_4 was minimized whereas both the production of VFA and biohydrogen were maximized. Methanogenic activity is automatically inhibited at a pH below 6.5 (Khan et al. 2016a), therefore operating AnMBR at pH 5.0 refers to a complete inhibition of methane production. It may be assumed that at this stage of operation, the total carbon present in the AnMBR was mainly converted to different VFA components and CO_2. The produced hydrogen in the biogas mixture was mainly produced through acidogenesis and acetogenesis. Section 3.2.10 shows the equations to measure specific hydrogen production rate and yield.

3.2.8 VFA extraction

The produced VFA was separated from the AnMBR effluent using the solvent extraction method. The collected samples were acidified to a pH level between 1.8 to 2.0 to stop any further microbial activity for biodegradation. To make sure no suspended particles were present in the sample, the sample was centrifuged for 20 minutes at 3500 rpm. The supernatant was then filtered using a 0.45 µm syringe filter and then NaCl was added at a ratio of 1g in a 4 ml sample. Methyl Tert-Butyl Ether (MTBE) was used as the organic solvent for this process. 2 ml of MTBE was added in every 4 ml of the sample. The sample was centrifuged again at 3900 rpm for 5 minutes to separate the organic phase from the emulsion.

Liquid from the organic layer was collected using a syringe. For the same sample, MTBE was added again followed by the centrifuging and separation process. The liquid recovered from the organic phase was collected in a test-tube and anhydrous Na_2SO_4 was added to remove any residual water from the sample. Finally, the sample was filtered using a 0.22 µm syringe and left in a freezer for 4-5 hours before it was subjected to Gas Chromatogram Mass Spectrometry (GC-MS). Figure 3.3 shows the major steps to extract VFA sample from AnMBR.

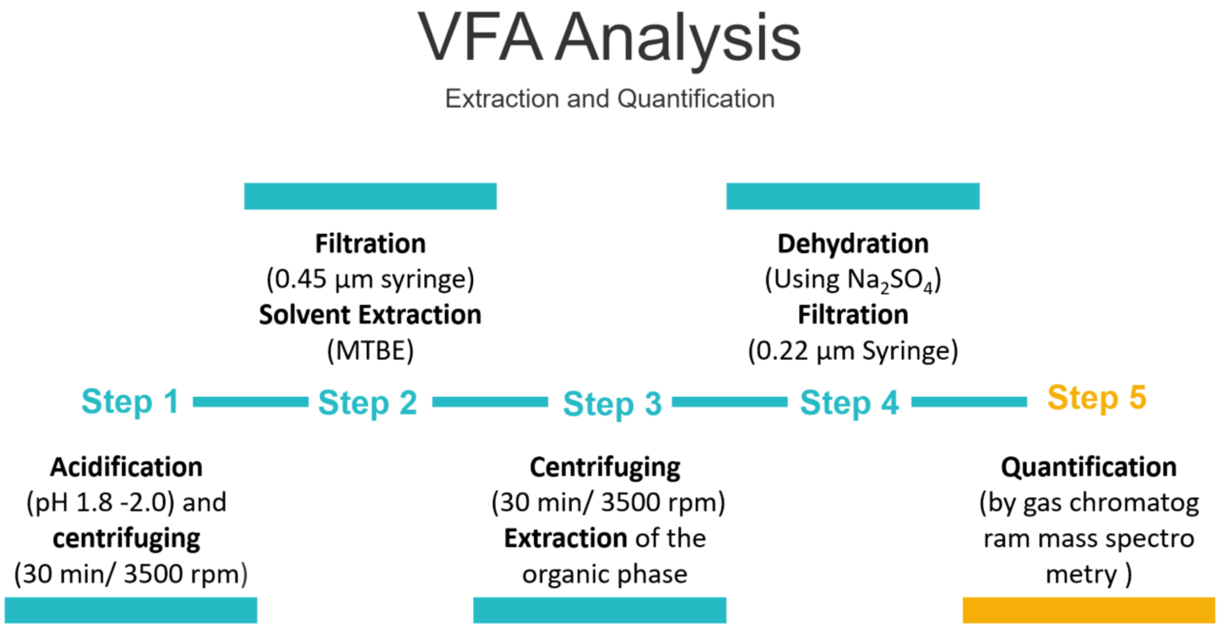

Figure 3.3: Steps for VFA extraction from AnMBR

3.2.9 Quantification of VFA from GC-MS

Individual VFA concentrations were measured by gas chromatogram mass spectrometry method (GC-MS TQ8040, Shimadzu, Japan). For each measurement the open tubular analytical column was used (VF-WAXms, Agilent, U.S). Helium was used as career gas with a flow rate of 2.05 mL/min. The temperature program started at 50˚C and was held for 5 min before ramping to 250˚C at 10˚C/min and was then held for 10 min. Electron impact ion source was set at 230˚C while the injection port and transfer line temperatures were held at 230˚C. Mass spectrometer (MS) operated in a selected ion monitoring (SIM) mode and in a full scan mode (m/z 15-550). Ions for detection of individual VFA in SIM mode were selected using the mass spectra of standards generated in SCAN mode.

$$\text{Total VFA production} = \sum Y_i \times M_i \qquad (1)$$

Where,

Y_i = Concentration of individual VFA component I mili-mole/L

M_i = Molecular weight of individual VFA component

$$\text{VFA yield} = \frac{\sum Yi \times Mi}{COD_{feed}} \text{ mg VFA/ COD}_{\text{feed}} \qquad (2)$$

3.2.10 Gas analysis and measurement

The produced biogas was analyzed using a portable biogas analyzer (Geotech BIOGAS 5000, UK). The volume of produced hydrogen was quantified using the water displacement method. Overall Hydrogen Yield and Specific Hydrogen Production Rate (SHPR) were calculated based on the following equations:

$$\text{HY; mL/g COD}_{\text{feed}} = (\text{H}_2 \text{ production rate; mL/d}) / (\text{Total COD; g/d}) \qquad (3)$$

$$\text{SHPR; mL/L/d} = [\text{H}_2\text{; mL/d}] / (\text{reactor volume; L}) \qquad (4)$$

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Chapter 4

Optimization of hydraulic retention time and organic loading rate for volatile fatty acid production from low strength wastewater in an anaerobic membrane bioreactor

4.1 Introduction

4.1.1 Background

In past decades, anaerobic bioreactors have been utilized to recover value-added chemicals and bioenergy from different waste materials (Khan et al., 2016a; Wang et al., 2018). The recent development in this technology includes coupling a membrane module with conventional an anaerobic digestion system for treating industrial and municipal wastewater (Liu et al., 2018). Although biogas has been considered as the primary resource to be recovered from wastewater, research studies have proven the technical and economic feasibility for recovering VFA and biohydrogen from anaerobic bioreactors (Liu et al., 2018; Xin et al., 2017).

So far, industrial application for anaerobic membrane bioreactors has been limited because the product revenue earned from this process is not constant. This is because of the variable production rate of VFA, biohydrogen, and methane due to the frequent change of carbon content in feed wastewater (Khan et al., 2016b; Pretel et al., 2016). In addition to this discussion, recovering VFA and biohydrogen, where biogas production can improve revenues earned from anaerobic processes and eventually improve the economic feasibility of large-scale production. Although for treating municipal wastewater, the influent COD typically varies from 150 to 350 g/L, it can be concentrated further for resource recovery purposes (Ji et al., 2014; Zheng et al., 2018).

A few studies have analyzed the economic feasibility of VFA production in contrast to biogas. The results demonstrated that production difference between VFA and the methane-containing biogas from the anaerobic process for VFA produced higher revenue compared to biogas (Khan et al., 2016b; Kleerebezem et al., 2015). VFAs have been identified as raw materials for biopolymers like Polyhydroxyalkanoate (PHA). It can also be used as a potential precursor for valuable organics, for example, alcohols, ketones, and aldehydes, biogas, biohydrogen and biodiesel (Khan et al., 2016a; Lee et al., 2014; Tao et al., 2016). VFAs are short-chain fatty acids that are produced through initial hydrolysis and the acidogenic phase in anaerobic digestion. During this process carbohydrates, proteins and fats are hydrolysed into amino acids, sugar, and fatty acids. The hydrolysis process is followed by acidogenesis where VFAs, $BioH_2$, and CO_2 are produced. VFAs produced from initial two stages are consumed by the methanogens at the final anaerobic stage to produce biogas (Begum et al., 2018; Scoma et al., 2016).

Production of VFA can be undertaken in two major ways: Firstly, VFA can be produced as the main product of anaerobic digestion (Aydin et al., 2018). This production type involves the inhibition of the methanogenesis process so that the methanogens cannot consume the VFA during the final stage of the anaerobic process to convert them into biogas. The selective inhibition of methanogenesis is mainly carried out by heat shock and load shock treatment. Additionally, acidic and alkaline pH treatments are also applied for selective inhibition of methanogens (Khan et al., 2016a). These methanogens have been reported to have optimum microbial growth at a pH range of 6.5 to 8.2 (Mao et al., 2015), therefore reducing the pH below 6.5 or above 8.2 can be applied to inhibit the activity of methanogens. Secondly, VFA can be simultaneously produced with biogas. This production technology involves the anaerobic process where the initial stage of acidogenesis and final stage of methanogenesis are separated through a multiple stage bioreactor design (Li & Yu, 2011; Schievano et al., 2014). In this experiment, the first type of production scheme was used to produce VFA without any selective inhibition of methanogenesis process. The reason for this approach is to improve the industrial application of the AnMBR as following the operating conditions any existing AnMBR model can be tuned to produce VFA without any design alterations.

For a specific bioreactor design, production of VFA directly depends on temperature, pH, Hydraulic Retention Time (HRT), Organic Loading Rate (OLR), pre-treatment methods of the sludge and chemical additives (Garcia-Aguirre et al., 2017; Jankowska et al., 2018; Khan et al., 2016a; Peces et al., 2016). Among these conditions, hydraulic retention time and organic loading rate can both be changed directly based on the feed composition. Kuruti et al., (2017) states that a general decrease in HRT increases the VFA production through anaerobic acidification (Kuruti et al., 2017). However, the value at which the highest VFA yield and production rate of VFA would be achieved depends on bioreactor design, microbial community, and feed characteristics. In contrast, it has been identified that an initial increase in the loading rate increases the VFA production but at the same time can affect membrane fouling and bioreactor performance in terms of COD and nutrients removal (Khan et al., 2016b; Mao et al., 2015). An optimum value for OLR for any anaerobic process depends on the bioreactor design, and an OLR above the optimum value reduces the production of VFA significantly. Slezak et al., (2017) studied the effect of organic loading rate for VFA production in dark fermentation and identified the VFA concentration increases only up to the initial OLR of 48.2g VS/L (Slezak et al., 2017). Increasing the OLR also effects the VFA composition in the product stream. Wijekoon et al. (2011) identified that the predominant VFA component changed from acetic acid to n-butyric acid with an overall increase in VFA concentration when OLR was increased from 5 to 12 kg COD m^{-3} d^{-1} in a two-stage thermophilic anaerobic membrane bioreactor (Wijekoon et al., 2011). Although numerous studies were carried out to optimize the production of VFA, most of them utilized anaerobic digestion (AD) process. A very limited number of researches performed to produce VFA from ANMBR where potential membrane fouling is an important area of concern. Additionally, the available studies aimed to optimize VFA production mainly used different inhibition process for methanogenesis.

4.1.2 Objectives

The experimental investigation aims to find out the optimum hydraulic retention time and loading rate in the AnMBR treating low strength (synthetic) wastewater. The first stage of this experiment includes AnMBR operation in six different HRTs for 48, 24, 18,12,8 and 6 hrs. The production of VFA was observed at different HRT. In the second stage, the same bioreactor was used with three different organic loading rates using 350, 550 and 715 mg/L COD of synthetic wastewater. Production of VFA was investigated for each loading rate. Corresponding AnMBR performances like COD, nutrient removal efficiency and TMP were measured at each operating conditions.

A major part of Chapter 4 has been published as a journal article in ERA A-rated journal:

Khan, M.A., Ngo, H.H., Guo, W., Liu, Y., Nghiem, L.D., Chang, S.W., Nguyen, D.D., Zhang, S., Luo, G., Jia, H. 2019. Optimization of hydraulic retention time and organic loading rate for volatile fatty acid production from low strength wastewater in an anaerobic membrane bioreactor. *Bioresource Technology*, **271**, 100-108.

4.2 Materials and methods

4.2.1 Experimental setup

A 3.9 L column was used in this experiment with provisions for effluent recirculation, biogas spurging and nitrogen purging from the bottom (see Figure 1). The system had a working volume of 3.5 L. The pressure sensor at the top measured the pressure in the reactor. Hollow fibre membrane (PVDF, Pore size 0.07 – 0.1 µm) with the inner and outer diameter of 1.0 and 2.2 mm respectively was used for this experiment. The membrane had a surface area of 0.08 m^2.

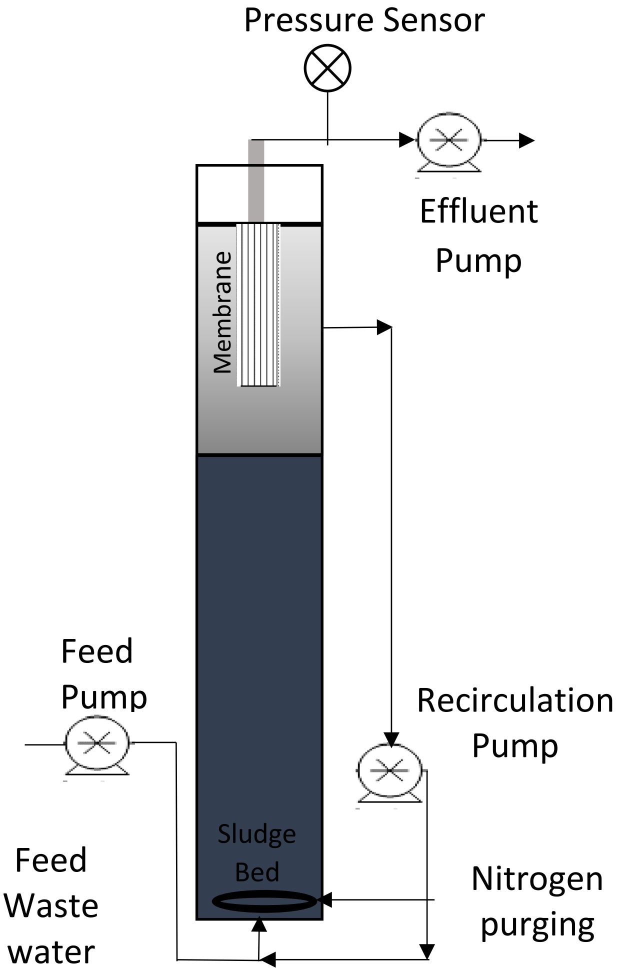


Figure 4.1: Experimental setup of AnMBR for VFA production

4.2.2 The operation of anaerobic membrane bioreactor

For this experiment, the operation of anaerobic membrane bioreactor can be divided into two different stages. The first stage involves the operation of AnMBR at different hydraulic retention times (HRTs). VFA samples were analyzed at 48, 24, 12, 8 and 6 hrs of HRT. The influent COD was kept constant at 550 mg/L with an OLR of 68.75 mg COD/l.hr. The second stage involved producing VFA at different organic loading rates.

The loading rates were changed by varying the influent COD in the feed wastewater. Influent COD was varied to 350, 550 and 715 mg/L with corresponding OLR of 43.75, 68.75 and 89.38 mg COD /l.hr, respectively, by keeping the HRT fixed at 8 hrs. Glucose, $NaNO_3$, and KH_2PO_4 were used as the main sources of carbon, nitrogen, and phosphorus respectively. The C: N: P ratio was kept constant at 100:5:1. Each trial for HRT and OLR involved 21 days of AnMBR operation in continuous mode. All relevant reactor operation conditions have been listed in Table 4.1.

Table 4.1: AnMBR Operating conditions for VFA production

Operating Parameter	Stage 1 (At different HRT)	Stage 2 (At Different OLR)
MLVSS (g/L)	10.1 ± .1	10.1 ± .1
COD in feed (mg/L)	550 ± 10	350 ± 10, 550 ± 10, 715 ± 10
HRT (h)	48, 24,18,12,8,6	8
Loading rate (mg COD /l. h)	68.75	43.75, 68.75, 89.38
DO (ppm)	0.01	0.01
Temperature (°C)	22 ± 1	22 ± 1
pH	7.0 ± 0.1	7.0 ± 0.1

Throughout the period of stage 1 and 2 of bioreactor operation, the was maintained at 7.0 ± 0.1 and the ambient temperature in the laboratory was kept constant at 22 ± 1 °C pH fixed at 7.0. Referring the information provided in the introduction section (optimum microbial growth of methanogens at pH 6.5 – 8), no inhibition process was applied to suppress methanogenic activity.

4.3 Results and discussion

4.3.1 AnMBR performance in COD and nutrients removal

For each stage of the AnMBR operation, bioreactor performance was analyzed in terms of COD and nutrient removal efficiency. Reactor effluents were added every 4 days during each trial for HRT and OLR. Figure 4.2 displays the efficiencies for COD, nitrate, and phosphorus removal at different HRTs and loading rates.

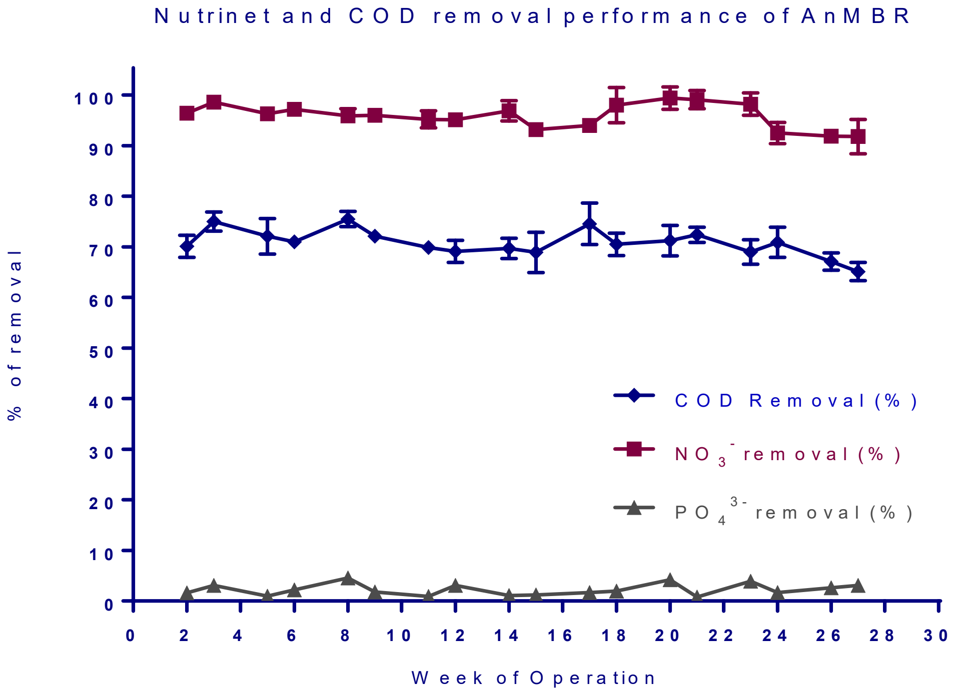


Figure 4.2: Nutrient and COD removal performance of continuous AnMBR

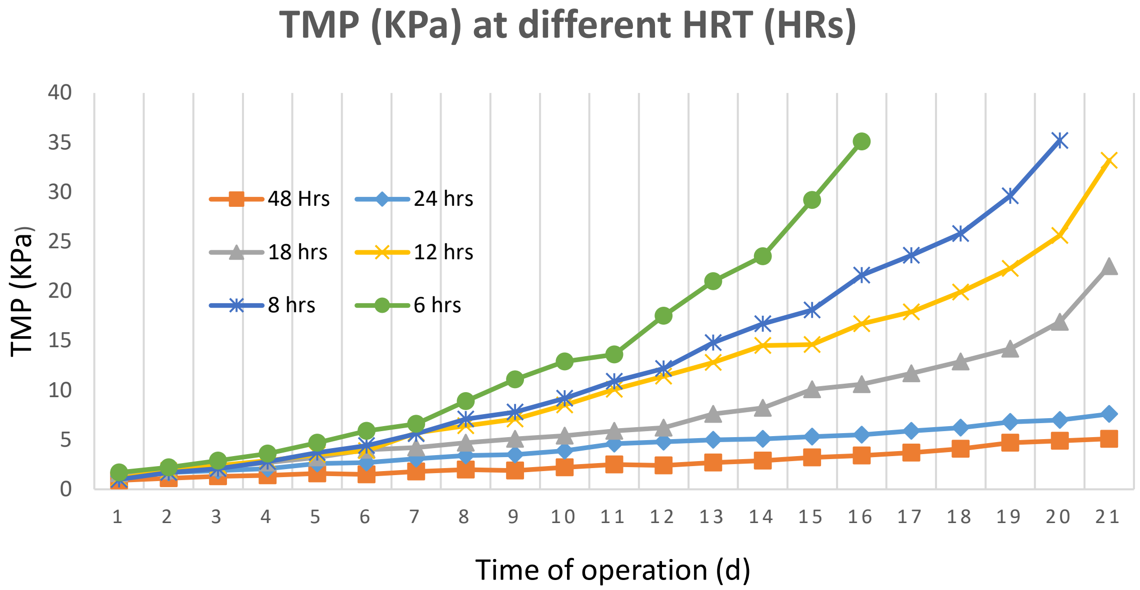
From Figure 4.2, it is evident that the COD removal performance was steady at approximately 70 % throughout the trials for both stages of operation. NO_3^- removal performance was maximum at the longest HRT (48 hrs) referring to the condition where the microorganisms were allowed enough time to undertake the denitrification process. As HRTs became shorter, a slight decrease in the NO_3^- removal was observed. The reason may be associated with the fact that the contact time between the feed wastewater and the denitrifying bacteria was lowered and shorter HRTs. Additionally, as the influent COD was kept constant at 550 ± 10 mg/L, the organic loading rate was also increased at shorter HRTs. As the denitrification process involved processing the high organic loading, the minor decrease in the nitrate removal was expected (Wang et al., 2018). However, a minimum removal efficiency of 93.2% indicates efficient denitrification process in the bioreactor. As expected, the anaerobic process had a steady PO_4^{3-}, the removal efficiency from 0.9 to 4.6% throughout the experiment.

