AMERICAN UNIVERSITY OF BEIRUT

ANAEROBIC MEMBRANE BIOREACTOR COUPLED WITH GAC FLUIDIZATION FOR WASTEWATER TREATMENT

by
LEA GEORGES ISSA

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Engineering
to the Department of Civil and Environmental Engineering
of the Faculty of Engineering and Architecture
at the American University of Beirut

Beirut, Lebanon
February 2020

AMERICAN UNIVERSITY OF BEIRUT

ANAEROBIC MEMBRANE BIOREACTOR COUPLED WITH GAC FLUIDIZATION FOR WASTEWATER TREATMENT

by

LEA GEORGES ISSA

Approved by:

Dr. Mutasem El- Fadel, Chairperson — Advisor
Department of Civil and Environmental Engineering, AUB

Dr. Pascal E. Saikaly, Associate Professor — Member of Committee
Biological and Environmental Science and Engineering Division, KAUST

Dr. Ibrahim Alameddine, Assistant Professor — Member of Committee
Department of Civil and Environmental Engineering, AUB

Date of thesis defense: February 13, 2020

AMERICAN UNIVERSITY OF BEIRUT

THESIS, DISSERTATION, PROJECT RELEASE FORM

Student Name: Issa Lea Georges

Master's Thesis Master's Project Doctoral Dissertation

I authorize the American University of Beirut to: (a) reproduce hard or electronic copies of my thesis, dissertation, or project; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

■ I authorize the American University of Beirut, to: (a) reproduce hard or electronic copies of it; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes
after: **One ---- year from the date of submission of my thesis, dissertation, or project.**
Two ---- years from the date of submission of my thesis, dissertation, or project.
Three ---- years from the date of submission of my thesis, dissertation, or project.

Lea Issa 21-02-2020

Signature Date

ACKNOWLEDGMENTS

I would like to thank my advisor Prof. Mutassem El-Fadel for his great guidance, help, encouragement and advice through the preparation phase, experiment and writing process of this thesis.

I am also thankful for my thesis committee members Dr.Pascal Saikaly and Dr. Ibrahim Alameddine for their help and feedback throughout all this work.

I remain grateful to Dr.Krishna Katuri for his help in setting up the system and teaching me how to operate it and his continuous help and support throughout all the experimental process.

I would like to thanks Mr.Helmi El Khatib , Mr.Ramez Zayyat , Mr.Joseph Dawoud , Ms. Dima El Hassaniah and Ms. Rania Shatila for their help and assistance during the experimental program.

Special thanks goes to Ms.Olga El Kik, my experimental and writing partner whom without none of this could be possible.

Also, I want to thanks my family for their continuous moral support and understanding through the duration of my study.

Lastly, special thanks are extended to Dar Al-Handasah (Shair & Partners) for its support to the graduate programs in Engineering at the American University of Beirut and the support of the Center Competitive Funding Program (FCC/1/1971-05-01) from King Abdullah University of Science and Technology (KAUST) to P.E.S for this work.

AN ABSTRACT OF THE THESIS OF

Lea Georges Issa for Master of Engineering
Major: Environmental and Water Resources Engineering

Title: Anaerobic Membrane bioreactor coupled with GAC fluidization for wastewater treatment.

An experimental program consisting of the operation of an AnFMBR and AnFMBR-MEC reactors was conducted for a period of 273 days. The work done consisted of gas, fouling, energy, effluent and microbial analysis. The first part of the results which includes the two AnFMBR reactors performance will be presented in Lea Issa's thesis titled Anaerobic Membrane bioreactor coupled with GAC fluidization for wastewater treatment. The second part of the results which includes the AnFMBR-MEC reactors performance will be presented in Olga El Kik's thesis.

The membrane technology has evolved into an effective treatment technology, with the anaerobic membrane bioreactor (AnMBR) offering a potential for energy sustainability, pollution control, and waste management. However, these systems are still associated with several operational challenges such as membrane fouling and loss of energy through dissolved methane. This paper aims at improving the AnMBR process by proposing a new configuration with a hollow fiber membrane (HFM) and Granular Activated Carbon (GAC) fluidization. The configuration was operated at room temperature and was fed with synthetic wastewater for a period of 264 days. Two identical AnFMBRs were operated at a hydraulic retention time (HRT) of 1.5 day and an organic loading rate (OLR) of 0.43-0.31 Kg substrate/m^3.day. The membrane flux was set to 6.5 L/m^2/h throughout the experiment and the transmembrane pressure (TMP) was around 5 KPa after 87 days of start-up. TMP increased slightly after that period and reached a TMP value of 10 kpa by day 134 for AnFMBR1 and day 166 for AnFMBR2 without any chemical cleaning for the membrane which is considered a long period compared to reported literature. The methane yield of the two AnFMBRs was on average 0.26 and 0.13 L/g.COD removed at an OLR of 0.43 and 0.31 Kg substrate/m^3.day respectively. The COD removal ranged between 80-99 % for both reactors. The energy needed to operate the AnFMBR (0.06 kWh/m^3) was significantly less than that required in an AnMBR system that uses gas sparging as a fouling control mechanism.

CONTENTS

ILLUSTRATIONS

Figure Page

TABLES

ABBREVATIONS

AnFMBR	Anaerobic Fluidized Membrane Bioreactor
AnMBR	Anaerobic Membrane Bioreactor
AnFMBR-MEC	Anaerobic Membrane Bioreactor coupled with Micro Electrolysis Cell
CAS	Conventional Activated Sludge
COD	Chemical Oxygen Demand
CSTR	Completely Stirred Tank Reactor
DNA	Deoxyribonucleic Acid
GAC	Granular Activated Carbon
GC	Gas Chromatographer
HFM	Hollow Fiber Membrane
HRT	Hydraulic Retention Time
MBR	Membrane Bioreactor (aerobic)
OLR	Organic Loading Rate
SEM	Scanning Electron Microscope
SRT	Solid Retention Time
TMP	Transmembrane Pressure

CHAPTER I
INTRODUCTION

Treatment technologies as the Membrane Bioreactors (MBR) attracted remarkable interests during the last two decades (Lin et al., 2013, Judd, 2008) due to their effectiveness in removing pollutants and degrading very small particles (Malaeb et al., 2013, Santos et al., 2011). MBRs are made up of two parts, a bioreactor similar to a Conventional Activated Sludge (CAS) and a membrane which replaces the settling tank in CAS. The biodegradation of organic waste occurs inside the bioreactor and along the membrane biofilm with filtration of the treated water from biomass and microorganisms happening through physical processes at the membrane level (Ahmed and Lan, 2012). While additional costs have reportedly been attributed to the operation and maintenance of MBRs (Hashisho and El-Fadel, 2016), they exhibit several advantages that can offset these costs including greater biomass retention and consequently better quality effluents, faster loading rates, reduced reactor size, growth of slow-developing microorganisms and less residual sludge due to lower solid retention times (SRTs). MBRs can be categorized based on the type of membrane used or based on its location with respect to the bioreactor. Regarding the membrane type, it is usually in the form of a flat sheet, hollow fiber, or tubular structure among which hollow fiber is reportedly the most efficient economically (Hashisho and El-Fadel, 2016; Lin et al., 2013). As for the membrane location with respect to the bioreactor, two arrangements are common 1) side-stream arrangement, where the membrane is outside the reactor, that needs a high crossflow velocities using a recirculation pump to overcome the decline in flux due to fooling and 2) submerged arrangement, where the membrane is fully immersed in the bioreactor and is less demanding in terms of energy and space(Ahmed and Lan, 2012; Bohdziewicz et al., 2008; Hashisho and El-Fadel, 2016; Lin et al., 2013). MBRs can also be categorized based on material of the membrane: ceramic, metal, or polymeric material (Lin et al., 2013).

In the context of water reclamation and reuse, MBR systems have evolved to encompass the anaerobic MBR (AnMBR) that consumes less energy than the aerobic system and produces methane as a renewable energy source (Yoo et al., 2012). Anaerobic processes tend to be less popular because their corresponding microorganisms have a slower growth rate and are difficult to retain inside conventional bioreactors. However, in AnMBR systems, the microorganisms can be better retained by the small pores of the membrane (Lin et al., 2013, Chen et al., 2016). In AnMBRs, the advantages of anaerobic processes and the MBR technology are combined to improve biomass retention and effluent quality resulting in a smaller footprint, lower sludge generation (low biomass yield), and greater net energy production (Lin et al., 2013).

The effectiveness of the AnMBR technology has been tested on various types of wastewater including synthetic wastewater, food processing wastewater, industrial wastewater, high-solids-content waste streams, and municipal wastewater (Ozgun et al., 2013, van Lier, 2008). In recent years, the use of AnMBRs has increased (Lin et al., 2013, An et al., 2009, Lew et al., 2009, Chang, 2014) in treating municipal wastewater, normally characterized with a low organic strength (Ozgun et al., 2013, van Lier, 2008). Yet, the long-term operation of AnMBR for the treatment of municipal wastewater under ambient temperature needs further evaluation and optimization (Yoo et al., 2012).

While the AnMBR has several advantages over other aerobic and anaerobic treatment techniques, biofouling caused by the deposition of macromolecules (organic, inorganic and microbiological substances) either on the membrane surface or inside its pores remains a major limitation (Wiszniowski et al., 2006, Trzcinski and Stuckey, 2016). Many fouling control strategies have been tested on membrane bioreactors such as using vibrating membranes (Kola et al., 2012), applying ultrasonic waves (Sui et al., 2008), or adding chemicals or adsorbents to reduce soluble foulants concentration (Akram and Stuckey, 2008). Scouring techniques have also been developed for the same purpose including pulse gas scouring (Aslam et al., 2019) and intermittent gas scouring (Buer and Cumin, 2010). In general, the energy consumption to reduce fouling can be high (Aslam et al., 2014). The use of granular activated carbon (GAC) as a fluidized media to support the active biofilm and control membrane fooling through its scouring effect on the membranes has shown promise (Aslam et al., 2014, Kim et al., 2011). Unlike gas-sparging or cross-flow filtration mode, membrane fluidization by GAC particles in the reactor results in a relatively low energy requirement (Aslam et al., 2014).

In the same context, the treatment of low strength wastewater in a two stage system consists of an anaerobic fluidized bioreactor (AFBR) followed by an anaerobic fluidized membrane bioreactor (AFMBR) has been shown to be successful (Shin et al., 2014, Aslam et al., 2018, Lee et al., 2015). Two stage system are used to help meet stringent effluent requirements (Kim et al., 2011). However, the two stage system requires a higher foot print and energy for operation. A single stage AFMBR have reportedly performed as well as the staged AFMBR system offering the advantage of reducing costs for construction and maintenance (Bae et al., 2014). However, fluidization for a single stage system is done for the whole reactor volume, therefore a higher energy demand is needed for the reactor's operation as compared to two stage systems. As such, Gao et al. (2014) maintained GAC fluidization in the outer chamber of a single stage integrated AFMBR with the membrane positioned in the inner chamber (in the middle) to reduce both foulants and energy consumption. GAC souring of the membrane was not used in that study (Gao et al., 2014).

The aim of the current study was to develop a new AnFMBR configuration to **mitigate fouling** and test its **reproducibility** by running two identical systems in parallel under the same operating conditions. The proposed system is simple and occupies a small footprint. Also, the energy demands for the reactor operation is expected to be low compared with a two/single stage system reported for AFMBR systems in the literature. The outer loop of the reactor is designed to perform as an anaerobic biofilm bed reactor (ABBR) and the inner loop is designed to serve as an anaerobic fluidized membrane bioreactor (AnFMBR) with GAC as a carrier. Hence, GAC fluidization is restricted to the inner loop for effective membrane scouring and to minimize the energy needed for GAC fluidization. The system was tested under two different organic loading rates (OLRs) and operated in a continuous mode using an acetate-rich synthetic medium, the typical precursor for methanogenic bacteria (Zhang et al., 2018). DNA sequencing was applied at two sampling events (two different OLRs) on the GAC and the suspension liquid, and on the HFM by the end of operation to explore the bacterial community.