During the second stage, at loading rates of 350 and 550 mg COD/l, the lowest COD removal efficiency was steady at about 70.9 ± 1.1%. NO_3^- removal was observed above 98.2 ± 1.7% with a maximum removal efficiency of 99.4 ± 0.1%. PO_4^{3-} removal was steady within the range of 0.9 ± 0.2% to 1.8 ± 0.1%. Instead, at a loading rate of 715 ± 10 mg COD/l, the COD removal efficiency dropped to 65.1 ± 2.2% and consequent NO_3^- removal efficiency dropped at 91.9 ± 0.5%. The deterioration in the general AnMBR may be associated to multiple facts like momentary pH drops due to VFA accumulation and less contact time between the biomass and feed solution (Khan et al., 2016a; Mao et al., 2015)

4.3.2 Membrane fouling of AnMBR

Properties of the membrane, sludge characteristics, wastewater properties, and operating conditions are the key factors that control the membrane fouling in a bioreactor (Guo et al., 2012). For this experiment, the same type of membrane, sludge, and synthetic feedwater were used throughout the experiment except for changes in the HRT (stage 1) and OLR (stage 2). Instead of discussing the mechanism of membrane fouling, this section includes discussions on how different hydraulic retention times and organic loading rates change the fouling pattern in the AnMBRs. MLVSS was fixed at 10 g/L at different HRTs and membrane fouling was measured in terms of Trans Membrane Pressure (TMP). All data have been plotted in Figure 4.3 (a) and (b).

4.3 (a) TMP at different HRT (hrs)



4.3 (b) TMP at different OLRs (mg/L)

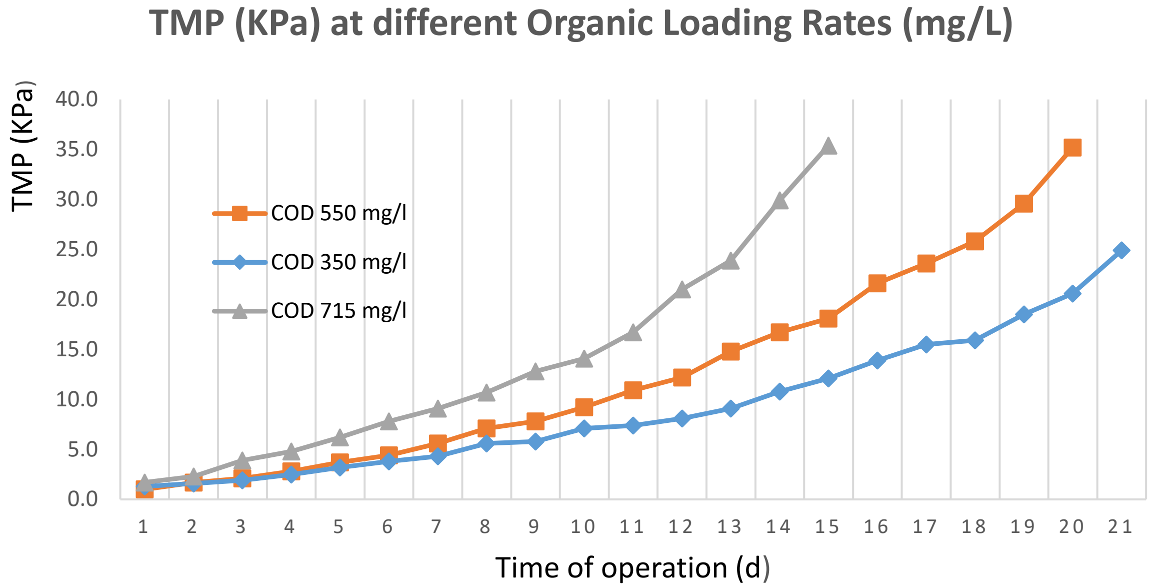


Figure 4.3: Variations of TMP at different operating conditions

In biological wastewater treatment, polysaccharides, EPS and organic colloids are the major contributors to membrane fouling in Membrane Bioreactors (MBRs). Additionally, the Natural Organic Matter portion in DOC, carboxylic acids, proteins, and amino acids have also been identified to have a significant effect on membrane fouling. At higher organic loading rates and shorter HRT, the trace nutrients in the synthetic wastewater (Ca^{+2}, Mg^{+2}, Fe^{+3}) are perhaps responsible for creating inorganic fouling in the membrane (Guo et al., 2012).

In the beginning, the little or no significant TMP development was observed (0 -7.6 kPa) for 48 and 24 hr HRT. The results indicated that the soluble organics, trace nutrients and carboxylic acid concentration was not high enough to develop the TMP above 7.6 kPa. However, TMP developed at a faster rate (22.5 and 33.2 and kPa after 21 days) for 18 and 12 hrs. This is because at this loading rate the soluble organics and the nutrients present in the feed water started to foul the membrane surface. The manufacturers' recommendation was to change/clean the membrane module once the TMP exceeds 30 kPa, therefore during these 21 hrs of operation membrane cleaning was not performed.

At shorter HRTs (8 and 6 hrs) a steady increase in TMP development was observed until day 11 (up to 13.6 kPa). The steady increase was followed by a rapid TMP development for both 8 and 6 hr HRT (35.2 kPa at the end of day 20 and 35.1 kPa at the end of day 16 respectively. The first stage of fouling (up to day 11) behaviour suggests the deposition of the foulants on the membrane surface. Later, the rapid rise in the TMP indicates blockage of the membrane pores by the formation of biofilms. Note that the results after membrane cleaning are not included in Figure 4.3 (a).

At low HRT, bacterial cell releases extracellular polymeric substances that eventually increases the SMP content and deflocculates the sludge. Additionally, at very low HRT, oversized and irregular flocs may have been formed in addition with the production of filamentous bacteria. The combination of these factors might have been responsible for the membrane fouling observed in 8 and 6 hr of HRT.

Membrane material also plays an important role in anaerobic wastewater treatment. Clark & Heneghan, (1991) mentioned that hydrophobic membrane materials are to suffer more membrane fouling than hydrophilic membranes (Clark & Heneghan, 1991). For this experiment, polyvinylidene fluoride (PVDF) was used as the material for membrane fabrication. As PVDF is chemically hydrophobic, a higher extent of membrane fouling was expected in this experiment.

The results for membrane fouling at different organic loading rates correlated closely with the results observed at different HRTs. These different organic loading rates (43.75, 68.75 and 89.38 mg COD/l.hr) were applied using synthetic wastewater having COD of 350, 550 and 715 mg/L. As maximum VFA concentration was found at 8 hrs of HRT at the first stage of this experiment, for all the organic loading rates, HRT was kept constant at 8 hrs.

The results found in this step of this experiment were interesting. When the influent COD was dropped to 350 mg/L (corresponding loading rate of 43.75 mg/L .h) TMP only went up to 24.9 kPa at the end of day 21 compared to than that of 35.2 kPa at the end of 20 days of operation using 550 mg/L COD in the feed. The lower organic loading at 350 mg/L COD involved less amount of organic acid, EPS and organic colloids deposit in the membrane surfaces. Therefore, membrane fouling was not severe at this organic loading.

In contrast, for 715 mg COD in the feed wastewater (loading rate of 89.38 mg/L .h), a rapid 35.4 KPa TMP was developed at the end of 15 days of operation. At this operating condition, high amounts of SMP and bound EPS were generated that resulted in a decrease in sludge filterability and filtration index. As the concentration of different foulants were high due to the high loading rate, a combination of these factors may be responsible for membrane fouling during this condition (Chen et al., 2018; Guo et al., 2012).

4.3.3 VFA concentration at different HRT

The major components of VFA include acetic acid, propanoic acid, butyric acid, and valeric acid. The components are mainly produced in the acidogenic phase of anaerobic digestion. Among these VFA components, acetic, propanoic and butyric acids are predominant during VFA production from the anaerobic process. According to literature, 65 to 95% of methane present in biogas is directly produced from butyric and acetic acid (Khan et al., 2016a; Mamimin et al., 2017; Morgan-Sagastume et al., 2011). Table 4.2 shows the concentration of individual VFA components at different HRT.

Table 4.2: Concentration of VFA components at different HRT

VFA Component	**Concentration (mili-mole/L)**					
HRT (hrs)	**48**	**24**	**18**	**12**	**8**	**6**
Acetic Acid	0.4922 ± 0.0134	0.8321 ± 0.0160	0.8753 ± 0.0062	1.1451 ± 0.0175	1.1844 ± 0.0165	1.0090 ± 0.0081
Propanoic acid	0.2172 ± 0.0126	0.4376 ± 0.0198	0.4632 ± 0.0035	0.3185 ± 0.0431	0.5160 ± 0.0141	0.5293 ± 0.0300
Isobutyric acid	0.1128 ± 0.0008	0.2836 ± 0.0005	0.2880 ± 0.0212	0.2801 ± 0.0141	0.2283 ± 0.0117	0.2398 ± 0.0406
Butyric Acid	0.0084 ± 0.0011	0.0001 ± 0.0000	0.0035 ± 0.0013	0.0051 ± 0.0007	0.0148 ± 0.0009	0.0155 ± 0.0034
Isovaleric acid	0.0003 ± 0.0001	0.0044 ± 0.0008	0.0164 ± 0.0002	0.0103 ± 0.0002	0.0108 ± 0.0008	0.0093 ± 0.0008
n-Valeric acid	0.0193 ± 0.0025	0.0019 ± 0.0004	0.0113 ± 0.0005	0.0119 ± 0.0004	0.0108 ± 0.0013	0.0143 ± 0.0023
Isocaproic acid	0.0110 ± 0.0008	0.0030 ± 0.0001	0.0033 ± 0.0002	0.0023 ± 0.0003	0.0041 ± 0.0008	0.0049 ± 0.0002
n-caproic acid	0.0971 ± 0.0075	0.0253 ± 0.0011	0.0040 ± 0.0008	0.0005 ± 0.0004	0.0037 ± 0.0006	0.0085 ± 0.0023
Heptanoic acid	0.0286 ± 0.0015	0.0129 ± 0.0009	0.0029 ± 0.0012	0.0017 ± 0.0001	0.0023 ± 0.0001	0.0038 ± 0.0018
Overall VFA Yield	**13.39 ± 1.21%**	**32.88 ± 2.56 %**	**35.35 ± 1.89 %**	**38.83 ± 3.25 %**	**48.20 ± 1.21%**	**42.32 ± 2.32 %**

From the experiment, it has been observed that the acetic acid concentration was nearly doubled (from 0.4922 ± 0.0134 to 0.8321 ± 0.0160 mili-mole/L) when the HRT was reduced from 48 hrs to 24 hrs. Production of acetic acid was increasing gradually when HRT was shortened and the maximum concentration was achieved at 8 hrs (1.1845 ± 0.0165 mili-mole /l). The change from 48hrs to 24 hrs indicates a shift in microbial activity from methanogenesis to acidogenesis. A gradual increase in acetic acid concentration was observed when the HRT was reduced further to 18,12 and 8 hrs. These trials with shorter HRT involved higher organic loading rates as the COD of influent wastewater was kept constant at COD of 550 ± 10 mg/L.

Although the increase in acetic acid concentration had the highest degree of increase during the first change from 48hrs to 24 hrs, the following increase in the trend for acetic acid was associated with the high amount of organics and nutrients loading the bioreactor at a fixed MLVSS of 10.1 ± 0.1.

A further decrease in the HRT (from 8 to 6 hrs) reveals a drop in acetic acid concentration to 1.0095 ± 0.008 mili-mole/l. Although the initial decrease in HRT supported acetic acid production, an HRT below 8 hrs indicates an imbalance in the initial hydrolysis and acidogenesis process. More explicitly, the high amount of organics fed into the reactor at this HRT had a faster rate of initial hydrolysis whereas the acidogenic bacteria could not perform their action by consuming amino acids, sugar and other fatty acids that are produced through the initial hydrolysis process in anaerobic digestion.

For isobutyric acid, the concentration initially increased from 0.1128 ± 0.008 to 0.2836 ± 0.0005 mili-mole/l when the HRT was shortened from 48 to 24 hr period. Similarly, for acetic acid, the results suggest that the methanogens could not convert the produced VFA into methane and CO_2 during this change (Braguglia et al., 2018). However, no significant rise in the butyric concentration was observed when in shorter HRTs (18,12,8 and 6 hrs). The reasons may be associated with operating pH factors in the reactor. According to the literature, pH values of 6.0-7.0, 4.0-5.0 and 11.0 have been referred as optimum for acetic acid, propanoic acid, and butyric acid production respectively (Begum et al., 2018; Lin & Li, 2018; Yu & Fang, 2003). As the experiment involved maintaining the reactor pH level to 7.0 ± 0.1, the butyrate type fermentation was not predominant during this experiment.

A gradual increase in the concentration of propanoic acid has been observed when the HRT was shortened from 48 hrs to 6 hrs. The highest concentration was observed at 6 hrs (0.5293 ± 0.03 mili-mole/l). Propanoic acid, unlike acetic and butyric acid, remains unconverted during the final stage of anaerobic digestion as the conversion is thermodynamically less favourable compared to the other two major VFA components (Yu et al., 2016). As a result, it accumulates in the bioreactor at high organic loading rates or shorter HRT. The literature explains that this single VFA component is responsible for rapid acidification in anaerobic bioreactors that eventually leads to the conditions of microbial stress, sharp pH drop and reactor instability (Wang et al., 1999). For this study, the reactor pH was maintained to 7.0 ± 0.1 by adjusting the pH of the feed solution. Therefore, the possibility of propanoic acid accumulation was very small during this experiment.

The remaining components present in our analysis included isovaleric, n-valeric, iso-caproic, n- caproic and heptatonic acid. Although no particular trend was observed in their concentration, an overall decrease in their concentration was observed when the HRT was shortened. The results may be associated with the fact that shorter HRT encouraged the production of major VFA components like acetic, butyric and propanoic acid. Consequently, VFA production shifted towards acetic, butyric and propanoic acid at shorter HRTs.

Table 4.3 also shows the overall percentage of VFA yield in mg VFA/mg COD in the feed solution. A change from 48hrs to 24 hrs records a rapid increase in VFA yield from 13.39 ± 1.21% to 32.88 ± 2.56%. In unit time, the carbon content in the feed solution increased in shorter HRTs, the VFA yield increased up 48.20 ± 1.21% at 8 hrs HRT. A further decrease in the HRT (6 hrs) caused a drop in the overall VFA yield 42.32 ± 2.32% (mg VFA/mg COD_{feed}) indicating insufficient contact time between the microbes and the feed solution. As discussed previously, the predominant VFA components (acetic, propanoic and butyric acid) only had a rise in concentration up to the HRT of 8 hrs. The drop in their individual concentration triggered an overall decrease of the VFA yield at 6 hrs operation.

Experiments have shown that, for VFA production, at pH 5.5 acetic acid is the major VFA component whereas at pH -11 butyric acid is the predominant VFA component (Begum et al., 2018; Jankowska et al., 2017). In this case, an alteration of reactor pH to acidic (pH 5.5) or alkaline (pH 11.0) can be beneficial for acetic and butyric acid production. Therefore, maximizing the concentration of individual VFA component can improve the overall VFA yield from this process.

4.3.4 VFA concentration at different OLR

Three different organic loading rates were applied (43.75, 68.75 and 89.38 mg COD /l.h) using an influent COD of 350, 550 and 715 mg/L at 8 hr HRT period. For 350 mg/L COD in feed solution, acetic, propanoic and isobutyric acid concentrations 0.7602 ± 0.014, 0.2707± 0.011 and 0.2393± 0.007 mili-mole/l respectively with an overall VFA yield of 35.39% (± 3.52%)

mg VFA/ 100 mg COD feed. At this loading rate, the soluble organics and nutrients were not enough for the acidogenic bacteria present in the reactor. Therefore, both individual VFA concentrations and overall VFA yield were relatively low during this condition.

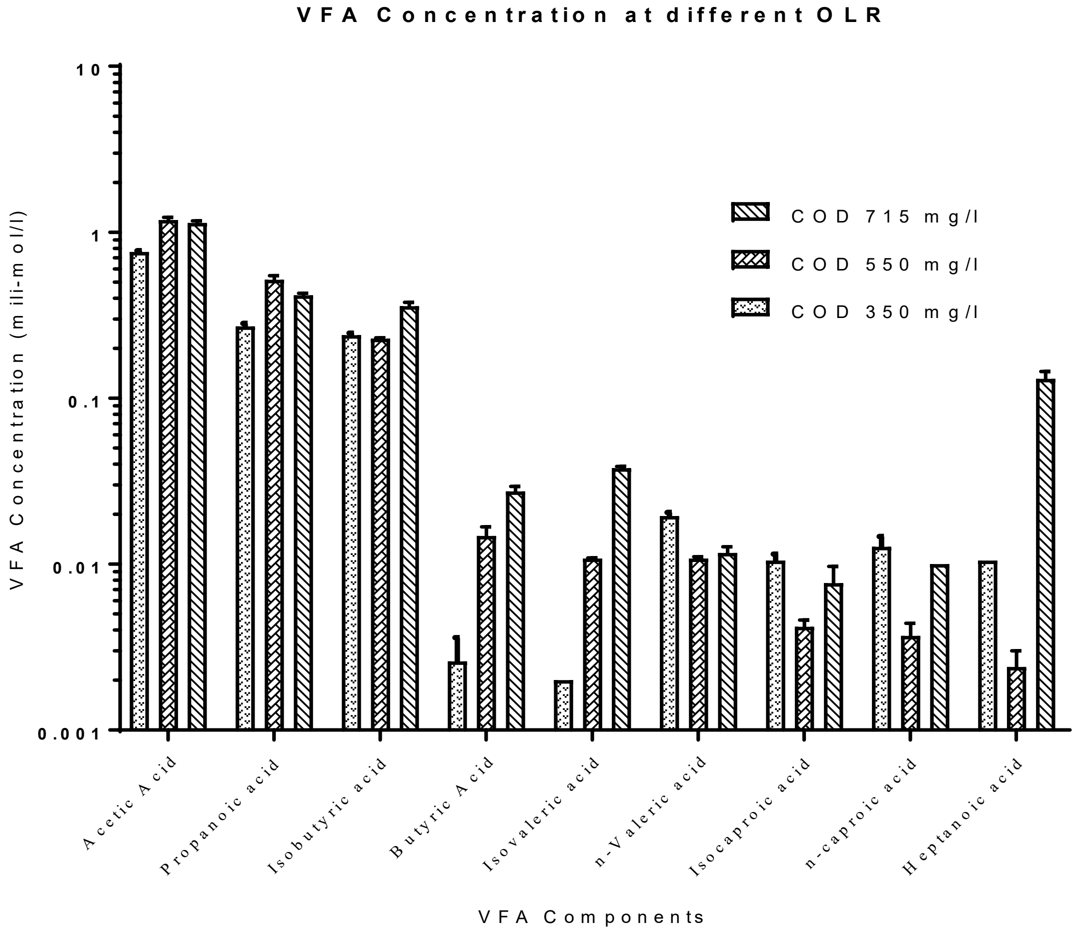


Figure 4.4: VFA concentration at different OLR

At a loading rate of 550 mg/L COD in feed, highest acetic acid concentration was observed (1.1845 ± 0.0165 mili-mole/l) along with an increase for propanoic acid (from 0.27070 ± 0.03 to 0.5160 ± 0.0104 mili-mole/l). This initial increase in the influent COD increased the supply of organics and nutrients to the microbes that were performing acidogenesis. However, at this loading rate the butyric acid concertation dropped 0.2393 ± 0.0406 to 0.2284 ± 0.0023 mili-mole/l. The subsequent drop in butyric acid concentration may be associated with the fact that it was degraded to acetic acid by acetic acid producing bacteria (Shen et al., 2018). Another reason for this drop in the butyric acid concentration is linked to the fact that, the system was not supported with the optimum pH (5.5 to 6.5) for butyrate type fermentation (Kuruti et al., 2017). An increase in the propanoic acid concentration was observed at this loading rate due to the reason that it was not consumed by any other acidogenic bacteria or methanogenic archaea (Khan et al., 2016a).

Finally, for 715 mg/L COD at the influent, there was a decrease in acetic acid and propionic acid concentration (from 1.1881 ± 0.0081 mili-mole/l to 1.1385 ± 0.0081 and 0.5160 ± 0.03 to 0.4167 ± 0.03 mili-mole/l respectively). In addition to this decrease, an overall drop in AnMBR performance was also observed (COD removal rate dropped to 65.1 ± 2.2% and the NO_3^- removal rate dropped to 91.9 ± 0.5%). In contrast, an increase in the trend of isobutyric acid concentration was observed (0.2284 ± 0.0117 to 0.3580 ± 0.0407 mili-mole/l) in this condition. A possible reason may be at this loading rate VFA accumulation in the reactor triggered a momentary drop of the reactor pH below 6.5 that encouraged butyrate type fermentation. In summary, the high organic loading rate can be referred to as a trade-off between AnMBR performance and maximizing butyric acid production.

A possible future improvement opportunity can be operating the bioreactor by altering the pH condition into the acidic zone (5.5 to 6.5). Where the acetic acid and propanoic acid production can be maximized at this pH range (Begum et al., 2018), it would be interesting to see the possibility of overall VFA concentration exceeding the values that are obtained in the loading rate of 550 mg COD /l.

4.3.5 Advantages of VFA production from continuous AnMBR

The current study utilizes a continuous AnMBR to produce VFA from low-strength synthetic wastewater. Over the past few years, there have been a lot of experiments to extract VFA using anaerobic digestion, but most of these researches involve anaerobic digestion process in batch operation. For example, Begum et al. (2018) used anaerobic batch reactors to produce VFA using landfill leachate. The reactor was operated at different pH conditions (pH: 5.5 / 11.0) and at a temperature of 37 ± 2 °C.

The highest VFA yield from this research was 48% VFA/ COD feed. Garcia-Aguirre et al. (2017) investigated the production of VFA using different carbon sources (Slaughterhouse wastewater, Paper mill wastewater, and glycerol) using batch fermentation process. The range of overall VFA yield from paper mill wastewater and glycerol were 32 - 47 %. Both studies used adequate inhibition of the methanogenic activity of microorganisms through pH and temperature control in the batch mode of operation. The research by Li & Li (2017) carried out an experiment to produce VFA using iron-flocculation batch reactor using wastewater and food waste and achieved a conversion of 66% of food waste into VFA. Yarimtepe, Oz & Ince (2017) achieved a 68% overall VFA yield in an anaerobic sequencing batch reactor using olive mill wastewater. Therefore, the currently available research studies for VFA production have achieved a high percentage of VFA yield through utilizing inhibition of methanogenic activity in the batch mode of operation. There has been a limited no of research that involves VFA production from low strength municipal wastewater in a continuous AnMBR.

Hence, a continuous AnMBR can offer many advantages over the currently available VFA production processes. Firstly, VFA production in the continuous mode of operation makes it more applicable for wastewater treatment whereas the batch mode of operation is more practical for anaerobic digestion of organic waste. Secondly, this study has achieved a maximum VFA yield of 48.20 ± 1.21% (mg VFA/mg COD in feed solution) without the inhibition of methanogenic activity.

Therefore, the result can be used in the simultaneous production of VFA and methane can increase the amount of revenue earned from the AnMBR. Thirdly, acidification is a major operational problem in AnMBR that are primarily caused by VFA accumulation (Khan et al., 2016b). Throughout this experiment, the reactor pH was maintained at 7.0 ± 0.1. Recovering VFA from this process offered an operational benefit by reducing the chance of rapid acidification. Finally, the membrane fouling profile during VFA production under different operating conditions has not yet completely discovered. Therefore, the findings from this research study could be beneficial to reduce/eliminate membrane fouling in the future.

4.4 Conclusions

The experimental results concluded that the highest individual VFA concentration was observed at HRT 8hrs with a corresponding yield of 48.20 ± 1.21% without any selective inhibition of methanogenesis. From different organic loading rates, highest acetic and propanoic acid concentration were 1.1845 ± 0.0424 and 0.5160± 0.0322 mili-mole/l respectively at 550 mg/L COD $_{feed}$. An increase in high organic loading at 715 mg COD/feed suggested a future research option by operating the AnMBR at different pH levels. Additional operating conditions like reactor pH and temperature can be altered to maximize the production rate and yield of VFA.

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Chapter 5

Selective production of volatile fatty acids at different pH in an anaerobic membrane bioreactor

5.1 Introduction

5.1.1 Background

Anaerobic Membrane Bioreactor (AnMBR) has proven to be effective in treating wastewater from different sources (Cheng et al., 2018a; Song et al., 2018). Although the operational and maintenance costs are the major issue for AnMBR wastewater treatment, the past few years have shown significant developments regarding AnMBR design, resource recovery and membrane fouling control (Cheng et al., 2018c; Jeong et al., 2018; Song et al., 2018). So far, the major research theme on resource recovery from anaerobic bioreactors has tended to focus on the production of methane-containing biogas (Cheng et al., 2018b; Lu et al., 2018; Song et al., 2018; Wang et al., 2018a). Methane is favoured as the main AnMBR product because it does not require any downstream processing and can be readily used as fuel for different purposes. More recent studies have shown that recovering VFA and biohydrogen from AnMBR can be both technically and economically feasible (Khan et al., 2018; Khan et al., 2016a; Khan et al., 2016b; Kleerebezem et al., 2015; Romero Aguilar et al., 2013). Moreover, the production of methane leads to various technical and environmental issues like process inhibition, greenhouse gases, ecotoxicity, fugitive methane emissions, etc. Process optimization, different pre- and post-treatment processes along with chemical and biological additives have been used to improve the production of biogas from the anaerobic process (Ngo et al., 2019). In contrast, production of VFA from the anaerobic process offers technical and economic advantages, for example, bioreactor stability, less expensive operation, and a relatively higher profits compared to methane (Hassanein et al., 2017; Khan et al., 2019; Khan et al., 2016b).

VFAs are used as a precursor for carbon-based biopolymers such as Polyhydroxyalkanoate (PHA), fuels like biodiesel, organic chemicals like alcohols, aldehydes and even for the production of biogas (Esteban-Gutiérrez et al., 2018; Jankowska et al., 2018; Khan et al., 2019). During anaerobic digestion, the initial stage of hydrolysis involves the production of VFA along with amino acids and sugar through the conversion of proteins and carbohydrates. The second stage of the anaerobic process also produces VFA along with hydrogen, ammonia and carbon dioxide (Adekunle & Okolie, 2015). For a given anaerobic system, production of VFA can be maximized through optimizing common process conditions, for instance Hydraulic Retention Time (HRT), Organic Loading Rate (OLR), and pH. Our previous studies demonstrate that a low HRT (8 hrs) at a loading rate of 600 mg COD/l HRT produced the highest overall VFA yield (48.20 ± 1.21%) without any selective inhibition of a microbial community (Khan et al., 2019).

The economic feasibility of producing VFA from an anaerobic process largely depends on the percentage of major VFA components, for example, acetic, propanoic and butyric acid present in the VFA mixture. According to Esteban-Gutiérrez et al. (2018) the market size for acetic acid is the biggest one (up to 3,500,000 t/year), followed by propionic and butyric acid (180,000 and 30,000 tonnes per year respectively) (Esteban-Gutiérrez et al., 2018). The majority of current research has shown VFA mixture produced from the anaerobic process contains acetic acid as the predominant VFA component. However, butyric acid has the highest market value and is priced at approximately US$2,000–2,500 per tonne (Esteban-Gutiérrez et al., 2018; Zacharof & Lovitt, 2013). Furthermore, it is necessary to optimize the operating conditions of an anaerobic process to maximize different VFA components. For example, altering the pH of an anaerobic process can cause a shift in microbial activity and cause a different type of fermentation. A research study has identified pH ranges of 4.0–4.5, 4.5–5.0, 5.0–5.5 and 5.5–6.5 to be ideal for ethanol, mixed acid, propionic and butyric acid-type fermentation, respectively (Zheng et al., 2010). Begum et al. (2018) recently carried out an experiment to show the effect of pH and OLR on VFA production using single and two-stage anaerobic digestion. Their study investigated the concentration of individual VFA components at acidic (pH 5.5) and alkaline (pH 11.0) conditions. The results showed the highest concentration of butyric acid was recorded at pH 11.0.

So far, no research study has observed the production of selective VFA components using low-strength synthetic wastewater in AnMBR. Membrane fouling can emerge as a serious problem in the AnMBR especially when the system is operated at acidic or alkaline conditions. A variation in operating pH directly affects the cell morphology along with a major alteration in adhesion and flocculation phenomena. Also, a variation in the production of Extracellular Polymeric Substances (EPS) and Soluble Microbial Products (SMP) can affect the membrane fouling at different pH levels (Kunacheva et al., 2017). Improving the economic feasibility is another major challenge of VFA production from low-strength wastewater treatment because the separation and purification of VFA is still very expensive. Operating an AnMBR at different pH conditions can contribute to determining the optimum pH level for individual VFA components and eventually improve the economic feasibility of VFA production based on the anaerobic process.

5.1.2 Objectives

The technical study that aims to optimize selective production of VFA by changing the pH of an AnMBR using low-strength synthetic wastewater. During this study, no selective inhibition was applied for the methanogens, meaning that the findings can be applied to a generic AnMBR model producing both VFA and biogas. Concentrations of individual VFA members were measured at different pH levels to find the optimum values for different VFA components. Another objective is to study the performance of AnMBR in terms of membrane fouling for the removal of nutrients and COD. The findings of this experiment have been charted to ascertain the predominant type of fermentation occurring at each pH level.

A major part of Chapter 4 has been published as a journal article in ERA A-rated journal:

Khan, M.A., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Varjani, S., Liu, Y., Deng, L., Cheng, C. 2019a. Selective production of volatile fatty acids at different pH in an anaerobic membrane bioreactor. *Bioresource Technology*, **283**, 120-128.

5.3 Materials and methods

5.3.1 Experimental setup

A single stage Anaerobic Membrane Bioreactor (AnMBR) was used for this experiment. A hollow fibre membrane module was interned from the top of the reactor and the influent wastewater was fed from the bottom. The supernatant was recycled through a separate line at the bottom. The bioreactor was operated at continuous mode and had a working volume of 3.5 L (Figure 5.1).

The bottom of the reactor had a 1.5-inch layer of 850-900-micron glass microspheres for even flow distribution. Three Masterflex® L/S® Series easy-load II peristaltic pumps were used to control the flow of influent, recirculation and effluent streams (Figure 5.1). Samples were collected from: i) above the sludge bed; ii) top; and iii) bottom of the sludge bed. The objective was to obtain an average value of pH. Effluent samples were collected from the downstream of a Polyvinylidene Difluoride (PVDF) hollow fibre membrane (area – 0.08 m^2) with a pore size of 0.07 – 0.1 µm and 1.0 and 2.2 mm of the inner and outer diameter, respectively. Aeration tubes were assembled at the bottom of the reactor to supply the purged nitrogen gas when required.

5.3.2 The operation of anaerobic membrane bioreactor

For this experiment, the AnMBR was operated in continuous mode at six different pH values (5.0, 6.0, 7.0, 8.0, 10.0 and 12.0). Each level of pH condition was maintained for 3 weeks followed by a one-week period to recover and stabilize the reactor to the new pH level. VFA samples were collected and analyzed at 4 day intervals and each time two different samples were analyzed simultaneously to obtain the most accurate results. At the end of each trial, Mixed Liquor Volatile Suspended Solids (MLVSS) were measured and the value was maintained at 10000 ± 500 mg/l. The COD and nutrient removal performance was recorded at every two operating days. SRT for this experiment was maintained at 60 days, excess sludge was collected from the reactor at the end of every week.

According to our previous study, the COD in the influent was maintained to at 550 ± 20 mg/l with a Hydraulic Retention Time (HRT) of 8 hrs (Khan et al., 2019). Minimum dissolved oxygen in the reactor was 0.01 ppm and the temperature was kept constant at 22 ± 1 °C.

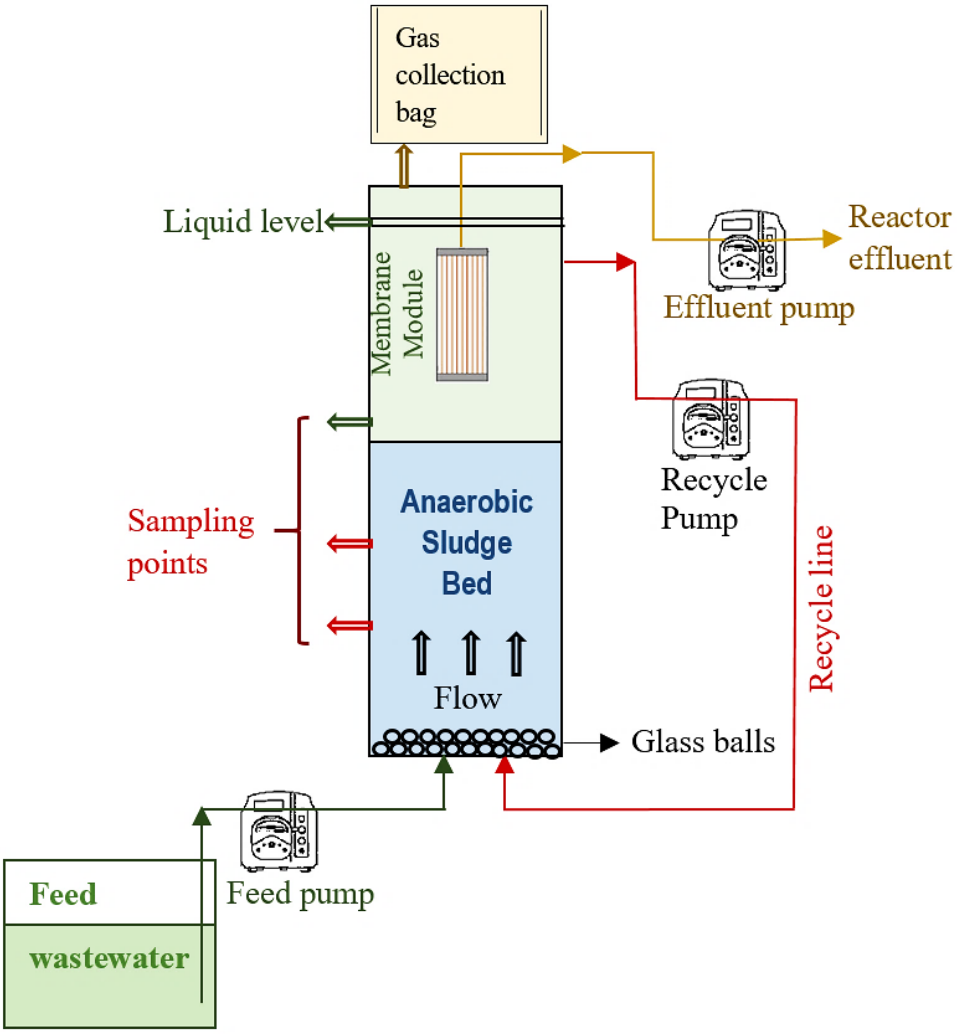
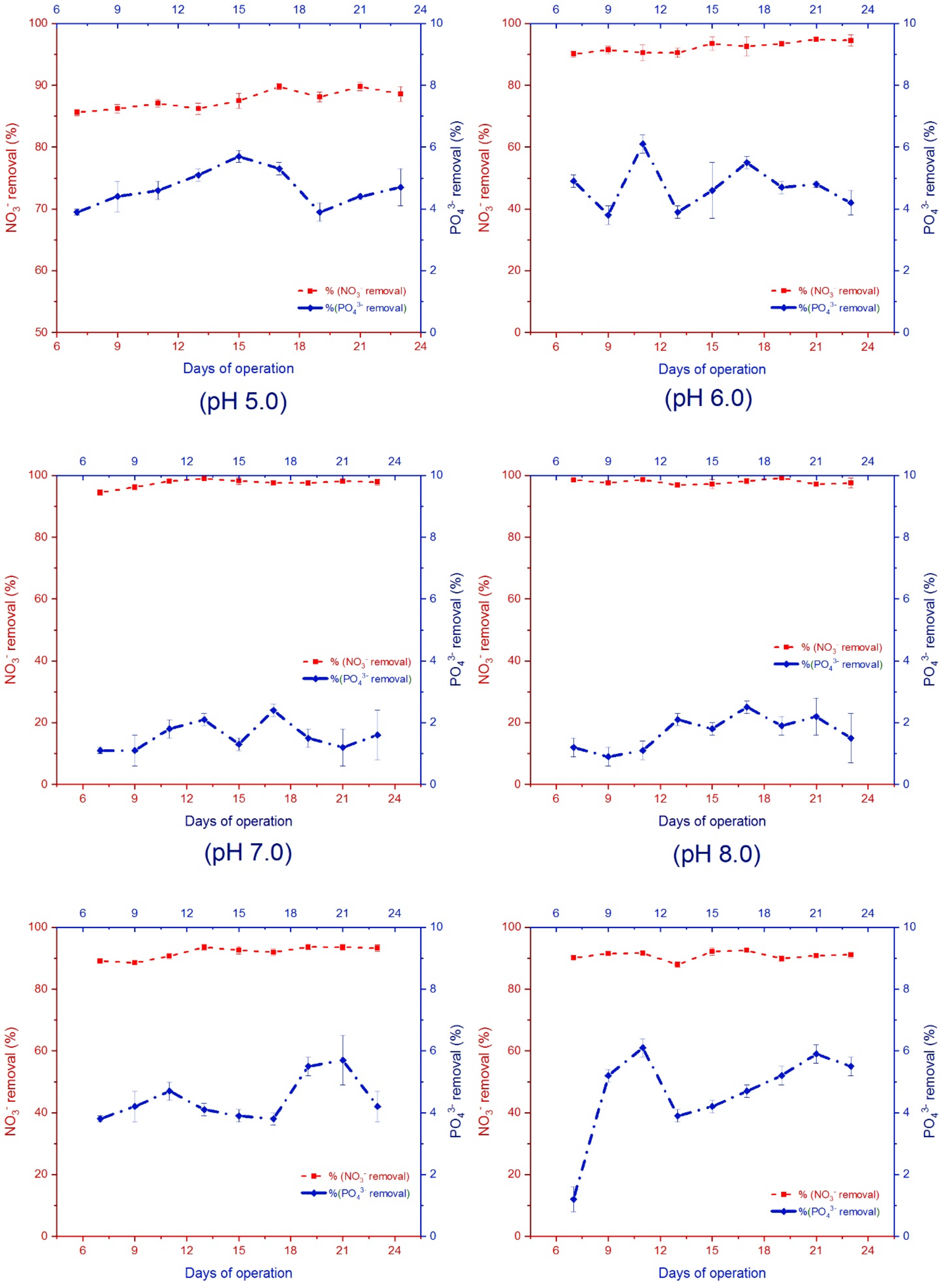


Figure 5.1: Schematic of experimental setup for selective VFA production

5.3 Results and discussion

5.3.1 AnMBR performance in nutrient removal

The removal efficiency of nitrate and phosphate at different pH levels were measured in second and third weeks of operation. Fig. 5.2 shows the nitrate and phosphate removal efficiency at different stages of AnMBR operation. The removal efficiencies fluctuated during the second week and this indicates that the microbial activity did not adjust sufficiently to the new pH level. Stable NO_3^- and PO_4^{3-} removal efficiencies were achieved during the third (final) week of the AnMBR operation.



(pH 5.0) (pH 6.0)

(pH 7.0) (pH 8.0)

(pH 10.0) (pH 12.0)

Figure 5.2: Nutrient removal performance of AnMBR

In general, a high percentage of nitrate removal was achieved through this experiment (highest 99.2 ± 0.2%) indicating high microbial activity for the purpose of denitrification. The reason may be linked to the fact that the single major source of NO_3^- in the feed was $NaNO_3$ which is readily soluble in water. As all the nitrate in this system was present in the liquid, the removal efficiency was higher compared to the system releasing NO_3^- from solid waste (Tang et al., 2019).

The NO_3^- removal efficiency was lowest at a level of pH 5.0 showing a value of 85.6 ± 0.5%. The percentage went up to 90.1 ± 1.0% when the AnMBR was operating at pH 6.0. A further increase in the pH to 7.0 indicated the highest removal efficiency rate of $NO_3^{\,-}$ (98.9 ± 0.2%). Therefore, the initial increase in the pH levels from 5.0 to 7.0 improved the rate of denitrification in the reactor. According to literature the optimum pH range for hydrogenotrophic denitrification has been identified as 7.6-8.6 (Karanasios et al., 2010). The highest rate of denitrification was observed at pH 8.0, referring to a value of 99.2 ± 0.1%.