CHAPTER II

MATERIALS AND METHODS

A. Reactor configuration and operation

The experiment consisted of two identical Anaerobic Fluidized Membrane Bioreactors (AnFMBR) run in parallel under the same operating conditions as shown in Figure 1. The Plexiglas reactors had a total volume of 1.5 L and a working volume of 1.43 L. The configuration of the system (see Figure 2) consisted of two concentric cylinders of 40 cm height with respective inner diameters of 6.4 and 3.5 cm. The internal tube, perforated from top with 5mm holes, enclosed the hollow fiber membrane and 55 g of Granular Activated Carbon (GAC) (10 cm packed height). The PVDF hollow fiber membrane (2.0 mm outside diameter, 0.8 mm inside diameter, 0.1 µm pore size, 40 cm height, Kolon Inc., South Korea), having a total membrane surface of 0.005715 m2, was submerged in the reactor. The top of the membrane was connected to a peristaltic pump (model no. 7528-30, Masterflex, Vernon Hills, IL), giving an effluent of 1L/day. The GAC (Coconut Activated Carbon Granules, 0.8 mm diameter, Calgon Carbon (Catalog # 207C), USA) that served as support for bacterial growth and fluidization medium was soaked in water overnight before use to remove any residuals. GAC fluidization at a recirculation rate of 0.75 L/min resulted in a 60-70% bed expansion which can help mitigate fouling.

The reactors were filled with a synthetic feed (Table 1) containing 820 mg/l sodium acetate as a source of carbon and energy. Each reactor was seeded with 1 L of cow manure solution and 0.1 L of anaerobic sludge (from a laboratory scale AnMBR). Manure solution was prepared by mixing 500 g of cow manure (collected from a dairy farm) with 2 L of distilled water. The reactors were operated at room temperature (25 °C) in a continuous flow mode at an HRT of 1.5 days with a low-organic strength synthetic wastewater (COD equivalent of 640-470 mg/L) to mimic a COD concentration close to municipal wastewater. The feed tank was purged with pure nitrogen gas for 30 min to remove oxygen, then stored in the refrigerator (at 4°C) and isolated from the light to avoid changes in the feed characteristics and algal growth. The feed was pumped using a peristaltic pump at a feeding rate of 1L/day. After 88 days of startup, the sodium acetate concentration in the feed was set at 600 mg/l for the remaining operation period.

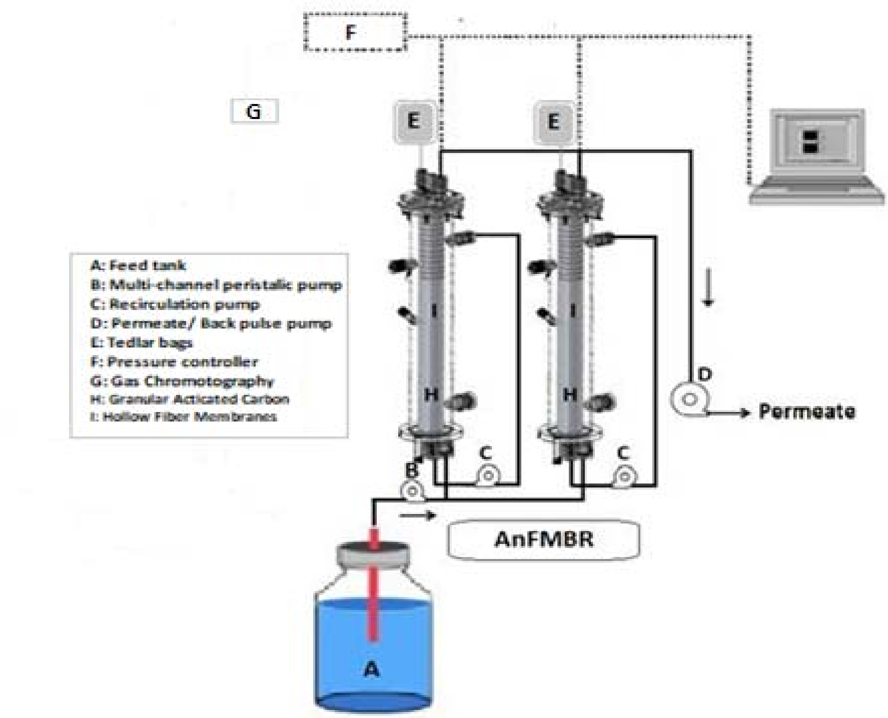

Figure 1: AnFMBR Setup

Table 1: Synthetic wastewater composition (Wang et al., 2013, Katuri et al., 2010)

Composition	Concentration
Ammonium Chloride (NH4Cl)	1.5 g/L
Sodium Phosphate Dibasic (Na2HPO4)	0.6 g/L
Potassium Chloride (KCl)	0.1 g/L
Sodium Acetate (C2H3NaO2)	0.82 or 0.6 g/L
Sodium Bicarbonate (Na2HCO3)	2.5 g/L
Trace Elements [a]	10 ml/L
Vitamin Solution [b]	10 ml/L

[a] Composition of the Trace Elements solution (in g/L): Nitrilotriacetic acid:1.50, $MgSO_4 \cdot 7H_2O$:3.00, $MgSO_4 \cdot H_2O$:0.50, NaCl:1.00, $FeSO_4 \cdot 7H_2O$:0.10, $CoSO_4 \cdot 7H_2O$:0.18, $CaCl_2 \cdot 2H_2O$:0.10, $ZnSO_4 \cdot 7H_2O$:0.18, $CuSO_4 \cdot 5H_2O$:0.01, $KAl(SO_4)_2 \cdot 12H_2O$:0.02, H_3BO_3:0.01, $Na_2MoO_4 \cdot 2H_2O$:0.01, $NiCl_2 \cdot 6H_2O$:0.03, $Na_2SeO_3 \cdot 5H_2O$:0.3mg, Distilled water:1000mL