Therefore, the findings from this experiment support the values suggested in the literature. However, a further increase in the pH level to a more alkaline zone decreased the rate of denitrification. In this experiment, the lowest nitrate removal at pH 10 and 12 went down to 88.5 ± 0.7% and 89.9 ± 0.8%, respectively. Consequently, this could be associated with the fact that, in alkaline conditions, nitrites can accumulate inside the reactor and cause a significant decrease in the denitrification process (Karanasios et al., 2010). For this experiment, KH_2PO_4 was used as a single major source of phosphate. It may be assumed that the addition of KH_2PO_4 contributed to maintaining a neutral pH state by creating a phosphate buffer (Rust et al., 2000; Xu et al., 2018).

At different stages of the bioreactor operation, the efficiency to remove phosphorus varied between 0 to 5% as expected. Fig. 5.2 shows the PO_4^{3-} removal efficiency at different pH levels. The figure shows, both at acidic and basic pH level, the removal efficiency was slightly higher compared to the effects observed at pH 7.0 and 8.0. At acidic levels of pH (5.0 and 6.0) and basic pH (10 and 12), the removal efficiency of PO_4^{3-} varied between 1.2 ± 0.4 and 6.1 ± 0.3, whereas a more neutral pH (7.0 and 8.0) meant that the overall efficiency dropped between 0.9 ± 0.3 and 2.5 ± 0.2. It may be assumed that at pH 7.0 and 8.0, more phosphorus was released compared to the amounts released in acidic or alkaline conditions.

5.3.2 Membrane fouling at different levels of pH

Membrane fouling mainly depends on the membrane material, characteristics of feed wastewater, sludge properties and operating parameters of the reactor (Guo et al., 2012). A change in pH levels can affect the cell metabolism and cell lysis of the microbes as well as the concentration of proteins and carbohydrates in the reactor. A variation in the amount of proteins and carbohydrates can be a major contributing factor in membrane fouling. For this experiment, the same membrane module was used at different stages of the AnMBR operation with the same bioreactor arrangement, feed composition, and sludge characteristics. Optimum HRT and OLR were maintained at 8 hrs and 550 ± 20 mg/l, respectively, based on the results found in our previous study (Khan et al., 2019).

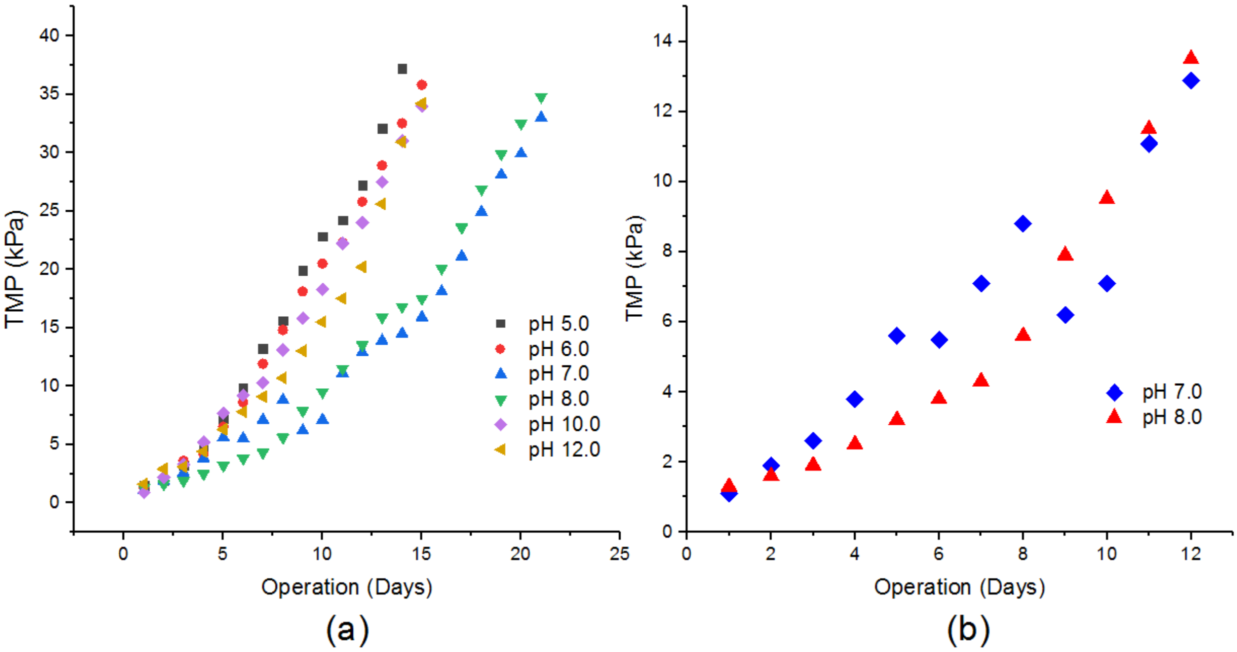


Figure 5.3: (a) TMP at different operating pH (b) TMP at two short operating periods for two optimum pH levels

Figure 5.3 (a) shows the development of Trans Membrane Pressure (TMP) at different pH levels. Among six different pH rates, membrane fouling was worse at pH 5.0 with reference to a TMP of 37.2 kPa at the end of 14 days of operation. pH 6.0 demonstrated a superior result indicating a TMP of 35.2 kPa at the end of 15 days of stable operation. The reason may due to the fact that the production of carbohydrates in an anaerobic process is usually higher in acidic conditions (pH ~ 5.0-6.0). Additionally, larger proteins can increase the amount of colloidal particles through the process of cell lysis (Kunacheva et al., 2017). A combination of these factors might be responsible for a higher rate of membrane fouling in this scenario.

The membrane fouled at a slower rate when the pH increased to a neutral value. At pH 7.0 and 8.0, 33.0 and 34.8 kPa developed, respectively, at the end of 21 days of the AnMBR operation. However, an increase in the fouling rate was observed again at pH 10.0 and the trend continued at pH 12.0 as well. It may be assumed that, in alkaline conditions there is a general increase in protein-like compounds which can be responsible for fouling the membrane surface. On this topic, Zhou et al. (2016) showed that the supernatant and foulants in an AnMBR contains more protein-like compounds compared to polysaccharides at higher operating pH. They also noted that the proteins are more likely to attach to the membrane surface when the ratio of proteins to carbohydrates is as high as 3.1 (Zhou et al., 2016).

A short HRT in the anaerobic system can be a potential reason for membrane fouling. As this experiment had a short HRT of 8 hrs, it triggered the release of EPS and eventually the amount of SMP increases at different pH conditions. The low HRT is also responsible for sludge deflocculation, the formation of large and irregular flocks and overgrowth of filamentous bacteria (Guo et. al., 2012). As a result, a low HRT can indirectly contribute to an increase in the concentration of membrane foulants through increasing the SMP content in a reactor. Qian et al. (2019) demonstrated that the release of EPS was significantly higher at pH 5.5 compared to pH 7.0 and 8.5. They also found a high rate of SMP being released at pH 8.5 compared to neutral and acidic pH conditions. Therefore, it can be stated that the release of EPS and SMP were both less at pH 7.0 and 8.0 compared to acidic and alkaline pH conditions applied in this experiment.

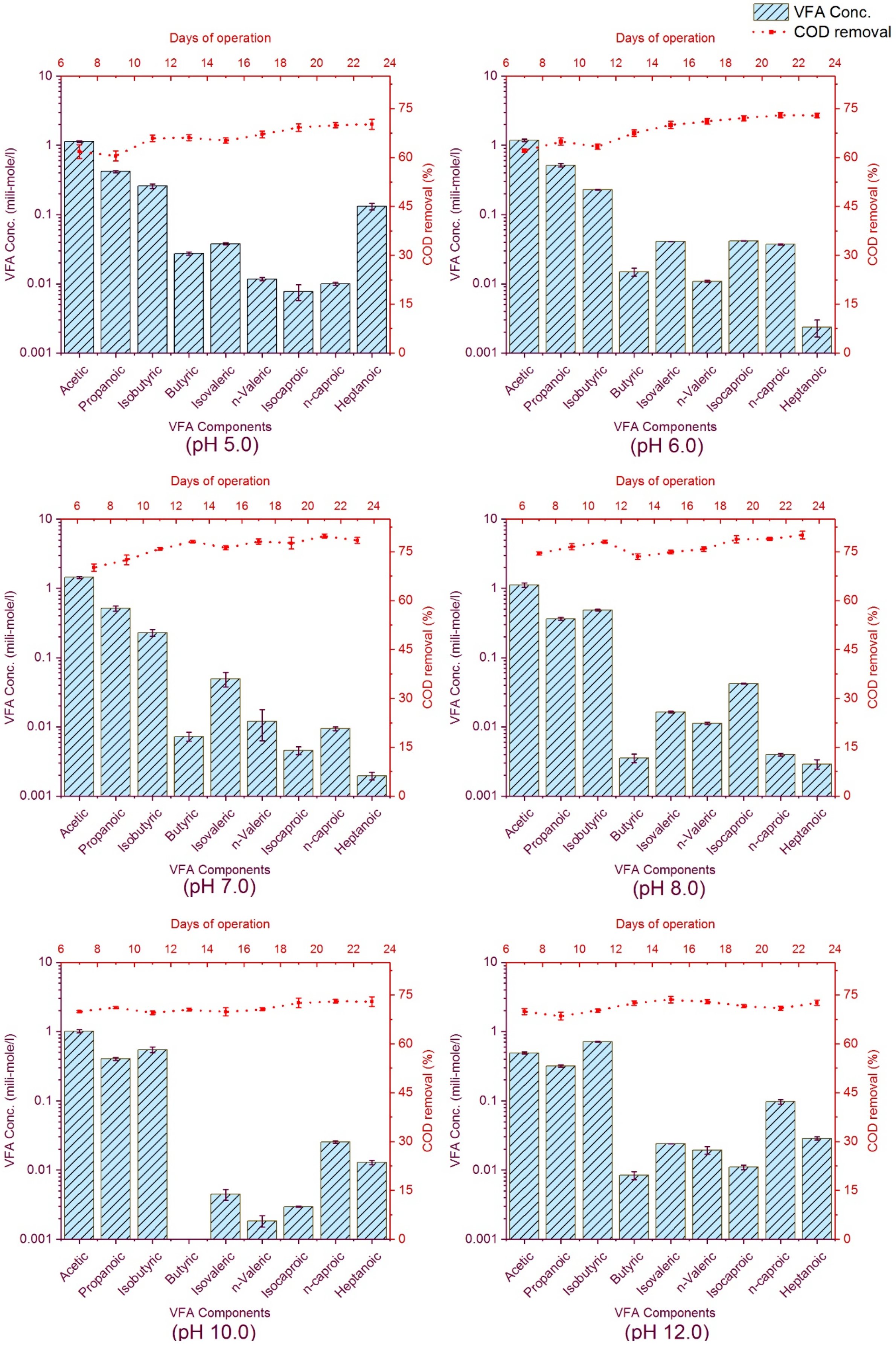
Polysaccharides and proteins are the two major contributors in biological membrane fouling. Research studies have shown that the cake layer formation on membrane surface is aided by an intermediate layer that has a high concentration of carbohydrates. The research performed by Zhou et al. (2016) involved photometric analysis that showed that the supernatant and membrane foulants in a Submerged Anaerobic Membrane Bioreactor (SAnMBR) were dominated by 90% of the total proteins and polysaccharides. At alkaline condition, the rate of initial hydrolysis of organic components is higher compared to the rates in neutral to acidic pH condition (Wang et al., 2019). The result of a high rate in initial hydrolysis can be a potential reason to increase the soluble proteins and carbohydrates in the reactor and finally accelerate the membrane fouling process (Li et al., 2019b).

Figure 5.3 (b) shows the development of TMP in the first 12 days of operation for pH 7.0 and 8.0. Both pH values have shown a significantly lower rate of TMP development compared to acidic or more alkaline pH conditions used in this experiment. From the figure, it is also evident that at the end of the first week of operation pH 8.0 showed a lower fouling rate compared to pH 7.0. But at the end of the 12-day period, the development of TMP was slightly lower at pH 7.0 (12.9 kPa) compared to 13.5 kPa at pH 8.0. As the present study has only performed the experiment at fixed pH values of 7.0 and 8.0, future research study can be done at around pH 8.0 o find the optimum pH for this process.

Measurement of polysaccharides and proteins can be useful to characterize the membrane fouling at different pH levels. As the results obtained from this experiment showed a higher membrane fouling rate at acidic and alkaline conditions, additional measures can be applied for controlling the membrane fouling. For example, Bio Electrochemical Systems (BES) coupled with membrane bioreactors, mechanical scouring or chemically enhanced backwashing can be effective in reducing the rate of membrane fouling (Li et al., 2019a; Wang et al., 2018b; Wu et al., 2017; Yue et al., 2018).

5.3.3 Selective VFA production at different pH

In this experiment, seven major VFA components (Acetic, Propanoic, Isobutyric, Butyric, Isovaleric, n-Valeric, Isocaproic, n-caproic and Heptanoic acid) were analyzed at six different pH conditions. In each stage of the operation, the concentration of individual VFA components was measured using GC-MS.



(pH 5.0) (pH 6.0)

(pH 7.0) (pH 8.0)

(pH 10.0) (pH 12.0)

Figure 5.4: Concentration of major VFA components and COD removal at different pH

Accumulation of VFA can affect the AnMBR performance in terms of COD and nutrient removal and make the operation unstable (Khan et al., 2016a). Therefore, COD removal efficiency was measured for each stage of the bioreactor's operation.

Figure 5.4 depicts the concentration for individual VFA components in the overall VFA mixture at different pH. The concentration of acetic acid was found to be 1.132 ± 0.034 and 1.184 ± 0.042 mili-mol/L at pH 5.0 and 6.0, respectively. The highest concentration of acetic acid was 1.444 ± 0.051 mili-mol/L at pH 7.0. A further increase in pH resulted in a drop in acetic acid concentration, referring to 1.115 ± 0.086 and 1.014 ± 0.053 mili-mol/L at pH 8.0 and 10.0, respectively. However, pH 12.0 confirmed the lowest acetic acid concentration of 0.492 ± 0.016 mili-mol/L. Figure 5.5 (a) illustrates the percentage of major VFA components, i.e. acetic, propanoic and isobutyric and n-butyric acid. It shows that acetic acid was the predominant VFA component at acetic to neutral pH range but the percentage of acetic acid dropped significantly at the alkaline condition. The composition of VFA mixture showed the amount of acetic acid covered 61, 62 and 66% in the total VFA mixture at pH 5.0, 6.0 and 7.0, respectively. In contrast, the percentage of acetic acid dropped to 57, 52 and 32% when the pH was increased to 8.0, 10.1 and 12.0, respectively. Therefore, it can be concluded that acetate-type fermentation in VFA production derived from the anaerobic process has an optimum pH range of 6.0 – 7.0.

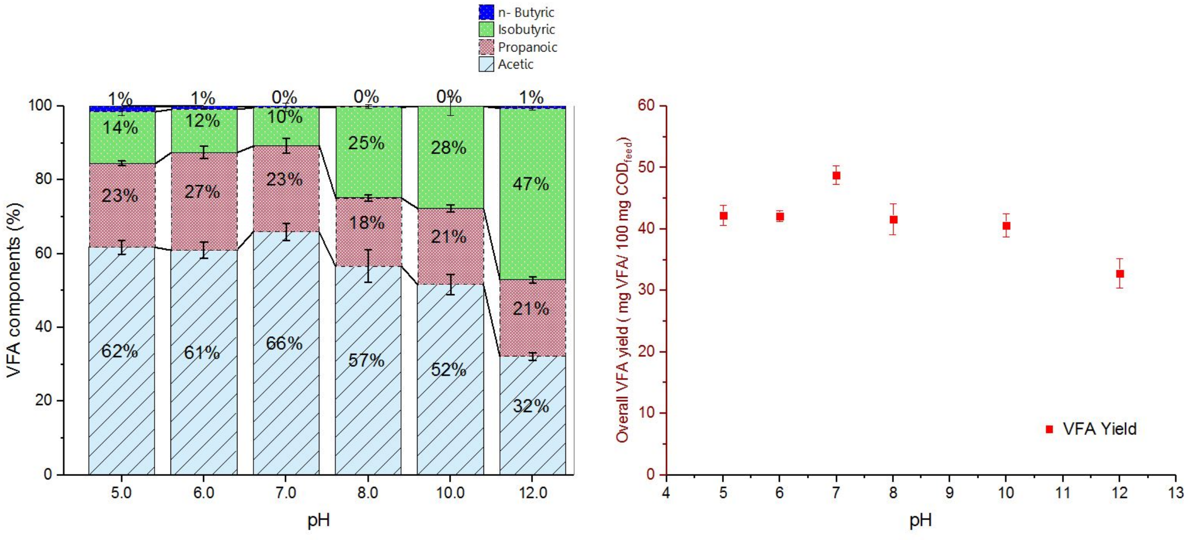


Fig 5(a)

Fig 5(b)

Figure 5.5: (a) Percentage of major VFA components produced at different pH (b) Overall VFA yield (mg VFA/ 100 mg COD $_{feed)}$ at different pH

Propionic acid is another predominant VFA component at lower pH levels. At pH 5.0, 6.0 and 7.0, the concentrations of propanoic acid were 0.417 ± 0.012, 0.516 ± 0.032 and 0.512 ± 0.043, respectively. Like acetic acid, propanoic acid concentration also dropped in alkaline conditions and the lowest concentration was found to be 0.317 ± 0.013 at pH 12.0. It may be assumed that the acidic pH conditions favored propionate-type fermentation in the reactor. At pH 6.0 and 7.0, the accumulation of propanoic acid was higher compared to alkaline conditions. Additionally, the conversion of acetic and butyric acid is thermodynamically favorable when the methanogens consume VFA for biogas production (Khan et al., 2018; Khan et al., 2016b).

This may possible explain the high concentration of propanoic acid at these pH conditions. According to the literature, propionate can be more rapidly degraded at pH 8.4 and above in comparison with the acetate (Boone & Xun, 1987). It explains the gradual decrease in the propionic acid concentration after pH 8.0. Figure 5 shows, in the overall VFA mixture, 23, 27 and 23% of propanoic acid were present at pH 5.0, 6.0 and 7.0. The percentages dropped to 18%, 21% and 21% at pH 8.0,10.0 and 12.0, respectively.

Unlike acetic and propanoic acid, the concentration of butyric acid did not change at acidic to neutral pH conditions. The lowest concentration of isobutyric acid was found to be 0.228 ± 0.002 mili-mol/l at pH 6.0 which was only 12% of the total VFA mixture. Therefore, it may be assumed that most of the butyric acid produced at these conditions was degraded through the process of acetogenesis and methanogenesis (Khan et al., 2016b). Additionally, another reason for the low concentration of butyric acid may be that butyrate-type fermentation was not predominant at these pH conditions.

However, a general increase in isobutyric acid concentration was observed when the pH rose to above 7.0. The highest concentration of isobutyric acid was 0.712 ± 0.008 mili- mile/l at pH 12.0. A general increase in the percentage of isobutyric acid was also observed at alkaline conditions as it increased to 25, 28 and 47% at pH 8.0, 10.0, and 12.0, respectively. These results indicate that butyrate-type fermentation was predominant at pH 12.0. The findings can be applied to an industrial process that aims for the selective production of isobutyric acid through an anaerobic process.

No specific trend was observed in the concentration of the remaining VFA components like n-butyric, isovaleric, n-valeric, iso-caproic, n-caproic and heptatonic acid throughout the experiment. Figure 5.5 (b) shows the overall VFA yield per 100 mg of COD_{feed} at different stages of AnMBR operation. At pH 5.0 and 6.0 overall VFA yields were 42.24 ± 1.6 and 42.1 ± 0.9%, respectively. The yield increased up to 48.74 ± 1.5% at pH 7.0. The results may be associated with the fact that there might be possible VFA accumulation inside the reactor at lower pH levels (5.0 and 6.0).

At pH 7.0 the system performed better in the initial hydrolysis and acidogenesis stage of anaerobic digestion. The yield dropped gradually at pH 8.0 and 10.0 and there was a significant decrease in overall VFA yield at pH 12.0 (32.81 ± 2.4%). Gao et al. (2010) carried out an experiment to observe the effect of elevated pH shock on AnMBR-treated thermochemical pulps. The study identified a total VFA concentration of 721 mg/l in the supernatant which was higher compared to 608 mg/l present in the permeate. Referring to these findings, it may be assumed that at higher pH levels, the membrane fouling layer retained a certain amount of VFA that caused an overall decrease in the VFA yield. Several studies showed that decoupling the initial hydrolysis/acidification process from the final stage of methanogenesis by using a multiple stage AnMBR can improve the overall yield of VFA from low-strength waste (Pathak et al., 2018; Robles et al., 2018).

The COD removal efficiency of AnMBR was significantly affected at different pH levels. Figure 4 shows the COD removal rate at different pH conditions. At the beginning of each trial, the pH removal efficiency fluctuated and during this experiment, variable rates in COD removal were observed at the second week. However, the rate steadied in the third week of AnMBR operation at a certain pH level.

Although pH 5.0 and 6.0 encouraged the production of acetic acid, the overall COD removal efficiency of AnMBR dropped to 60.5 ± 1.5% at pH 5.0. At pH 6.0 there was a slight improvement in COD removal efficiency and this led to the lowest removal rate of 63.4 ± 0.8%. A low operating pH can cause VFA accumulation inside the bioreactor and cause a change in microbial activity. The methanogens in this scenario cannot perform well as they require an optimum pH of 6.5 to 8.2 (Ngo et al., 2019).

A low operating pH can lead to an increase in SMP production due to enhanced cell lysis (Yue et. Al 2018). Additionally, during this experiment excessive growth of filamentous bacteria was observed when the AnMBR was operated at pH 5.0. The filamentous bacteria can break down the flocks and finally affect the reduction of COD and nutrient removal efficiency of the AnMBR. Therefore, the efficiency of COD removal is expected to be low at this pH condition. The AnMBR performed well at pH 7.0 and the highest COD removal efficiency here was 79.8 ± 0.6%.

In alkaline conditions, the overall COD removal efficiency dropped and at pH 12.0 it reached its lowest point (68.5 ± 1.2%). The low rate of COD removal may be a result of the possible accumulation of acetate and propionate inside the reactor that inhibits microbial activity. Kunacheva et al. (2017) investigated the effect of pH change on the performance of AnMBR. According to this study, the concentration of low Molecular Weight (MW) SMP at the supernatant increased from 0.17 mg/l to 0.32 mg/l at pH 7.0.

An increase in the SMP concentration reduced membrane fouling and also eventually reduced the overall COD removal efficiency to 50%. Although the third week of each operating stage showed the highest TMP, the AnMBR did manage to achieve the steady COD removal rate at this period. Therefore, it may be assumed that the membrane fouling layer was effective in COD removal and can remove most of the low MW components from the supernatant. Finally, it is a trade-off between the bioreactor performance and the amount of individual VFA components produced in the reactor.

Economic feasibility assessment for individual VFA components is important for large scale industrial production of VFA from wastewater. Based on the results obtained from this experiment, the production of any particular VFA component can be maximized by altering the pH. The highest overall VFA yield in this study was 48.74 ± 1.5 mg VFA/ 100 mg COD_{feed} without inhibiting the activities of VFA- consuming microbes (Methanogens). It would be interesting to see the maximum VFA yield that can be achieved through the selective inhibition of the methanogens.

5.4 Conclusions

The experimental result shows that acetic acid is the predominant VFA component at pH 7.0 whereas the concentration of propanoic acid was maximum at pH 6.0. Percentage of acetic acid in the overall VFA mixture decreased with an increment in pH above 7.0. The lowest acetic acid concertation was observed at pH 12.0 while the same pH showed highest isobutyric acid production. As the type of VFA component can be controlled by altering reactor pH, results from this experiment can be utilized for the selective production of VFA from anaerobic wastewater treatment.

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Chapter 6

Production of VFA and biohydrogen from AnMBR using low-strength synthetic wastewater

6.1 Introduction

6.1.1 Background

Anaerobic membrane bioreactors (AnMBRs) are currently favored for domestic and industrial wastewater treatment due to their potential in recovering energy from biodegradable organics present in water (Chen et al., 2017; Mahat et al., 2018). Over the past few years, production of methane-enriched biogas from organic waste streams has been the most common method of energy production in anaerobic membrane bioreactors. Research studies have been carried out to improve the production of anaerobic methane production through: firstly, different integrating pre- and post-treatment processes (Khan et al., 2019b); secondly, different chemical and biological additives; and thirdly, optimizing process conditions (Khan et al., 2019b; Mao et al., 2015). Despite these efforts, there is no net profit in an AnMBR because the revenue earned from AnMBR products is less than the cost involved in their operation and maintenance (Khan et al., 2016; Kleerebezem et al., 2015; Pretel et al., 2016).

Several issues are responsible for the small amount of energy, and resources recovered from an AnMBR. Firstly, an anaerobic membrane process designed to produce biogas as the single main product incorporates different technical issues. In the anaerobic process biogas is produced at the final stage of methanogenesis. This stage has been identified as the slowest among the four different stages of anaerobic digestion. As a result, even if the initial three anaerobic stages - hydrolysis, acidogenesis, and acetogenesis have higher rates - the slow rate of methanogenesis always restricts the production rate of methane (Adekunle & Okolie, 2015). Additionally, the process conditions at which methane production can be maximized is entirely different from the optimum conditions for the initial three anaerobic stages.

For example, the growth rate of the methanogens requires a pH range of 6.5-8.2 whereas the optimum pH range for acidogenesis is between 5.5 and 6.5 (Mao et al., 2015). Consequently, for process optimization, it is not possible to apply the optimum conditions separately for each anaerobic stage. Economically, the energy density from the produced biogas of an anaerobic process is low compared to the biogas used for domestic and industrial purposes (95-96% methane) (Ngo et al., 2019). Apart from the technical and economic limitations, AnMBR producing methane-containing biogas generates serious environmental outcomes such as, global warming, aquatic ecotoxicity, human toxicity, and abiotic depletion, etc. (Pretel et al., 2016; Pretel et al., 2013a; Pretel et al., 2013b). All the above-mentioned issues have contributed to making large-scale industrial application of AnMBR very challenging to carry out.

An alternative approach has been developed to produce Volatile Fatty Acids (VFAs), and biohydrogen from the AnMBR. During anaerobic digestion, VFAs are produced after the first stage when acidogenic bacteria convert the insoluble organic components present in the waste stream into soluble organic components like fatty acids, monosaccharides, etc., through the process of hydrolysis. Hydrolysis has been identified as the rate limiting step since it involves the production of toxic heterocyclic compounds. At the same time the produced VFA can directly affect the production of biogas through lowering the pH level of the bioreactor rapidly (Adekunle & Okolie, 2015; Khan et al., 2016).

According to Kleerebezem et al. (2015) a proposed alternative model is one where VFA is considered as the single main production of anaerobic digestion. VFAs have been identified as the raw material for the production of biodegradable polymers mainly Polyhydroxyalkanoate, valuable organic compounds like aldehydes, ketones, alcohols and alternative carbon sources for microbial fuel cell production, and even for biogas production (Khan et al., 2019b; Singhania et al., 2013). An economic analysis was done aiming to compare the revenue earned from Polyhydroxyalkanoate (PHA) and methane in a wastewater treatment facility processing 5000 m^3/day of cardboard wastewater. Results indicated a daily revenue of 20.2 k€ from PHA compared to 3.6 k€ from methane (Kleerebezem et al., 2015). Therefore, recovering PHA offers both technical and economic advantages over the production of methane from an AnMBR.

Biohydrogen is another intermediate product of an AnMBR that is produced in two different ways: firstly, through the process of acidogenesis where biohydrogen is created from monomers and soluble organic compounds; and, secondly, through acetogenesis where VFAs produced during the first two stages are converted into acetate and biohydrogen (Khan et al., 2018). To date, simultaneous production of biohydrogen and methane has been researched using two-stage anaerobic bioreactors (Algapani et al., 2019; Nathao et al., 2013; Salem et al., 2018). Two-stage anaerobic bioreactors have the technical advantage of optimizing process conditions separately for each step. They can also contribute to increasing the energy density as well as product revenue; however, the high cost of initial installation, operation and maintenance does not improve net profit gains. Since the final product methane of an AnMBR is responsible for damaging the environment, the multiple stage assembly method is not a useful alternative for reducing such environmental impacts. The following set of reactions show how VFA can be converted to biohydrogen and methane (Wang et al., 1999):

$$CH_3COO^- + H_2O \rightarrow HCO^-_3 + CH_4 \quad (6.1)$$

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \quad (6.2)$$

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 \quad (6.3)$$

$$CH_3CH_2CH_2CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CH_3CH_2COO^- + H^+ + 2H_2 \quad (6.4)$$

$$4H_2 + HCO^-_3 + H^+ \rightarrow CH_4 + 3H_2O \quad (6.5)$$

$$4CH_3CH_2COO^- + 3H_2O \rightarrow 4CH_3COO^- + HCO^-_3 + H^+ + 3CH_4 \quad (6.6)$$

$$2CH_3CH_2CH_2COO^- + HCO^-_3 + H_2O \rightarrow 4CH_3COO^- + H^+ + CH_4 \quad (6.7)$$

So far, VFA and biohydrogen have been produced in a single stage anaerobic bioreactor using the direct inhibition of methanogenesis (Atasoy et al., 2018; Begum et al., 2018; Huang et al., 2016). One major disadvantages of this approach is that the product spectrum is not flexible as a bioreactor model designed to produce VFA or biohydrogen cannot be configured to produce biogas. Another is that a designed process can treat only one specific type of pollutant present in the waste stream. Therefore, to improve the technical and economic feasibility of the AnMBR, a generic model should be developed where controlling the product spectrum can be controlled by altering the operating conditions only.

6.1.2 Objectives

This research study is aiming to simultaneously produce VFA and biohydrogen without any selective inhibition of microbial activity. To investigate the production of VFA and biohydrogen, five different HRTs were applied (24,18,12,8 and 6 hr). The optimum OLR was investigated based on the results found at different HRTs. In each operating condition, the membrane fouling rate was investigated. The bioreactor's effectiveness in removing COD and common nutrients have also been measured at different HRT and OLR.

6.2 Materials and methods

6.2.1 Experimental design

An anaerobic submerged membrane bioreactor was employed in this experiment with a working volume of 3.5 L. A submerged hollow fibre membrane module was used in this experiment (PVDF, 0.04 m^2, 0.07–0.1 µm pore size, 1.0 and 2.2 mm of the inner and outer diameter, respectively) (Figure 6.1).

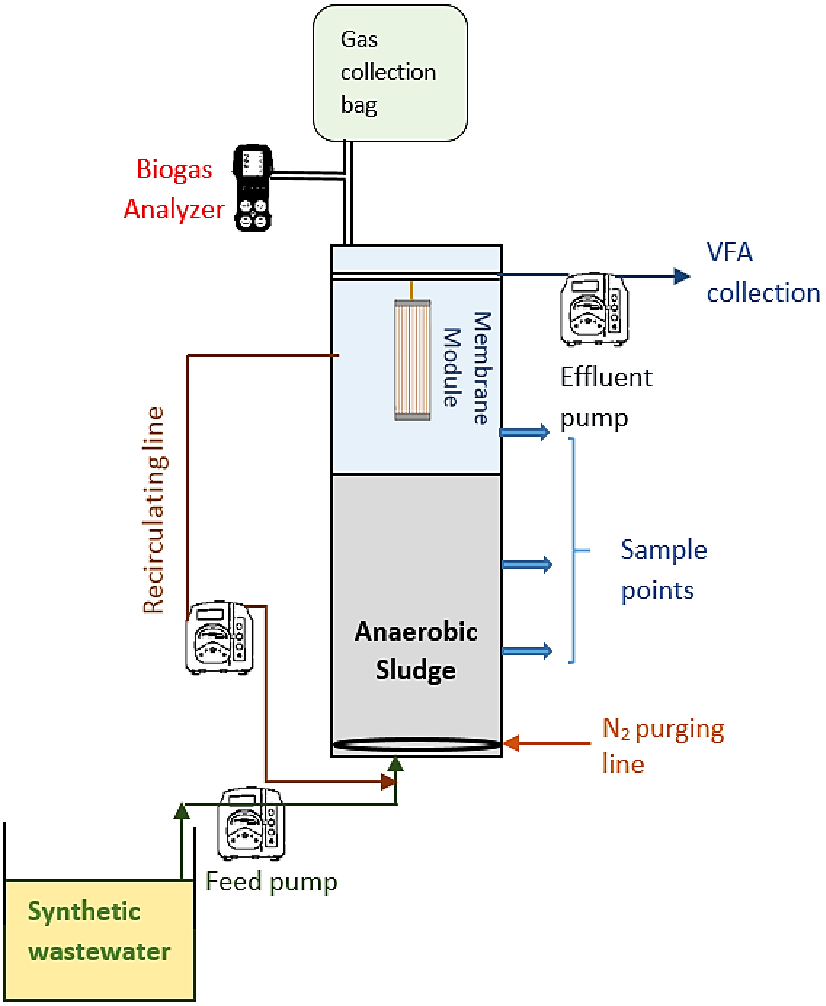

Figure 6.1: Schematic of experimental setup for VFA and biohydrogen production

6.2.2 AnMBR operation

Continuous mode of operation was applied in the membrane with 8 minutes of suction and 2 minutes of relaxation time. The production of biohydrogen and VFA was observed by altering two different operating parameters. The HRT of the system was maintained at 24, 18, 12, 8 and 6 hrs. For each trial, the AnMBR's performance was documented in terms of nutrient removal and membrane fouling rate. Secondly, at optimum HRT, the production of VFA and biohydrogen was measured at 350, 600 and 715 mg $COD_{feed.}$ The operating conditions were maintained for 3 weeks, and average values were used in the analysis of the results.

6.3 Results and discussion

6.3.1 AnMBR performance

Figure 6.2 illustrates the NO_3^- and PO_4^{3-} removal efficiencies at different COD levels and HRT. A gradual increase in the removal efficiency of nitrate was observed at the second and third week in each operating condition. In general, different COD levels in the AnMBR did not reveal any significant change in the NO_3^- removal efficiency.

The lowest NO_3^- removal efficiency was observed to be 92.1± 0.5% when influent COD was set at 600 mg/L from 350 mg/L. This indicated that the denitrification process was not recovered under the new operating conditions. The same operating conditions showed 98.5 ± 0.2% rate of denitrification on the 16th day of operation. The phosphate removal efficiency varied from 0.5 ± 0.2% to 5.6 ± 0.2%, but did not show any particular trend when the COD level was changed in the feed solution.

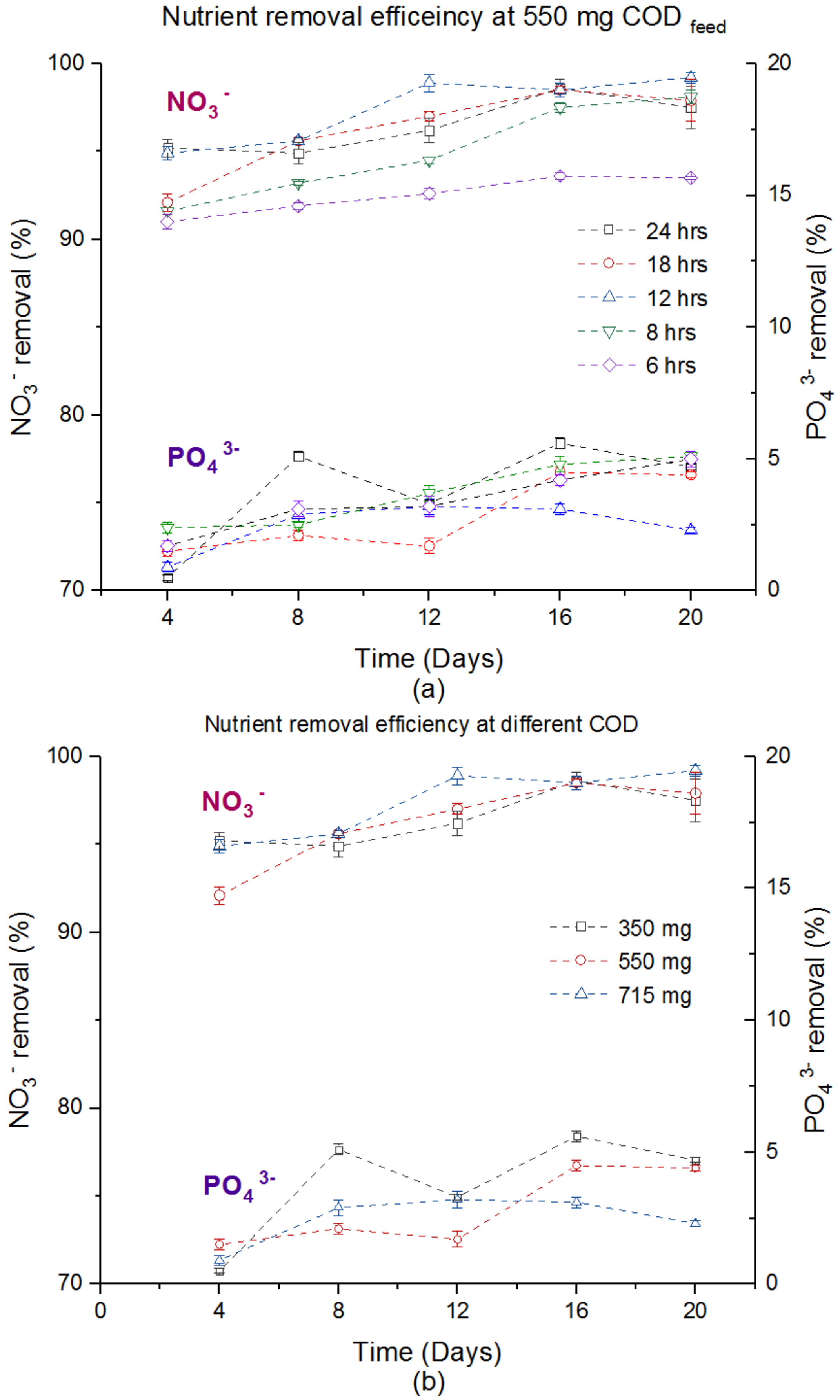

Figure 6.2: Nutrient removal performance of AnMBR at (a) different HRT (b) different COD

Figure 6.2 illustrates the NO_3^- and PO_4^{3-} removal efficiencies at different COD levels and HRT. A gradual increase in the removal efficiency of nitrate was observed at the second and third week in each operating condition. In general, different COD levels in the AnMBR did not reveal any significant change in the NO_3^- removal efficiency. The lowest NO_3^- removal efficiency was observed to be 92.1± 0.5% when influent COD was set at 600 mg/L from 350 mg/L. This indicated that the denitrification process was not recovered under the new operating conditions. The same operating conditions showed 98.5 ± 0.2% rate of denitrification on the 16th day of operation. The phosphate removal efficiency varied from 0.5 ± 0.2% to 5.6 ± 0.2%, but did not show any particular trend when the COD level was changed in the feed solution.

The pattern of NO_3^- removal efficiency revealed a general decline when the HRT was reduced from 24 hrs stepwise to 6 hrs. It dropped from 97.5 ± 1.2% to 92.1 ± 0.5% when the HRT was curtailed from 24 to 18 hrs. Although the system recovered in nitrate removal efficiency at the end of each operating HRT (97.5 ± 1.2, 97.9 ± 1.2, 99.2 ± 0.3, and 98.1 ± 0.4% for 24, 18, 12 and 8 hrs, respectively) the poorest nitrate removal efficiency was observed at 6 hrs (91 ± 0.4%).

As this experiment involved no specific phosphate removal process such as precipitation, the overall phosphate removal efficiency varied between 0.5 ± 0.1% and 5.6 ± 0.2%. The result implies that a change in operating HRT or influent COD do not change the PO_4^{3-} removal level to a great extent. A post-treatment process for PO_4^{3-} might be used in this regard. Another recommendation from the graph can be made to extend the operating cycle for all operating conditions. Since the nutrient removal efficiency initially dropped after a change in operating conditions but recovered at the end of each operating cycle, an extension of the operating period might have been useful for producing more accurate results compared to the existing one.