[b] Composition of the Vitamin solution (in mg/L): Biotin:2.00, Folic acid:2.00, Pyridoxine:10.00, Thiamine-HCl·$2H_2O$:5.00, Riboflavin:5.00, Nicotinic acid:5.00, D-Ca-pantothenate:5.00, Vitamin B12:0.10, p-Aminobenzoic acid:5.00, Lipoic acid: 5.00, Distilled water: 1000mL

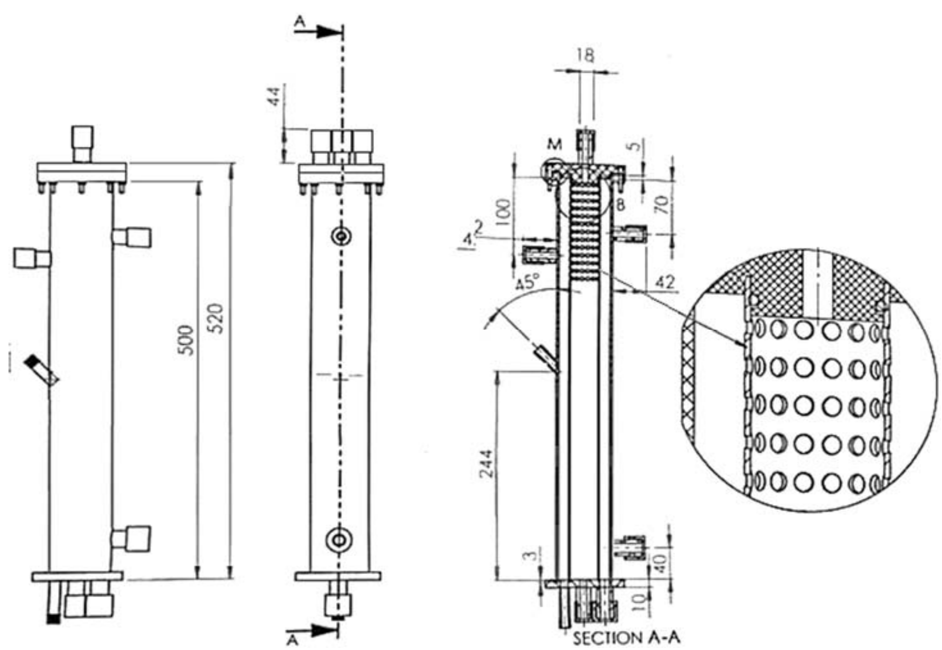

Figure 2: Reactor Configuration

B. Analysis

1. COD Analysis

The treatment efficiency was evaluated in terms of soluble COD (5220 D, HACH, Loveland, Co.) removal. Feed and permeate samples were collected on a regular basis. The samples were filtrated using 0.2 µm pore diameter syringe filter before the COD test was conducted (PTFE, Kinesis Ltd.).

2. Biogas Analysis

Biogas from the two reactors were collected in gas bags (Calibrated Instruments Inc.) that were tested 2-3 times per week using an SRI 310C Gas Chromatograph (GC) to detect H2, N2, CH4 and CO2 volumes. A 6´Molecular Sieve and 3´Silica Gel column with argon as carrier gas were used to detect H2, N2, and CH4 with an oven column temperature of 60 °C. For CO2, a 3´ HayesSep D was used with argon with an oven column temperature of 100 °C. Samples (200 µl volume) were taken using gas tight syringes from the headspace of each reactor, and from the gas bag before and after injection of 10 ml N2. At the end of each analysis, the gas bags were changed after sparging the reactors' headspace with N2 for 5-10 minutes to wash out any remaining gases that might affect the next reading. The average of the two or three weekly readings was presented.

3. SEM Imaging

Fouled and virgin porous hollow fiber membranes were sampled from the reactor and stored overnight in a glutaraldehyde fixative solution (2 % in 50 mM phosphate buffer, pH 7.0). Following fixation, samples were dehydrated using a series of graded alcohol solutions (10 to 100%; 10 min at each dilution). The samples were then oven dried for 30 min at 30 °C. Dried samples were mounted onto an aluminum stub using thin double-sided copper tape. Samples were sputter-coated with iridium layer (5 nm thick) for 40 s at 25 mA current (Quorum Q150T ES) in an argon atmosphere prior to SEM imaging (Quanta 600). Samples were analyzed for their surface topography and composition at an accelerating voltage of 5 KV at a spot size of 3 and beam current of 3 µA.

4. DNA Extraction and analysis

DNA samples were collected from the AnFMBR reactors suspension, the GAC, and the Hollow Fiber Membranes. They were stored at -20°C and then shipped to Denmark for subsequent analysis (DNASENSE, Denmark). The DNA extraction was performed using the standard protocol for FastDNA Spin kit for Soil (MP Biomedicals, USA) with the following exceptions: 500 L of sample, 480 L Sodium Phosphate Buffer and 120 L MT buffer were added to a Lysing Matrix E tube. Bead beating was performed at 6 m/s for 4x 40s (Albertsen et al., 2015). The forward and reverse tailed primers were designed according to Illumina (2015) and contain primers targeting the Archaea and Bacteria, 16S rRNA gene region V4: [515F] GTGYCAGCMGCCGCGGTA and [805R] GACTACHVGGGTATCTAATCC (Ye et al., 2016). PCR was conducted with the following program: Initial denaturation at 95 _C for 2 min, 8 cycles of amplification (95 _C for 20 s, 55 _C for 30 s, 72 _C for 60 s) and a final elongation at 72 _C for 5 min). The DNA concentration was measured using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using Tapestation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of sequencing libraries. The purified sequencing libraries were pooled in equimolar concentrations and diluted to 2 nM. The samples were paired-end sequenced (2x300 bp) on a MiSeq (Illumina, USA) using a MiSeq Reagent kit v3 (Illumina, USA) following the standard guidelines for preparing and loading samples on the MiSeq. >10% PhiX control library was spiked in to overcome low complexity issues often observed with amplicon samples. Forward and reverse reads were trimmed for quality using Trimmomatic v. 0.32 (Bolger et al., 2014) with the settings SLIDINGWINDOW:5:3 and MINLEN: 225. The trimmed forward and reverse reads were merged using FLASH v. 1.2.7 (Magoč and Salzberg, 2011) with the settings -m 10 -M 250. The trimmed reads were dereplicated and formatted for use in the UPARSE workflow (Edgar, 2013). The dereplicated reads were clustered, using the usearch v. 7.0.1090 -cluster_otus command with default settings. OUT abundances were estimated using the usearch v. 7.0.1090 -usearch_global command with -id 0.97 –maxaccepts 0 -maxrejects 0. Taxonomy was assigned using the RDP classifier as implemented in the parallel_assign_taxonomy_rdp.py script in QIIME (Caporaso et al., 2010) , using –confidence 0.8 and the SILVA database, release 132 (Quast et al., 2012). The results were analyzed in R v. 3.5.1 (R Core Team, 2017) through the Rstudio IDE using the ampvis package v.2.4.10 (Albertsen et al., 2015).