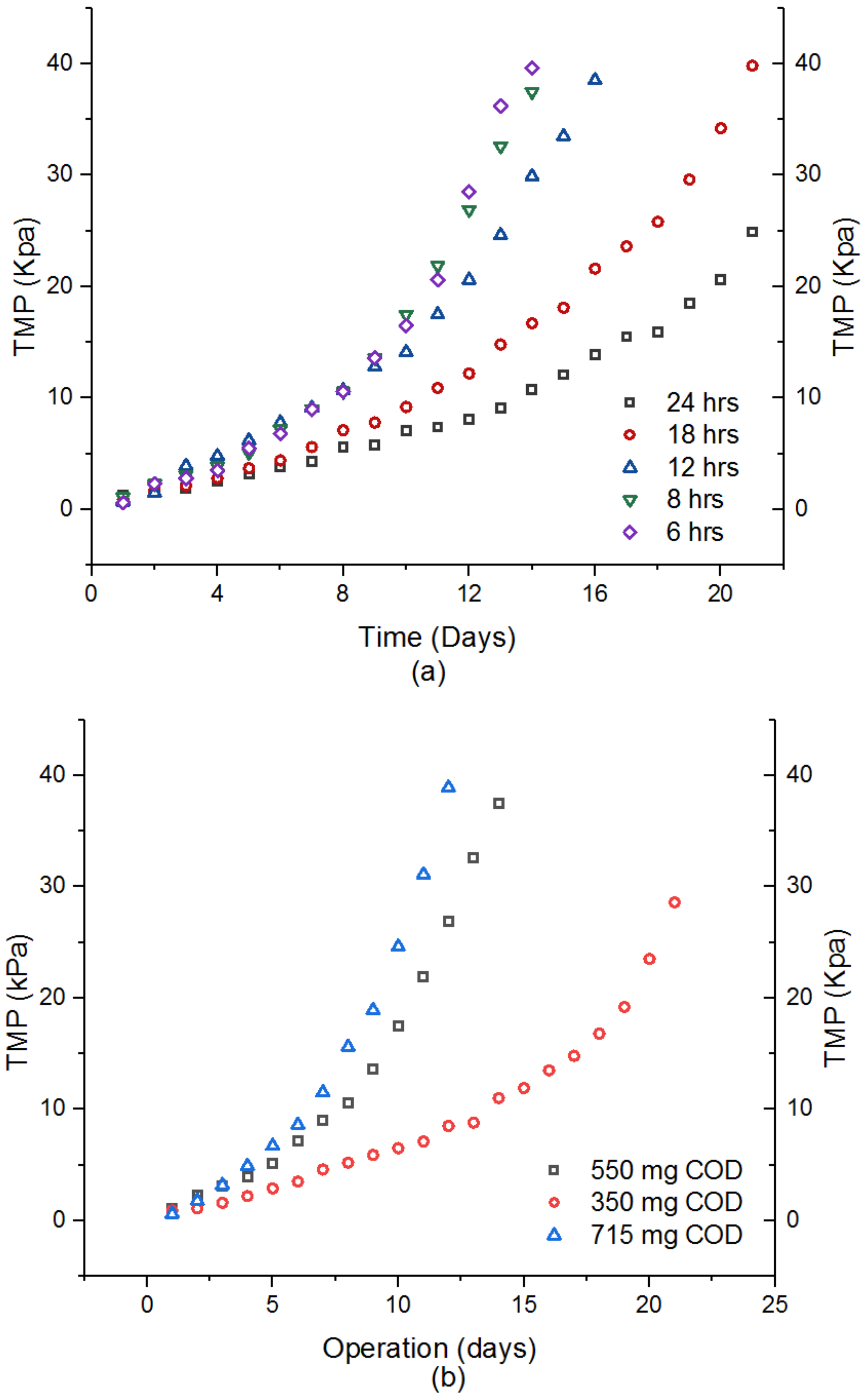

Figure 6.3: (a) TMP at different operating HRT (b) TMP at different influent COD

Figure 6.3 (a) depicts the development of TMP at different HRT. Operation of the AnMBR for the first 8 days showed similar TMP development in the 5.6 to 10.1 kPa range. At this stage, the permeate initially transports the compounds smaller than the membrane's cut-off size. The SMP present in the solution contributes to the increase in the adsorption of these molecules on the membrane surface. Although at lower HRTs, the SMP concentrations were higher, not a great variation in the fouling rate was observed in the first eight days of membrane operation. Nonetheless the fouling characteristics demonstrated significant variation in TMP development during the second and third week of membrane operation.

The EPS content present in the AnMBR affects the mechanism of pore blocking, and eventually contributes to reducing the membrane flux significantly. The results for this experiment show that at 6 hr HRT the 39.6 kPa developed across the membrane after only 14 days of operation. At the same time, 12 and 8 hrs of HRT showed 37.5 and 38.5 kPa, respectively, at the end of 14 days. On the other hand, the results for 24 and 18 hrs showed a promising 39.8 and 24.9 kPa TMP at the end of the 21day period.

The reason for this may be associated with the fact that the later stages of membrane fouling, specifically the pore blocking mechanism, were different in the various operating HRT. At low HRT like 6 or 8 hrs, the organic loading rates were higher compared to the ones applied in 24 or 18 hrs. Consequently, the production of EPS at lower HRT was higher when compared to 24 and 18 hr operations. A high value in EPS may have contributed to the pore blocking of the membranes. It can contribute to the sharp increase in TMP.

The initial adsorption on the membrane surface is typically followed by the formation of a biofilm as membrane washing was not carried out during this experiment. Typically, for a PVDF hollow fibre membrane, the formation of biofilm later involves clogging the membrane pores at a faster rate compared to the initial rate of adsorption. It may explain the rapid increase of TMP during the second and third week of operation. Figure 6.3(b) highlights the interesting behavior in the TMP development when the COD influent was reduced to 350 mg/L. A drop in COD concentration in the feed solution from 600 mg/L to 350 mg/L decreased the TMP from 37.5 kPa (at the end of 14 days) to 28.6 mg/L (at the end of 21 days). It indicates that a reduction in the organic loading rate can significantly reduce the membrane fouling rate. Waste streams such as low-strength synthetic wastewater typically contain a COD of 300 mg/L (Khan et al., 2019b). Hence, the issue of membrane fouling poses a smaller threat for low-strength municipal wastewater treatment. However, an increase in the COD influent from 600 mg/L to 715 mg/L wields a more adverse effect on membrane fouling. The TMP increased from 37.5 to 38.9 kPa and at the same time the membrane operation cycle was reduced from 14 to 12 days.

The TMP profile observed in this experiment can be useful to estimate the amount of membrane fouling during VFA and biohydrogen produced by the AnMBR. Especially for industrial applications, the fouling control strategies are important as the cost involved can contribute to the overall operational expenses and reduce the net profit margin from an anaerobic process. Throughout this experiment, the TMP development profile was observed at different conditions. Further research is needed to characterize the membrane fouling so that the fouling mechanism is better understood.

6.3.2 VFA production

Figure 6.4(a) and (b) shows the concentration of individual VFA components at different operating HRT and COD levels. A general increase in the VFA production rate has been observed when the HRT was reduced stepwise from 24 to 8 hrs. The reason for this may be linked to the fact that VFA is produced in the second and third stages of anaerobic digestion. The initial hydrolysis of the glucose present in the synthetic wastewater solution produced soluble compounds like amino and fatty acid components.

The hydrolyzed components go through the second stage of acidogenesis and produce VFA, alcohols, hydrogen, and CO2. VFAs can be produced at the end of the third anaerobic stage, i.e. acetogenesis (Adekunle & Okolie, 2015). However, the final anaerobic stage has been identified as the slowest among the four major anaerobic stages, and therefore the consumption of VFA through the final stage of methanogenesis is typically slow compared to rate at which VFA is produced (Khan et al., 2019b)

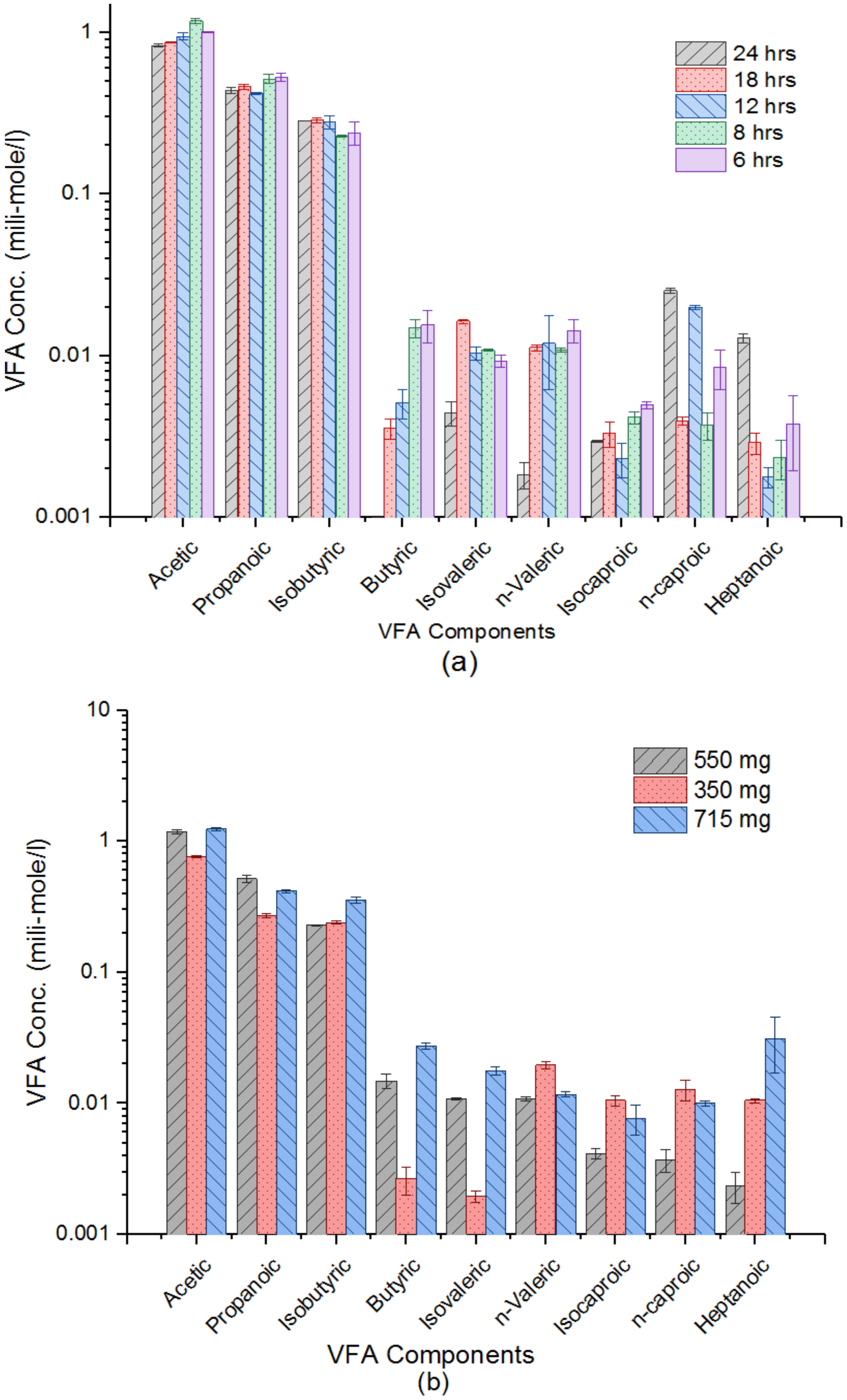

Figure 6.4: (a) TMP at different operating HRT (b) TMP at different influent COD

Therefore, reducing the HRT from 24 hrs decreased the exposure time of processed VFA to the methanogens present in the bioreactor. As the microbes did not get sufficient time to convert the VFA to methane, a general increase in the individual VFA components was observed during the stepwise reduction of HRT from 24 to 8 hrs. However, the production of VFA dropped slightly when the HRT was further reduced from 8 to 6 hrs, which suggests that the HRT was insufficient to complete the initial three anaerobic stages.

The major three VFA components in the mass spectroscopic analysis were found to be acetic, propanoic and isobutyric acid. At 8 hrs operating HRT, the overall VFA mixture contained 59.95, 26.12 and 11.55% acetic, propanoic and isobutyric acid, respectively. Based on our previous study (Khan et al., 2019a), pH 6.0-7.0 has been identified as optimizing the acetate type fermentation in an anaerobic process. Therefore, the experimental result implies that acetate type fermentation was predominant at this operating pH of 7.0. A change in pH to the alkaline zone (10.0 – 11.0) can alter the composition of the produced VFA through increasing the production of butyric acid. Therefore, in industrial application, altering the level of pH can be an option to improve butyric acid production.

The concentration of propanoic acid at different HRT varied between 0.4376 and 0.5293 mili-mol/L that covers about 27% to 28% of the total VFA. Propanoic acid can be responsible for causing bioreactor acidification when accumulating inside a bioreactor. It reduces the pH of the bioreactor sharply and makes the overall operation unstable (Khan et al., 2016). Given that the operating pH of the AnMBR throughout this experiment was maintained at 7.2 ± 0.1, the possible risk of reactor acidification propanoic acid was eliminated. The overall VFA yield also improved when the HRT decreased from 24 hrs to 18, 12 and 8 hrs. The maximum VFA yield was observed to be 37.08g / 100 g COD $_{feed}$ at 8 hrs whereas the yields for 24, 18, 12 and 6 hrs were 25.29, 27.19, 28.05 and 32.55 / 100 g COD $_{feed,}$ respectively. It means that the increase in overall VFA yield was observed from 24 to 8 hrs but a further decrease in VFA yield was observed at 6 hrs.

The reasons behind a low VFA yield at 6 hrs HRT can be varied. Firstly, the AnMBR performance in terms of COD and nutrient removal was compromised at this operating condition. A low removal of COD at 6 hrs can contribute to the reduction of overall VFA yield from the system. Secondly, membrane fouling was more severe at this operating condition. The fouling layer can retain a certain amount of VFA inside the bioreactor, and can cause an overall decrease in the VFA yield. Thirdly, 6 hr HRT can be deemed as too short a period to complete acetogenesis, and acidogenesis showed smaller individual acetic, propanoic and butyric acid concentrations compared to 8 hr HRT. As a result, a reduction in the major individual VFA components may have contributed to the overall reduction in VFA yield.

Figure 6.4 (b) illustrates the production of VFA at different influent COD levels. A general increase in the concentration of major VFA components was observed when the influent COD rose from 350 to 600 and 600 to 715 mg/L, respectively. As expected, an increase in the COD concentration increased the carbon content in the AnMBR feed. As a result, the initial hydrolysis, acidogenesis, and acetogenesis process produced a larger amount of VFA components and the individual concentrations went up although the COD, and nutrient removal performance declined to 715 mg/L COD. However, no particular trend was actually observed in the concentration of valeric, Caproic and Heptanoic acid at different HRT and COD levels. It can be concluded here that more research is required to optimize the production of these VFA components through anaerobic digestion.

The following points can summarize the optimization of VFA from an AnMBR:

- Membrane fouling is one of the major problems in optimizing VFA production. For our system, 8 hrs HRT resulted in the highest VFA yield but the membrane operation cycle was reduced from 21 to 14 days.
- The gradual development of the fouling layer at low operating HRT could retain a certain amount of VFA inside the reactor. In this case, fouling control strategies could produce a higher yield.
- The separation technique applied to extract VFA in this experiment involved MTBE as an organic solvent. For industrial scale applications, the cost involved in this process can increase the AnMBR operating costs significantly. Hence, more research initiatives are needed to understand how VFA can be extracted VFA from the AnMBR economically.

- The AnMBR's performance when taking into account COD, nutrient removal and membrane fouling was better when the feed solution contained 350 mg/L COD, compared to 600 and 715 mg/L COD. As most municipal wastewater has COD values between 300 to 350 mg/L, the observed results demonstrate the technical feasibility of VFA production without compromising the methanogenic activity.
- A comparative cost analysis of product revenue earned from VFA and methane production can be done in an AnMBR. An increase in product revenue through VFA production can improve the economic feasibility of the AnMBR in industrial applications.

6.3.3 Biohydrogen production

The effect of change in HRT was observed in overall hydrogen yields. Figure 6.5 demonstrates the overall hydrogen yield per gram of COD added to the system. As mentioned earlier, the AnMBR was operated for 21 days in each HRT and led to a 110 – day operating period for five different HRTs.

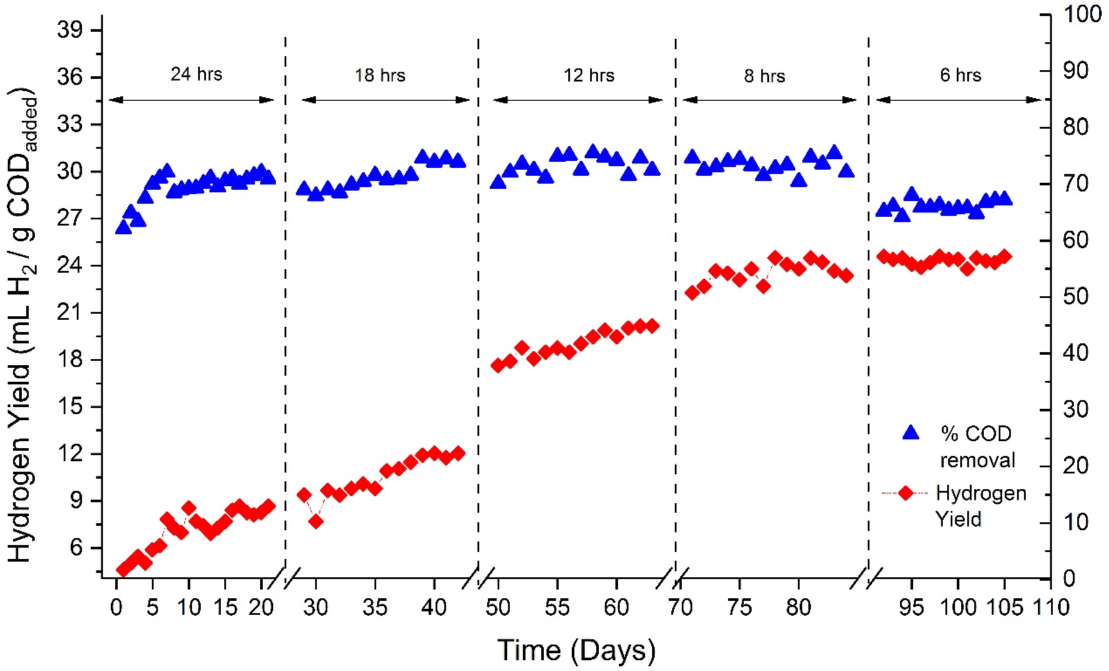

Figure 6.5: Overall hydrogen yield per gram of COD $_{added}$

Assuming that the system did recover from the change in HRT during the first week of operation, hydrogen production has been reported during the second and third week of each operating period.

6.3.4 Comparing yield of biohydrogen from different anaerobic processes

In general, an increase in overall hydrogen yield has been observed when the operating HRT was reduced from 24 to 18, 12, 8 and 6 hrs stepwise. Figure 6.5 shows that the first 21day period of the AnMBR operation involved operating at the 24 hr HRT and no significant hydrogen production was observed during this period. It may be assumed that in this operating scenario the produced hydrogen was consumed by the hydrogenotrophic methanogens to produce methane. Hydrogen acts as a proton donor during this process for the reduction of CO_2. As the operating pH for this experiment was maintained at 7.2 ± 0.1, it may be assumed that the methanogens functioned well at this operating HRT.

However, a gradual decrease in the HRT values increased the production of hydrogen per gram of COD $_{feed}$. Between 29 and 42 days, the AnMBR was operating at 18 hrs HRT and this slightly improved the hydrogen production from 6.2 to 8.6 mL H_2/ g COD $_{feed.}$
The graph shows a similar trend when the HRT was set to 12 hrs operation indicating a rise from previous figures of 8.6 to 14.4 mL H_2/ g COD $_{feed.}$ This change in hydrogen production implies that, without any inhibition of methanogenic microbes, a decrease in HRT reduces the rate of hydrogen consumption in an anaerobic process.

At 8 and 6 hr operating HRT, the yield of biohydrogen was maximized compared to higher HRT values. The results showed the maximum yield of hydrogen production was 18.6 and 17.5 mL H_2/ g COD $_{feed}$ at 8 and 6 hrs, respectively. At the same time, severe membrane fouling was observed at the 6 hr operating period where the membrane operating cycle was reduced from 21 to 12 days. In addition, it can be noted that the overall COD removal in the AnMBR system fell from approximately 79% to 68.6%. Therefore, although the overall hydrogen yield was highest at the 6 hr period the rate of hydrogen production might decrease due to a reduction in the removal of COD.

It has been observed that at 8 hrs operating HRT, the overall VFA concentration reached its maximum compared to the other three operating conditions. At the same time, acetic acid was the predominant VFA component at this condition with a concentration of 1.184 mili-mole/L. As a result, hydrogen-producing acetogens at this condition had more VFA as a raw material to produce biohydrogen and carbon dioxide. This outcome also suggests that acetate-type fermentation was the major microbial pathway of hydrogen production in this phase.

Table 6.1: Biohydrogen yield from different anaerobic process

System	**Substrate**	**Operating conditions**	**Hydrogen yield (mL H_2/g COD $_{added}$)**	**Reference**
Multi-phase anaerobic reactor	Saline industrial wastewater	2.34 g COD/L/d 25 ± 6 °C	75.70 ± 3.98	(Ali et al., 2019)
Multi-phase anaerobic reactor	saline industrial wastewater	1.17 g COD/L/d 25 ± 6 °C	100.81 ± 9.11	(Ali et al., 2019)
Two-stage anaerobic fermentation	beverage wastewater	pH 6.4 3500 mg COD/L HRT – 2 hrs	172.0 ± 65.0	(Lay et al., 2019)
Two-stage anaerobic batch + PAC	coffee husks	F/M ratio 0.7 25 °C	55.8	(Santos et al., 2018)
Two-stage anaerobic fermentation	beverage wastewater	pH 6.4 3500 mg COD/L HRT – 4 hrs	18.0 ± 17.0	(Lay et al., 2019)
Two-stage upflow anaerobic sludge blanket reactors (UASB)	cassava wastewater	pH – 5.5 37 °C COD loading rate 25 kg/m^{3}d	39.83 ml H_2/g COD $_{removed}$	(Intanoo et al., 2016)
Anaerobic membrane bioreactor	**Low-strength synthetic wastewater**	**pH – 7.0** **600 mg/L COD** **22deg C**	**24.6 ± 3.2**	**This study**

From Table 6.1, it is evident that a reduction in HRT from 4 to 2 hrs improves the production of biohydrogen significantly in a two-stage anaerobic fermentation. During this experiment, the highest yield was observed at 6 hrs and no further decrease in HRT was made. The major reason behind this strategy includes the severe membrane fouling, low COD, nutrient removal efficiency and a low overall efficiency in removing COD. A further reduction could decrease the membrane operation cycle much further and the AnMBR performance could be compromised in terms of the removal of nutrients and COD.