5. *TMP measurements*

A pressure transducer from Cole Parmer Instruments Inc. was installed in the permeate collection loop to measure the transmembrane pressure (TMP) for the membranes filters and the values were recorded on a computer every 10 seconds using a data acquisition device (LabJack U6, LabJack Corporation, Lakewood, CO).

6. Energy Requirements and Production

The AnMBRs offer the advantage of energy production in the form of biogas (Equations 1-2). Concurrently, AnMBRs require energy for recirculation and filtration (Equation 3) with energy efficiency expressed by Equation 4 (Katuri et al., 2014).

$$n = v/TR \quad (1)$$

$$W_{CH4}(Kj) = n_{CH_4} \Delta_{CH_4} \quad (2)$$

$$We(Kwh) = \left(\frac{\frac{Q1\delta E1}{1000} + \frac{Q2\delta E2}{1000}}{Q_2} \right) * V \quad (3)$$

$$n_e = \frac{w_{CH_4}}{W_e * 3600} \quad (4)$$

Where n_{CH_4}: number of methane moles produced, v: volume of gas (L), T: Temperature (K), R: Gas constant (0.08206 L.atm/K.mol), Δ_{CH4}: energy content based on the heat of combustion (891 kJ/mol), Q_1: Reactor Recycle Rate (m^3/s), δ: unit weight of water (9800 N/m^3), E_1: measured hydraulic pressure head loss through the system (m), Q_2: Permeate Flow Rate (m^3/s), E_2: Head Loss due to TMP (m), V: Total Volume pumped (m^3), n_e: energy efficiency, and 3600: conversion factor from Kwh to Kj.

CHAPTER III
RESULTS AND DISCUSSION

A. Operation

The two reactors were inoculated with cow manure and sludge and operated at room temperature at an initial sodium acetate equivalent concentration of 820 mg/l that was regulated down to 600 mg/l (OLR of 0.43 Kg of substrate / m^3.day) and an HRT of 1.5 days. During acclimatization (Figure 3), gas analysis was conducted for the headspace. When the volume of gas generation stabilized, gas bags were used with analysis twice. The reactors took 65 days to reach steady state in terms of CH_4 generation and COD removal, consistent with a period of 63 days reported by Gao et al. (2014) in a study treating synthetic wastewater (COD=320 mg/L) using an IAFMBR.

The monitored operational phase started on day 88 with substrate and gas removal performance presented in Figure 4. At start-up and during the first few days of operation, the organic loading rate (OLR) was 0.43 Kg substrate/m^3.day (equivalent to an acetate concentration of 600 mg/l) to sustain biomass growth. During this period (Phase B), COD removal reached 95% with a yield of 0.277 L/g.COD removed for AnFMBR 1 and 99% with a gas yield of 0.238 LCH4/g.COD removed for AnFMBR2, consistent with studies treating low strength wastewater having an OLR between 0.4-1 Kg COD/m^3.day that reported a gas yield of 0.24 L CH_4/ g of COD (Martinez-Sosa et al., 2011, Chang, 2014, Lin et al., 2013).

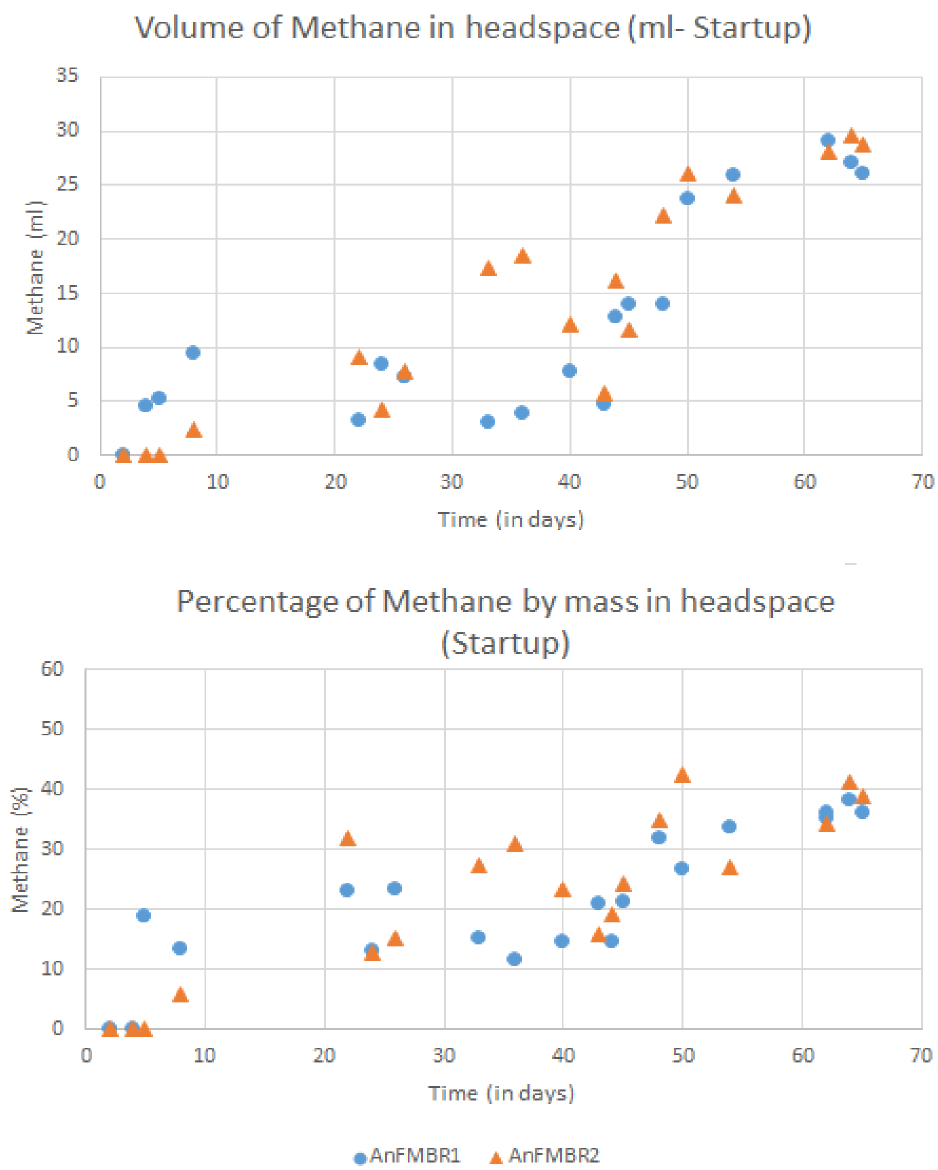

Figure 3: Biogas for AnFMBR ractors at start-up

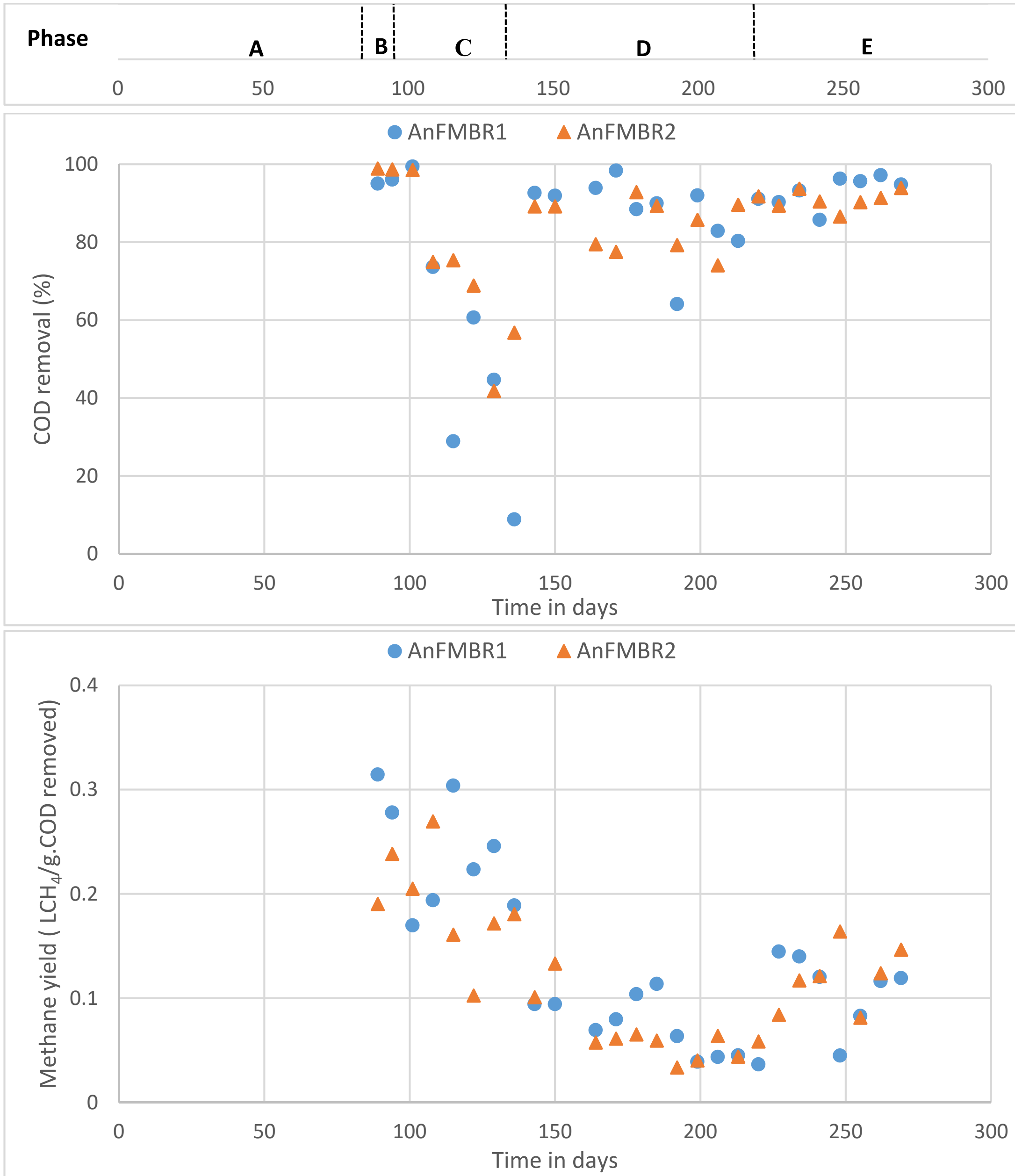

Figure 4: COD and Gas Generation at different stages:

Phase A: day 1-88; start-up at OLR = 0.43 Kg substrate./m^3.day
Phase B: day 89-98; operation at OLR = 0.43 Kg substrate./m^3.day
Phase C: day 99-147; OLR = 0.31 Kg substrate./m^3.day
Phase D: day 148-217; OLR varied; fluidization problem and unstable gas generation
Phase E: day 218-273; OLR 0.31 Kg substrate./m^3.day