Apart from HRT and COD in the influent, pH plays a critical role in hydrogen production. The optimum pH level has been referred to as being between 5.0 – 5.5 for different anaerobic processes. As the operating pH for this experiment was retained at 7.2 ± 0.1, the overall yield of biohydrogen is less compared to the actual optimized process yield. Furthermore, the activities of the hydrogen-consuming microbes were not suspended in this experiment for biohydrogen production. In summary, future research should investigate in more detail pH alteration and inhibition of methanogenesis.

6.4 Conclusions

The experiment confirms that a reduction in HRT can improve the production of VFA and biohydrogen in an AnMBR. At short HRT the rate of membrane fouling increased and COD and nutrient removal efficiency both decreased. Without inhibiting methanogenic activity, the highest VFA and hydrogen yields were 37.08g VFA / 100 g COD $_{\text{feed}}$, and 24.6 mL H_2/ g COD $_{\text{feed}}$, respectively. Economic assessment comparing the generated revenue from the produced VFA, biohydrogen with methane can contribute to improving the sustainability of the AnMBR.

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Chapter 7

Optimization of the production of biohydrogen from anaerobic membrane bioreactor (AnMBR) using low-strength synthetic wastewater

7.1 Introduction

7.1.1 Background

Anaerobic Membrane Bioreactors (AnMBRs) have been explored for energy and resource recovery from different waste streams. It involves the integration of conventional anaerobic digestion processes with a membrane module (Song et al., 2018). It utilizes the biological conversion of organic substances into methane-rich biogas. Like the anaerobic process, operation of an AnMBR is also dependent on strict operating conditions, these being low conversion rate of methanogenesis, and narrow range of pH operation. Additionally, the product revenue from the resources recovered from a conventional AnMBR is less compared to the costs involved in operation and maintenance (Ngo et al., 2019). As a result, the large-scale application of this process by the AnMBR industry is still very limited.

An alternative approach to change the product stream from methane-containing biogas to biohydrogen can be both technically and economically beneficial. Most recent research studies have shown that different bioreactor arrangements and substrates have been employed to produce biohydrogen from the AnMBR (Aslam et al., 2018; Bakonyi et al., 2014). The production rate and yield of biohydrogen are maximized through optimizing the process conditions and inhibition of the hydrogen-consuming methanogens. In general, a high rate of biohydrogen production is observed at the thermophilic condition due to a faster rate of sludge acclimatization, increase in enzymic activity and increased solubility of polymers present in the feed solution. The findings reported by Zhong et al. (2015) indicate 131.5 ml H_2/g-$COD_{removed}$ at 60 °C, whereas the rate dropped at 116.5 ml H_2/g-$COD_{removed}$ when the bioreactor was operated at 40 °C. Another operating condition, pH, controls the dynamics of fermentation and the metabolic pathway. pH has been identified as an important ecological factor of hydrogen-producing bacteria (Khan et al., 2018). Wang et al. (2011) on this issue have identified a pH value of 6.9 for maximum biohydrogen yield and 7.2 for average biohydrogen production rate. In contrast, pH in the 5.5 to 6.8 range has been identified as ideal for biohydrogen production through different anaerobic processes (Liu et al., 2011; Ruggeri & Tommasi, 2015).

Apart from temperature and pH condition, Hydraulic Retention Time (HRT), Solid Retention Time (SRT) and Organic Loading Rate (OLR) control the production rate and yield of biohydrogen produced through an anaerobic process. Although the HRT and SRT values are dependent on the design of the bioreactor, a typical low value of HRT can inhibit the activities of methanogens (Romero Aguilar et al., 2013). The research done by Kumar et al. (2016) investigated the HRT dependent performance of hydrogen production using galactose. The highest biohydrogen production rate and yield were observed to be 25.9 L H_2/L-d, and 2.21 mol H_2/mol galactose, respectively, when the HRT was varied between 3 to 6 hrs. For OLR, a general increase at the initial range increases the production of biohydrogen but at the same time a high OLR can be responsible for rapid acidification and severe membrane fouling in AnMBRs (Khan et al., 2016b;Aslam, 2018). It is consequently more practical to apply a range of loading rates for a specific bioreactor design, and discover the optimum OLR for a given process.

Currently available research studies have directly employed the inhibition of methanogenesis to produce biohydrogen through heat shock, load shock or pH regulation. The major drawback of this approach is that a process designed to produce biohydrogen cannot be modified later to produce methane or Volatile Fatty Acids. From the economic viewpoint it poses a difficult challenge as the energy density achieved through this process is too low to be considered for industrial application.

7.1.2 Objectives

The main objective of this investigation is to optimize the production of biohydrogen by optimizing the operating conditions and without any direct inhibition of hydrogen-consuming bacteria in an AnMBR treating low-strength synthetic wastewater. Through this experiment, the best operating conditions for biohydrogen production was found in a single stage AnMBR. The optimum pH to produce biohydrogen was investigated using the optimum values of HRT and OLR previously found in chapter 6. In addition to maximizing biohydrogen production, the AnMBR's performance is evaluated through membrane fouling, COD and nutrient removal, and finally the trend was observed in biogas production.

7.2 Analytical Methods

The seed sludge for an AnMBR was collected from a domestic sewage treatment plant in Sydney, Australia. The collected sludge was acclimatized for 60 days and COD and nutrient removal efficiencies were observed. At the start-up of each trial of this experiment, the value of Mixed Liquor Volatile Suspended Solid (MLVSS) was maintained at 8-10 g/L. The measurements of MLSS and MLVSS were carried out by following the procedure mentioned in chapter 3 section 3.2. The produced biogas was analyzed using Geotech Biogas 5000 gas analyzer. The volume of the biogas produced was measured using the water displacement method.

7.3 AnMBR setup and operation

The basic AnMBR arrangement with the major instrument has been illustrated in Figure 3.1 in chapter 3. Our previous investigation confirmed the yield of biohydrogen reached its maximum during the 6 hr operating period. For this experiment, the HRT was kept fixed at 6 hrs with an influent COD of 600 mg/L. Three different pH conditions were implemented at different operational stages. At each operating pH the AnMBR operated for four weeks in continuous mode. For multiple values in a single measurement, average values were used.

At the beginning of each operating stage, the concentration of Mixed Liquor Volatile Suspended Solids (MLVSS) was maintained at 10 g L^{-1}. The temperature of the AnMBR was controlled at 22 ± 2 °C. pH 7.0, 6.0 and 5.0 were applied for 4 weeks each to investigate the amount of biohydrogen produced. The pH level in the AnMBR was maintained at a desired value by adding Na_2CO_3 to the AnMBR feed. To eliminate any unexpected oxygen inside the reactor, nitrogen gas was purged from the bottom of the AnMBR. The maximum Dissolved Oxygen (DO) was maintained at 0.01 ppm.

7.4 Results and Discussion

7.4.1 AnMBR performance

During this experiment, the performance of the AnMBR was investigated in terms of COD removal, nutrient removal, and TMP development.

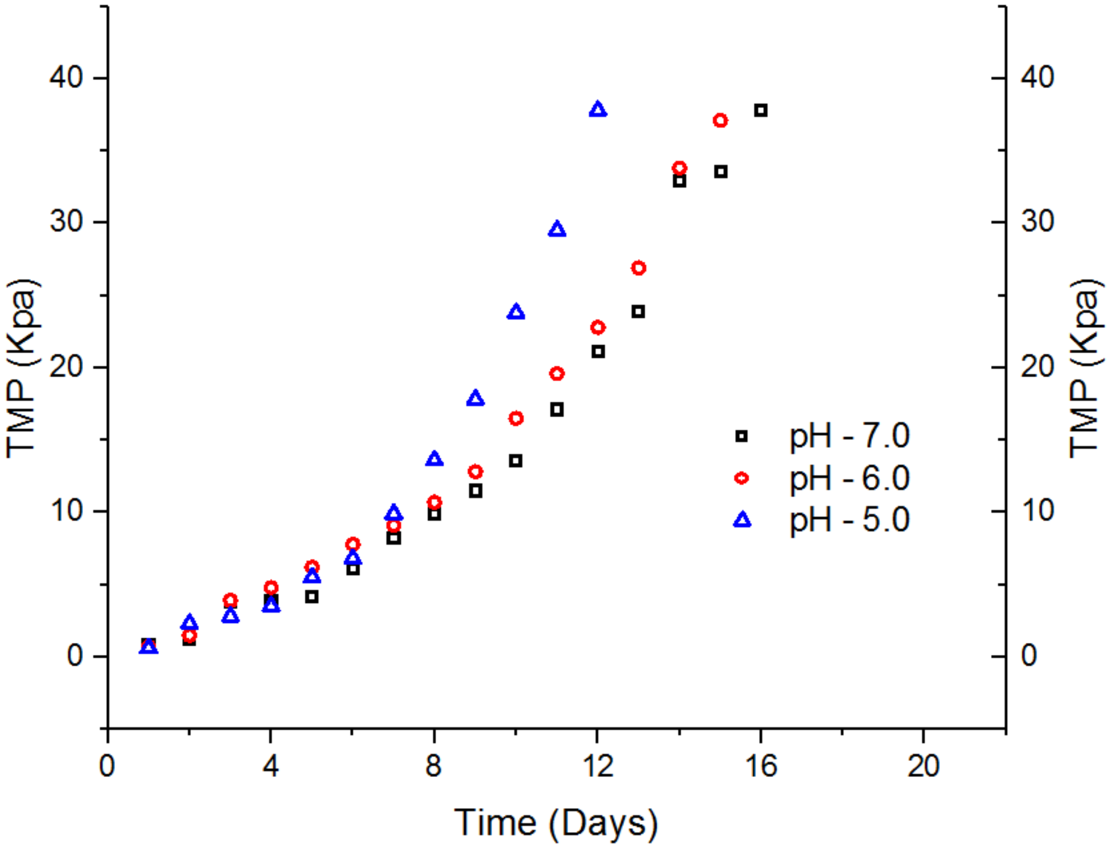


Figure 7.1: TMP developed at different pH levels

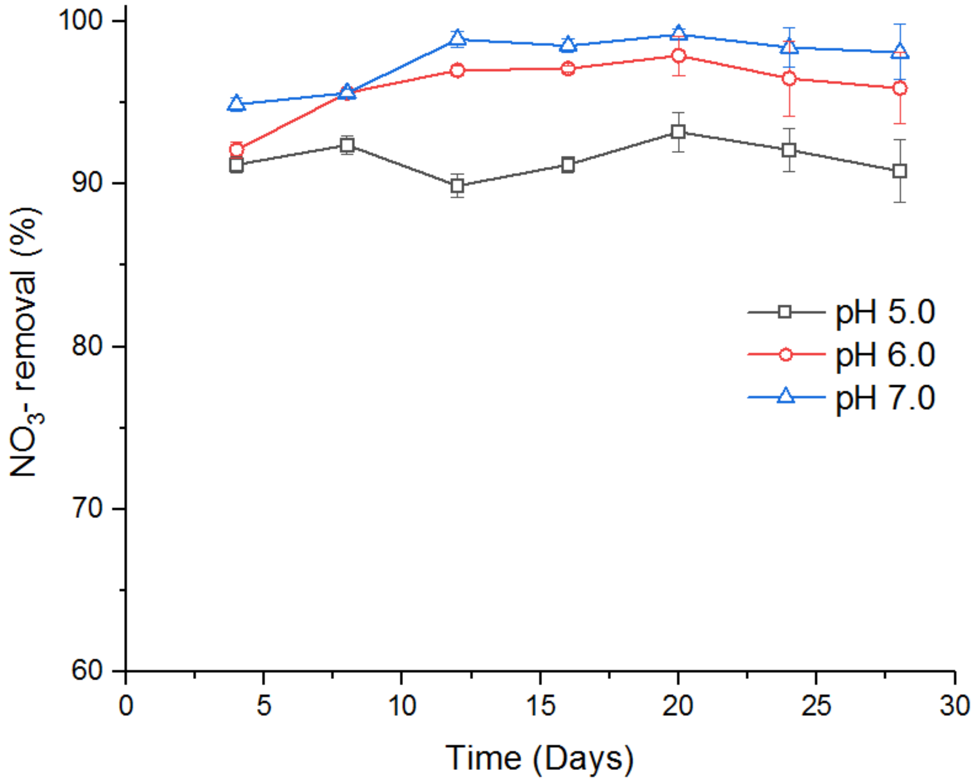
Figure 7.1 shows the rate of TMP development at different operating pH with a corresponding HRT of 6 hrs and 600 mg/L COD concentration in the bioreactor feed. The graph indicates a lower rate of TMP development at pH 7.0 compared to the rates observed at pH 6.0 and 5.0. At pH 7.0, the membrane operation cycle lasted 16 days, whereas it was reduced to 15 and 12 days for pH 6.0 and 5.0, respectively.

No significant difference was observed in TMP development at varying pH levels during the first week of AnMBR operation. Therefore, the findings suggest that the pore blocking mechanism at the initial stage of membrane fouling did not show any significant variation at different operating levels of pH. Typically, at acidic conditions, the concentration of TOC and

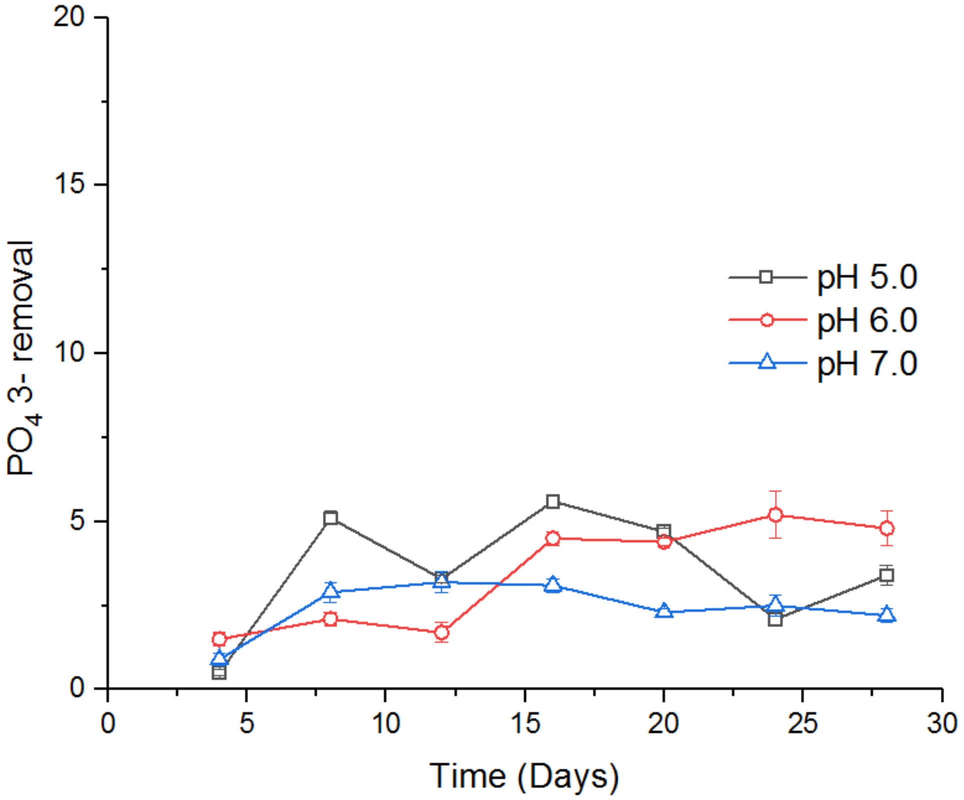
TN increases in the bioreactor. This can possibly result in an increase in large proteins or protein-like components from cell lysis (Kunacheva et al., 2017). These protein-like substances are attached to the membrane surface and likely to cause pore blocking during the first week of operation.

Adhesion of colloidal particles and deposition of biomass can be major contributing factors during fermentative hydrogen production when the level of pH is low. At high organic loading rates, the biomass concentration increases and this in turn contributes to increasing the colloidal proteins and polysaccharides. Shen et al. (2017) on this matter have investigated the membrane fouling behaviour during fermentative hydrogen production at pH 5.5. Their findings implied that the second stage consisted of a faster initial fouling rate followed by a slower rate in TMP development. Furthermore, they observed the pore clogging resistance exponentially increased when polysaccharides and colloidal proteins also increased. The current study was carried out at HRT of 6 hrs and the influent COD was fixed at 600 mg /L. The corresponding OLR of 2.4 g/L/d might have contributed to the increase in the proteins and polysaccharides, and increased the rate of pore blocking at pH 5.0, and 6.0.

Kunacheva et al. (2017) investigated the fouling behaviour in an AnMBR at different operating levels of pH. Their findings suggested that the production of alkanes and alkenes was higher at pH 5.0 compared to the amount present at pH 7.0. A microbial shock at pH 5.0 can kill the bacteria that degrade alkanes and alkenes in anaerobic conditions. Finally, unconverted alkanes and alkenes can contribute to the membrane fouling in acidic pH conditions. At low operating pH, colloids sized between 0.2 μm –1 μm are predominant and can contribute to the pore blocking of the membrane. In addition, a low level of pH can increase energy production through proton motive force because the cells try to maintain the balance of pH across the cell (Kunacheva et al., 2017). The ratio of proteins and carbohydrates can also be important since protein-like substances are more likely to attach to the membrane’s surface. As a result, the formation of the fouling layer after the pore blocking is typically accelerated by the generation of proteins inside the AnMBR. However, the membrane fouling layer at low pH can reject the SMP present in the effluent stream, and contributes to the removal of COD in the AnMBR. However, for large scale operation, the reduction of flux can emerge as a potential problem in operating AnMBR at low pH conditions.



(a)



(b)

Figure 7.2: (a) NO_3^- and (b) PO_4^{3-} removal efficiency at different pH levels

Different studies have identified that the production of SMP is higher in acidic conditions compared to the SMP content at neutral pH conditions (Khan et al., 2019a; Khan et al., 2019b). The carbohydrates present in SMP therefore may have contributed to the higher fouling rate at pH 5.0 and 6.0 in this experiment. Since the bioreactor was operated at an HRT lasting 6 hrs, the overall rate of TMP development was higher.

The different stages of operating the AnMBR at pH 7.0, 6.0 and 5.0 showed stable NO_3^- removal efficiency. The highest NO_3^- removal was recorded as 99.2 ± 0.3% during operation at pH 7.0. Figure 7. 2(a) shows the overall NO_3^- removal was higher at 7.0 compared to pH 5.0 and 6.0. Of the three operational stages, pH 5.0 indicated the poorest efficiency in removing NO_3^-. This outcome may be related to the fact that the denitrification process was most efficient at neutral pH condition. Consequently, at pH 6.0 and 5.0 the maximum rates of nitrate removal efficiency dropped to 89.9 ± 0.7 and 92.1 ± 0.5%, respectively. The pH shock experienced by the denitrifying bacteria may have affected the rate of denitrification, and eventually reduced the nitrate removal efficiency.

The rate of PO_4^{3-} removal did not show any particular trend during three operating pH levels. The AnMBR design involved in this experiment did not include any particular process for phosphate removal. Therefore, as expected, the phosphate removal efficiency was very poor in this experiment. From Figure 7.2(b) it has been observed that the phosphate removal efficiency varied from 0.5 ± 0.1% to 5.6 ± 0.2%. The removal of only small amounts of phosphate may also indicate that the anaerobic environment was maintained throughout the experiment. This is because the anoxic and aerobic treatment process can offer higher phosphate removal compared to anaerobic processes from different waste streams.

7.4.1 Biohydrogen production

At different operating pH, the production rate and yield of biohydrogen have been investigated along with the overall COD removal efficiency. Figure 7.3 illustrates the hydrogen production rate at various pH levels. The rate of hydrogen production was lower at pH 7.0, indicating a maximum production rate of 47.69 mL H_2 / L. d during this period. The rate of biohydrogen production gradually increased when the pH of the bioreactor decreased to 6.0 and 5.0, respectively, indicating the maximum rates of production to be 78.32 and 122.21 mL H_2 / L. d, respectively.