On day 99, OLR was decreased to 0.31 kg of substrate/m^3.day and for a period of 6 weeks (Phase C), COD removal varied between 9-74% for AnFMBR1 and between 41 - 75% for AnFMBR2. This decrease is mainly due to the time needed for methanogens to adapt and proliferate at this new organic loading rate. After this period and till the end of the experiment (Stage D and E), the COD removal was in the range of 80-97 % for both reactors (average value of 90% AnFMBR1 and 87% AnFMBR2). Studies treating low strength wastewater in an AnMBR reported a COD removal of 88% (Lew et al., 2009), 90% (Martinez-Sosa et al., 2011) and a range between 84-94% (Lin et al., 2013). During day 99 to 147 (Phase C) C, the yield decreased slightly (AnFMBR1: 0.3-0.18 L CH_4/ g of COD and AnFMBR 2: 0.26-0.18 L CH_4/ g of COD). After this period (Stage D), a further decrease in methane yield values occurred, probably due to time needed for the substrate-competing bacteria to adapt to this new OLR. However, this decrease was further aggravated due to a fluidization problem that happened between day 183 and 217. Following that decrease, the two reactors recovered with similar methane yield values averaging around 0.13 L CH4/ g of COD after day 220 (Stage E) (0.128 L CH4/ g of COD for AnFMBR1 and 0.134 L CH4/ g of COD for AnFMBR2). Table 2 compares the performance of AnFMBR1 and AnFMBR2 during operation. COD, gas, TMP, energy and microbial communities results of the two reactors are very close which proves the reproducibility of our system.

Table 2: Performance of AnFMBR1 vs. AnFMBR2

Reactor	***AnFMBR1***	***AnFMBR2***
COD removal (%)	90%	87%
Gas results (LCH4/g.COD)	OLR 1: 0.277 OLR 2: 0.128	OLR 1: 0.238 OLR 2: 0.134
TMP Results (without any type of chemical cleaning)	196 days (30 Kpa) 78 Kpa (@ the end of the experiment)	188 days (30 Kpa) 84 Kpa(@ the end of the experiment)
Energy needed (kWh/m^3)	0.0611	0.064
Microbial Community Structure	**Dominant bacteria** classes were *Proteobacteria, Bacteroidetes, Firmicutes, Synergistetes, and Actinobacteria.* For **archaea**, *acetoclastic methanogen*s dominated over *hydrogenotrophic methanogens*.	

B. Fouling

Membrane fouling is a concern in any membrane bioreactor as it increases energy consumption for filtration and its operating costs associated with membrane cleaning and maintenance. Figure 5 shows the transmembrane pressure of the two reactors over the period of reactors operation. Both AnFMBR reactors were operated at an effluent flux around 6.5 LMH and the TMP values were around 5 KPa followed by 88 days of operation at an OLR of 0.43 kg of substrate/m3.day. After switching the reactors operation to an OLR of 0.31 kg of substrate/m3.day, the TMP of AnFMBR 1 and 2 increased slightly to reach 10 Kpa at day 134 and day 166 respectively (Table 2). It has been reported that at a pressure of 30 Kpa, membrane cleaning processes, as backwashing or relaxation techniques, are used in order to mitigate fouling(Bae et al., 2014). However, this value was reached after 196 (AnFMBR1) and 188 (AnFMBR2) days. One study consisting of two stages having a wastewater similar to this study as it is a synthetic medium with an average COD of 513 mg/L and the membrane pore size was also 0.1 μm. The first stage was an anaerobic fluidized bed bioreactor (HRT 2-2.8 h) and was followed by another anaerobic fluidized bed reactor with membrane (HRT 2.2 h)(Kim et al., 2011). At first, when the flux rate was 7 L/m2/h, TMP stabilized at 3 Kpa and then the flux was increased to 10 L/m2/h, TMP values reached 5 to 7 Kpa during the first 20 days. After 40 days, TMP increased rapidly to 18 Kpa. In order to mitigate fouling, backwashing was done on day 40 and chemical cleaning was performed on day 54 and 87 (chemical cleaning performed when TMP increased above 35Kpa) (Kim et al., 2011). Although the membrane module in this study was placed in the second stage as a post-treatment, the TMP results were higher than that presented in our experiment which consisted of a single stage system. Those results proved that the new configuration of the AnFMBR system helped in controlling fouling without any type of chemical cleaning or backwashing. To further validate the system's contribution in fouling mitigation, the operation had been sustained until the effluent flux was compromised. AnFMBR 1 remained stable from day 166 till day 179 (including start-up), then started to increase linearly to reach 78 Kpa at the end of the experiment (day 269). As for AnFMBR 2, TMP increased linearly from day 166 (including start-up) till the end of experiment to reach a value of 84 KPa. Despite the linear increase in membrane pressure, COD and gas results were not affected which suggests that the degradation process is happening in suspension and on the GAC but not on the membrane surface. The fouling increase with time is mainly due to the carbon deposition on the membranes which leads to the blockage of pores (Aslam et al., 2018) and the microbial growth on the membranes. The AnFMBR system showed advantages over other AnMBR systems mainly due to a combination of factors as the scouring effect of the GAC and the new system configuration.