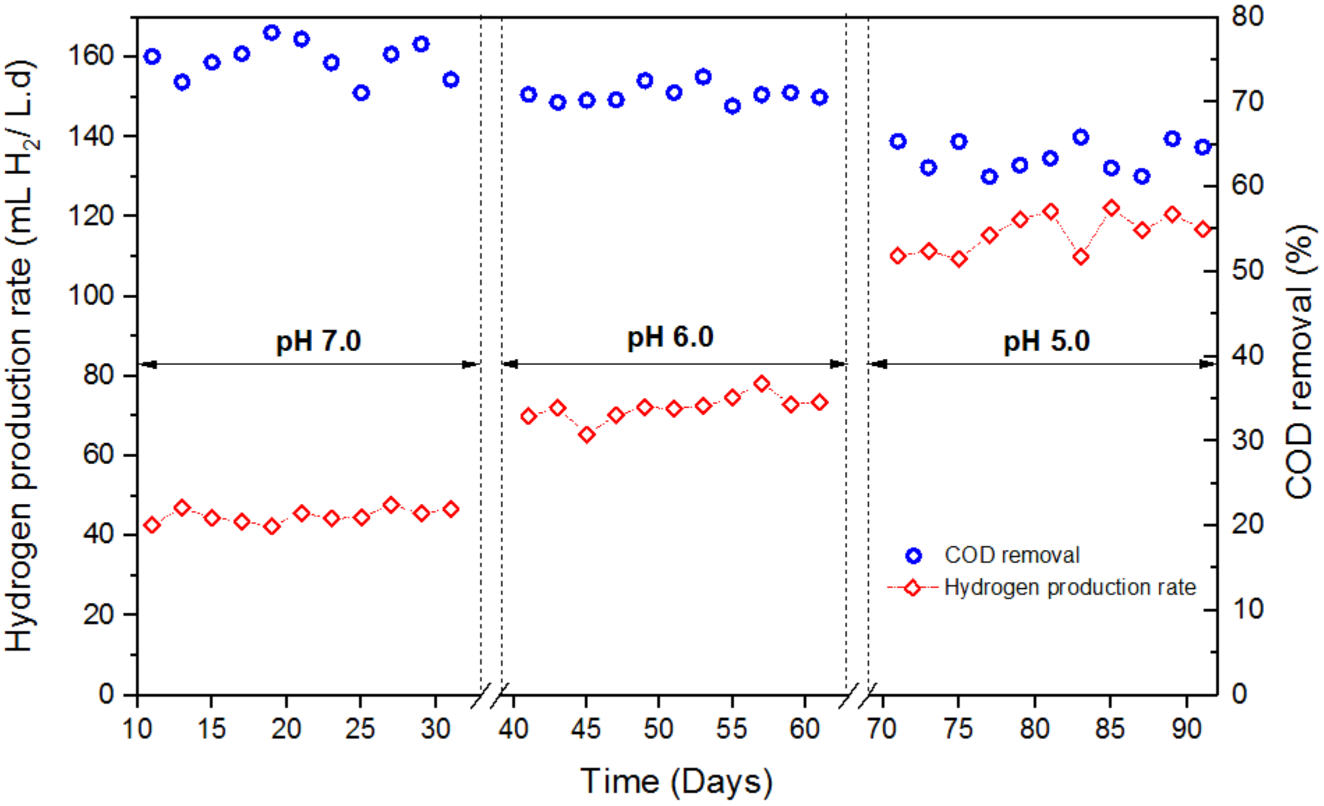


Figure 7.3: Specific hydrogen production rate at different pH

Figure 7.4 depicts a similar trend as the reduction in the AnMBR pH gradually increased the overall yield of biohydrogen produced per gram of COD added into the bioreactor. The graph shows that at pH 7.0 the overall hydrogen yield was 25.89 mL H_2 / g COD $_{added.}$ The overall yield has been observed to increase up to 39.58 and 65.38 mL H_2 / g COD $_{added}$ when the pH was reduced to 6.0 and 5.0, respectively.

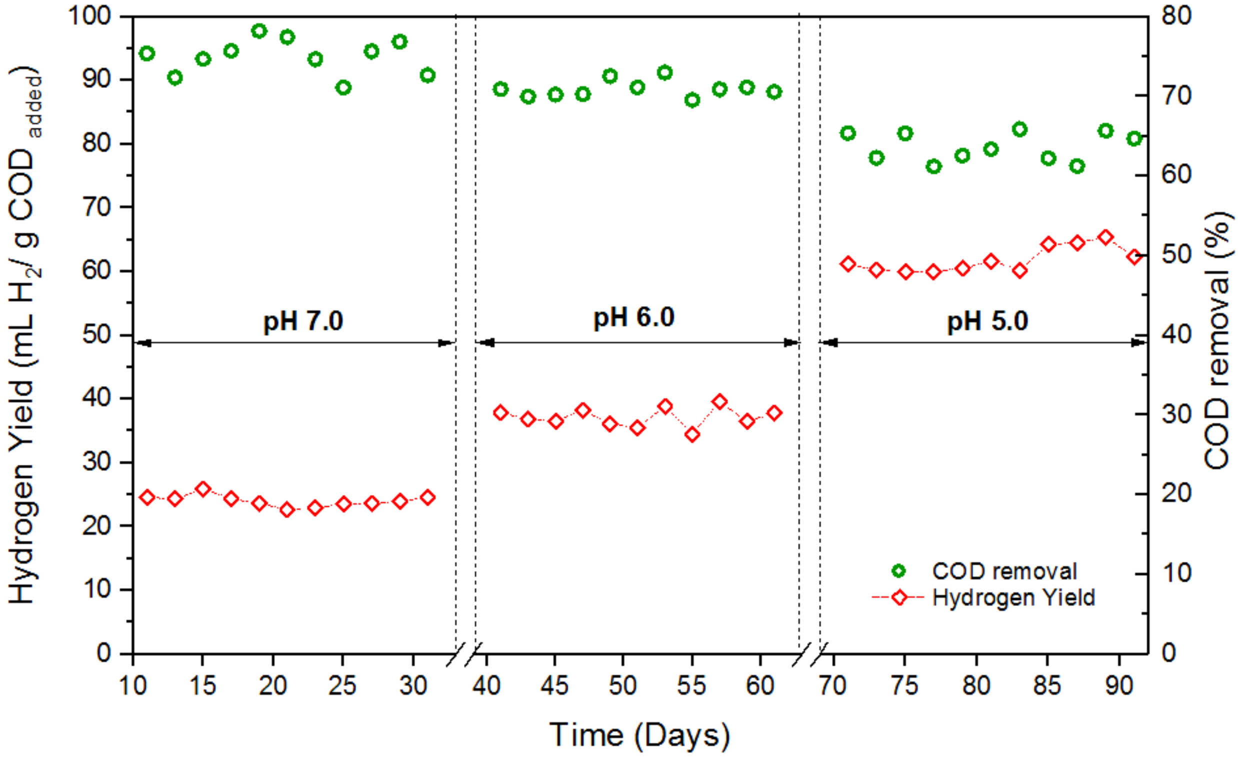


Figure 7.4: Overall yield of biohydrogen at different pH levels

The operating pH directly affected the overall COD removal efficiency of the AnMBR. The highest percentage of COD removal by AnMBR was observed to be 78.21% at pH 7.0. However, when pH was lowered to 6.0 the removal efficiency of COD was observed to be 72.54%. The efficiency further declined to 65.87% when the AnMBR was operating at pH 5.0. Both the amount of VFA production and microbial shock at different pH levels for the reduction of COD removal efficiency. As previously mentioned, the first week of AnMBR operation using a specific level of pH is not listed in Figures 3 and 4. However, it may be assumed the pH shock experienced by the microbial community can be responsible for small amounts of COD being removed.

In addition, the overall efficiency of anaerobic digestion does depend on operating conditions and mainly pH levels, temperature, HRT, and OLR. The overall COD removal efficiency depends on the reactions occurring in four different anaerobic stages. For example, pH 6.5–8.2 has been identified as the optimum value for the best efficiency during methane production.

Conversely, the 5.5 - 6.5 range has been identified as ideal for acidogenesis (Mao et al., 2015). Therefore, it can be concluded that the VFA produced in the second and third stage of the anaerobic process was not consumed by the methanogens at the final stage due to low pH conditions. The portion of VFA that remains soluble in the AnMBR effluent can contribute to increased COD value in the AnMBR effluent. As a result, the overall COD removal efficiency can decrease at pH 5.0 and 6.0. It is worth mentioning here that the reduction of COD does not necessarily reflect the decrease in VFA consumption rate at low pH conditions. The reason may be associated with the fact that the fouling layer created in low pH conditions can retain a certain portion of VFA produced by the system.

During anaerobic digestion, biohydrogen can be produced through the process of acetogenesis when volatile fatty acids are consumed by the acetogens to produce CO_2 and H_2. Consequently, the growth rate of the acetogens can be regarded as a critical factor for the production of biohydrogen in an anaerobic process. Additionally, the hydrogenotrophic methanogens are the major consumers of biohydrogen produced. Suppressing the methanogenic activity is also a critical factor when maximizing the production rate of biohydrogen.

It has been identified through different experiments that the growth rate of methanogens is negatively affected at a pH level below 6.6. As the initial run for the experiment involved operating the AnMBR at pH 7.0, it may be assumed that a certain portion of produced biohydrogen was consumed through the methanogenesis process. As the pH reduced to 6.0 the overall yield of biohydrogen increased up to 40 mL H_2 / g $COD_{feed.}$ This finding implies that the specific methanogenic activity was largely affected when the pH was reduced to 6.0. The highest production rate and yield of biohydrogen in this experiment have been observed at pH 5.0. On the 85th day of AnMBR operation the highest production rate of biohydrogen was observed (122.21 mL H_2/ L. d).

Three different types of acidogenic fermentation can be involved in the production of biohydrogen. CO_2 and H_2 are produced as the by-products along with butyric and acetic acid in butyrate type fermentation. Propionic type fermentation mainly produces propanoic acid with no significant contribution in biohydrogen production whereas ethanol type fermentation mainly produces ethanol an acetic acid along with small amounts of CO_2 and H_2 (Khan et al., 2018). However, the selective production of VFA components shows that butyrate type fermentation in mainly predominant at pH 11.0. As a result, it may be assumed that the high production rate of biohydrogen at pH 5.0 and 6.0 has not been produced through acidogenic fermentation.

For this experiment, acetogenesis may have contributed largely to the production of biohydrogen. The VFA produced in the second stage may have been converted to acetates, CO_2 and H_2. Hydrogen has been produced during this process since the protons act as the final electron acceptors. From our previous study, it has been observed the optimum HRT for biohydrogen production was 6 hrs. As methanogenesis is the slowest among all anaerobic stages, a low HRT worked against the conversion of biohydrogen to methane. Although no selective inhibition was applied, the growth rate of the methanogens may have been affected during the AnMBR operation at pH levels 5.0 and 6.0.

The highest production rate and yield of biohydrogen observed in this experiment have been compared with other anaerobic hydrogen processes in Table 2. It is clearly evident that the highest biohydrogen production rate and yield were observed in a multiple-stage anaerobic bioreactor with separate hydrolysis/acidogenesis and acetogenesis/methanogenesis processes. However, the maximum yield achieved in this study (65.38 ± 3.2 mL H_2 / g COD $_{added}$) can still be considered higher compared to a two-stage anaerobic batch process with PAC.

It has been observed that at an OLR of 2.34 g/L/d a multi-phase anaerobic bioreactor indicated the highest yield and production rate of 75.70 ± 3.98 mL H_2/g COD $_{added}$ and 177.30 ± 14.19 mL H_2 / L. d, respectively. Although the current study has achieved a lower biohydrogen production rate (122.21 ± 39.05 mL H_2/ L. d) and yield (65.38 ± 3.2 mL H_2 / g COD added) compared to multiple stage assembly, the result may be deemed technically successful considering the fact that no selective inhibition was applied for the hydrogen consuming microbes.

Table 7.1: Comparative analysis of hydrogen production rate and yield from different anaerobic processes

System	Substrate	Specific Hydrogen production rate (mL H_2/L/d)	Hydrogen yield (mL H_2/g COD_{added})	Reference
Multi-phase anaerobic reactor	Saline industrial wastewater	65.57 ± 6.03 (0.64 g/L/d OLR)	102.59 ± 7.13	(Ali et al., 2019)
		102.88± 11.01 (1.17 g/L/d OLR)	87.85 ± 6.16	
		177.30 ± 14.19 (2.34 g/L/d OLR)	75.70 ± 3.98	
Two-stage anaerobic fermentation	Beverage wastewater	115.00 ± 42	172.0 ± 65.0	(Lay et al., 2019)
Anaerobic membrane bioreactor	Low-strength synthetic wastewater	122.21 ± 39.05	65.38 ± 3.2	This study
Two-stage anaerobic fermentation	Beverage wastewater	3.20 ± 0.7	18.0 ± 17.0	(Lay et al., 2019)
Two-stage anaerobic batch + PAC	Coffee husks	N/A	55.8	(Santos et al., 2018)
Two-stage (UASB)	cassava wastewater	390	39.83 ml H_2/g $COD_{removed}$	(Intanoo et al., 2016)

Some experiments mentioned in Table 7.1 have been carried out at a HRT lower than 6 hrs. For example, a study involved in biohydrogen and methane production from low-strength beverage wastewater showed a HRT of 2 hrs period for initial hydrogen production stage (Lay et al., 2019). Therefore, a further reduction in HRT can be a future research option based on the current study. However, the application of fouling control measures is important for operating an AnMBR at a HRT shorter than 6 hrs. The current study described a membrane operation cycle of 12 hrs with the pH level at 5.0. A further reduction can cause severe membrane fouling and eventually lead to unstable AnMBR operation. Additionally, a multiple-stage AnMBR can be a potential solution for different HRT values separately for hydrogen and methane production. An economic feasibility assessment based on the initial cost of installation and product revenue earned from biohydrogen might be interesting before the findings are applied in full-scale operation.

7.5 Conclusions

The highest production rate and yield of biohydrogen have been observed at pH 5.0 and 6 hrs HRT. Acetogenic hydrogen production was the dominant hydrogen production pathway in this experiment. The nutrient and COD removal efficiency declined at pH 6.0 and 5.0 with a reduction in membrane operating cycle from 16 to 12 days. A further reduction in HRT and fouling control measures can be applied to maximize the production rate and yield of biohydrogen from low-strength wastewater.

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Chapter 8

Conclusions and Recommendations

8.1 Conclusions

The research study investigated the production of VFA and biohydrogen through the optimization of AnMBR operating conditions. Common operating parameters like HRT, OLR, pH have been optimized in order to focus on specific AnMBR products. As no specific microbial inhibition was applied through this process, the optimal operating conditions can be applied in full-scale operation. The specific findings from this research are:

- A general reduction in HRT can increase the production rate of VFA in an AnMBR. As methanogenesis has been referred to as the slowest among all the stages of anaerobic digestion, the produced VFA cannot be consumed completely by the methanogens in a short HRT. A low HRT limits the exposure of VFA to the methanogens and increases the accumulation of VFA inside the bioreactor. Unlike HRT, an initial increase in the OLR increases the production rate of VFA and the concentration of major VFA components (e.g. acetic acid, propanoic acid, and butyric acid). However, controlling the membrane fouling in a low HRT and high OLR can be particularly challenging as the rate of TMP development in used membrane module were significantly higher at these conditions.

- pH of the anaerobic process was regulated to maximize different VFA components. At low pH conditions, acetic and propanoic type of fermentation is predominant. The percentage of propanoic acid present in the overall VFA mixture was found to be an indication of possible AnMBR acidification. Although low pH conditions increased the production rate of acetic and propanoic acid, the overall VFA yield was maximum at pH 7.0. The reasons attributed to these result implies that at acidic conditions a certain amount of VFA was retained by the fouling layer present in the membrane. At pH 7.0, the membrane fouling rate was minimum, as a result, the least amount of VFA was retained by the fouling layer and the overall yield was maximum for the VFA mixture. An increase in pH in the alkaline zone shifted the predominant metabolic pathway to butyrate type fermentation. Altering pH from the neutral value increased the production of carbohydrates and protein like components which lifted the rate of TMP development and aid membrane fouling. It also reduced the nutrient and COD removal rate of AnMBR, Therefore, the findings from this research can be applied to enhance the concentration of the particular type of VFA production.

- VFA and biohydrogen were produced simultaneously by reducing the HRT of an AnMBR. A general decrease in HRT increased both the production rate of VFA and the yield of biohydrogen produced from each gram of COD. The concentration of individual VFA components increased with decreasing in HRT up to a certain value. Reducing HRT further indicated a lower concentration of major VFA components. The yield of biohydrogen increased slowly at the initial decrease of HRT whereas the overall yield of biohydrogen increased sharply at lower values in HRT. An initial increase in OLR improved both the VFA concentration and biohydrogen yield but reduced membrane operating cycle and nutrient removal efficiency.

- Production of biohydrogen can be optimized by lowering pH in an AnMBR. A reduction in pH into the acidic zone decreased the consumption of biohydrogen and caused an overall increase in the biohydrogen production rate and yield. A low COD and nutrient removal efficiency can be a potential issue for an AnMBR when it is operated at a low pH condition. The carbohydrates present as SMP contributed to the membrane fouling at low pH condition and reduced the duration of membrane operating cycle.

8.2 Recommendations

The research has determined the optimum operating conditions to produce biohydrogen and VFA using low-strength synthetic wastewater in a single stage AnMBR. Further investigations on this topic can provide a better insight into the production mechanism of VFA and biohydrogen and improve the performance of AnMBR in terms of COD and nutrient removal. The following points can be considered as future research direction on this topic:

[1] Further study on pilot and/or full-scale AnMBR for VFA and biohydrogen production using wastewater from municipal or industrial sources is essential;

[2] The bacterial community can be analysed along with the change in HRT, OLR, and pH to help in better understanding the metabolic pathway for VFA and biohydrogen production in an anaerobic process;

[3] Analysis of membrane fouling behaviour through the measurement of SMP and EPS will be useful in controlling the membrane fouling for full-scale industrial application;

[4] Effect of temperature on VFA and biohydrogen can be investigated in an AnMBR. The findings will be integrated with the membrane fouling performance and COD, the nutrient removal efficiency of the AnMBR;

[5] For municipal wastewater treatment, AnMBR performance should be investigated in mass transfer, film and intra-particle diffusion, and different surface reactions caused by trace organic contaminants.

[6] Different synthetic feed solution can be used by varying the ratio of C: N: P. The results will indicate the performance of AnMBR in industrial operation where the nutrient content is variable in the feed solution;

[7] Environmental impacts of an AnMBR should have been assessed through Overall Energy Balance (OEB) and Life Cycle Assessment (LCA) when it is configured to produce VFA or biohydrogen; and

[8] Production of VFA and biohydrogen can be investigated in a multiple-stage AnMBR with separate hydrolysis/ acidogenesis and acetogenesis/methanogenesis. The overall energy balance for single and multiples stage AnMBR could be compared along with the product revenue estimation.

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Appendix

Publications

1. **Book Chapter:** Ngo, H. H., **Khan, M. A**., Guo, W., Pandey, A., & Lee, D. J. (2019). Non-conventional Anaerobic Bioreactors for Sustainable Wastewater Treatment. In Water and Wastewater Treatment Technologies (pp. 265-295). Springer, Singapore.
2. **Journal – 1: M.A. Khan**, H.H. Ngo, W.S. Guo, Y.W. Liu, J.L. Zhou, J. Zhang, S. Liang, B.J. Ni, X.B. Zhang, J. Wang, Comparing the value of bioproducts from different stages of anaerobic membrane bioreactors, Bioresource Technology, Volume 214, 2016, Pages 816-825, ISSN 0960-8524, http://dx.doi.org/10.1016/j.biortech.2016.05.013
3. **Journal -2** : **M.A. Khan,** H.H. Ngo, W.S. Guo, Y. Liu, L.D. Nghiem, F.I. Hai, L.J. Deng, J. Wang, Y. Wu, Optimization of process parameters for production of volatile fatty acid, biohydrogen and methane from anaerobic digestion, Bioresource Technology, Volume 219, 2016, Pages 738-748, ISSN 0960-8524, http://dx.doi.org/10.1016/j.biortech.2016.08.073
4. **Journal – 3: Khan, M.A**., Ngo, H.H., Guo, W., Liu, Y., Zhang, X., Guo, J., Chang, S.W., Nguyen, D.D., Wang, J. 2018. Biohydrogen production from anaerobic digestion and its potential as renewable energy. Renewable Energy, 129, 754-768. http://dx.doi.org/10.1016/j.renene.2017.04.029.
5. **Journal -4: Khan, M.A**., Ngo, H.H., Guo, W., Liu, Y., Chang, S.W., Nguyen, D.D., Nghiem, L.D., Liang, H. 2018. Can membrane bioreactor be a smart option for water treatment? Bioresource Technology Reports, 4, 80-87. https://doi.org/10.1016/j.biteb.2018.09.002
6. **Journal - 5: Khan, M.A.,** Ngo, H.H., Guo, W., Liu, Y., Nghiem, L.D., Chang, S.W., Nguyen, D.D., Zhang, S., Luo, G., Jia, H. 2019. Optimization of hydraulic retention time and organic loading rate for volatile fatty acid production from low strength wastewater in an anaerobic membrane bioreactor. Bioresource Technology, 271, 100-108.
7. **Journal – 6: Khan, M.A**., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Varjani, S., Liu, Y., Deng, L., Cheng, C. 2019. Selective production of volatile fatty acids at different pH in an anaerobic membrane bioreactor. Bioresource Technology, 283, 120-128.

Awards

Best Student Oral Best Presentation Award for University of Technology Sydney at International Symposium on Advanced Membrane Bioreactors for Environmental Sustainability (IBA- AMBRES 2018) April 15-18, 2018, China.

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Best Student Oral Presentation Award

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International Symposium on
Advanced Membrane Bioreactors for Environment Sustainability

April 15-18, 2018
Tianjin, China

Prof. Huu Hao Ngo
Conference Chair

Prof. Yufeng Zhang
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