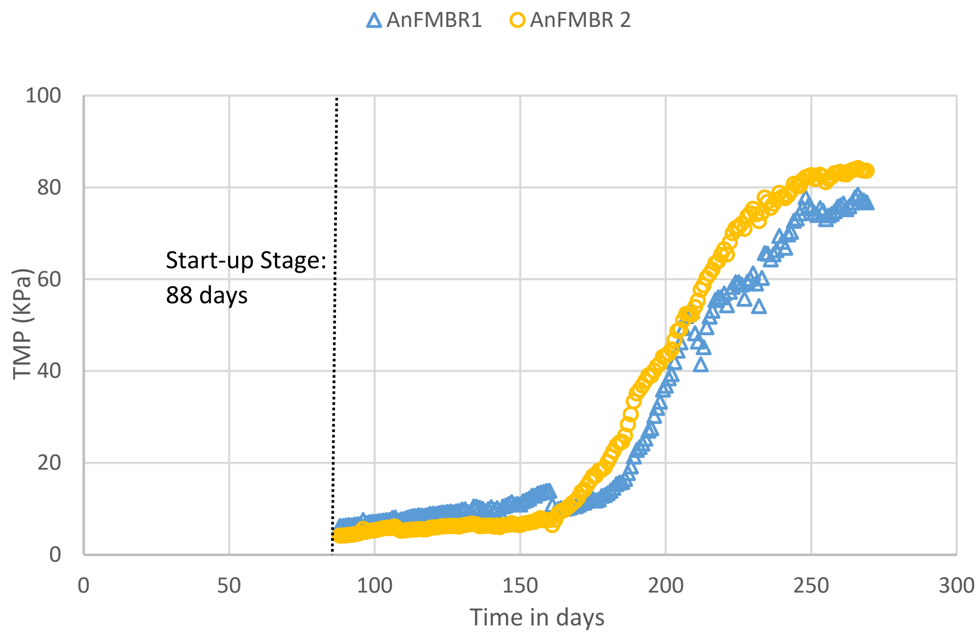

Figure 5: Transmembrane pressure (TMP) values

C. Energy Balance

In the AnFMBR configuration, methanogenesis was the only way to recover energy from synthetic wastewater. As acetoclastic methanogenesis produces one third carbon dioxide and two third methane from acetate, it yields a large fraction of carbon dioxide and subsequently less efficiency in substrate to energy recovery (less methane yield). Table 3 presents the detailed energy calculations using the equations already mentioned in the Material and Methods section. The energy consumption for the AnFMBR system was only attributed to the operation of pumps required for filtration and recirculation. Energy production calculated from recovered methane, excluding the concentration of dissolved methane, averaged 0.18 kWh/m^3. The average efficiency from the two reactors was calculated to be 150%. However, by combustion processes, methane can be recovered to electricity only with an efficiency of 33% (Malaeb et al., 2013, Kim et al., 2011). Accounting for this efficiency, the expected energy demand for AnFMBR operation is around 0.06 kWh/m^3 which was calculated by subtracting the recovered energy (i.e., 0.06 kWh/m^3) from the energy needed (0.12 kWh/m^3) for the reactor operation. This energy demand (0.06 kWh/m^3) reported in this study is much lower than that required in aerobic MBRs (1-2 kWh/m^3) (Malaeb et al., 2013) and the energy needed for gas sparging to prevent fouling (0.25-1 KWh/m^3) in in anaerobic membrane bioreactors (Liao et al., 2006, Kim et al., 2011). The potential energy advantage of the new configuration and GAC fluidization over other anaerobic and aerobic system is apparent even before any attempt to optimize the energy consumption of the system.

Table 3: Energy Calculations

		AnFMBR1	AnFMBR2
Input Parameters	Recirculation Rate Q1 (m^3/s)	9.72222E-06	9.72222E-06
	Permeate Rate Q2 (m^3/s)	1.15741E-08	1.15741E-08
	δ(N/m^3)	9800	9800
	E1 (m) Measured Hydraulic Pressue Head Loss	0.05	0.05
	E2 (m) Head Loss due to TMP	2.813	3.0861
Energy Demand	Energy for recirculation (kWh/m^3)	0.114333333	0.114333333
	Energy for filtration (kWh/m^3)	0.007657611	0.00840105
	Total Energy Demand (recirculation + Filtration + power supply) (kWh/m^3)	0.121990944	0.122734383
Energy Production	Energy Recovery or methane yield (kWh/m^3)	0.184654991	0.177078472
Efficiency	ηe (Wgas/total demand)	157.5922814	151.1261633
	Efficiency of converting methane to electricity (33%)	0.33	0.33
	System Efficiency (0.33 X ηe)	49.9513692	47.61167493
	Maximum electricity that could be generated from recovered methane (kWh/m^3)	0.060936147	0.058435896
Total Energy Required	Energy needed to operate the system (kWh/m^3)	0.061054797	0.064298488

D. Microbial Community Structure.

During the overall project, samples were collected from the reactor's bulk liquid and GAC at three different periods: after 3 months of start-up at OLR 0.43 kg substrate/m^3.day, then at OLR 0.31 kg substrate/m^3.day, and at the last day of operation. Also, HFM samples were collected at the end of the experiment. Library preparation was successful for all the samples and yielded between 69797 and 240804 reads after QC and bioinformatics processing. The most abundant genera were determined with the lowest assigned taxonomic classification that could be obtained (Figure 6). The two reactors presented very similar microbial community and this is further recognized in the principal component analysis whereby the sample dots are very close implying similarities between the two systems (Figure 7).

The most abundant bacteria phyla in the inoculum were *Firmicutes* (16.6%) and *Synergistetes* (3.8%), while the most abundant archaea were *Methanobacterium* (5.05%). After 3 months of start-up at an OLR of 0.43 kg substrate/m^3.day, the bacterial relative abundance in both reactors AnMBR1 and AnMBR2 differed from the inoculation, developing mostly *Proteobacteria* phylum on GAC (37% in AnMBR1 and 42.7% in AnMBR2), *Bacteroidetes* (14% in AnMBR1 and 11.3% in AnMBR2), *Firmicutes* (6.5% in AnMBR1 and 8.2% in AnMBR2), and *Synergistetes* (6.5% in AnMBR1 and 8.1% in AnMBR2), in accordance with previous anaerobic studies (Yi et al., 2014, Guo et al., 2014, Rivière et al., 2009, Ariesyady et al., 2007, Chouari et al., 2005). Same communities were present in suspension for both reactors with similar relative abundance, except for the *Proteobacteria* phylum which was 18.2% in AnMBR1 and 5.3% in AnMBR2. Other phyla were also found but in minor predominance as *Spirochaetea*, *Actinobacteria*, and *Chloroflexi.* At the class level, the most abundant classes in reactors 1 and 2 were *Deltaproteobacteria* belonging to the phyla of *Proteobacteria*, and *Clostridia* belonging to phyla of *Firmicutes;* both are commonly found in anaerobic digesters (Rivière et al., 2009, Deng et al., 2012, Zhang et al., 2013). At a lower taxonomic level, the *Desulfuromonadales sp*. were dominant on GAC, specifically the Geobacter genus (24% in AnFMBR1 and 24.5% in AnFMBR2) and in a lower abundance *Desulfuromonas* (3.1% in AnFMBR1 and 4.9% in AnFMBR2).

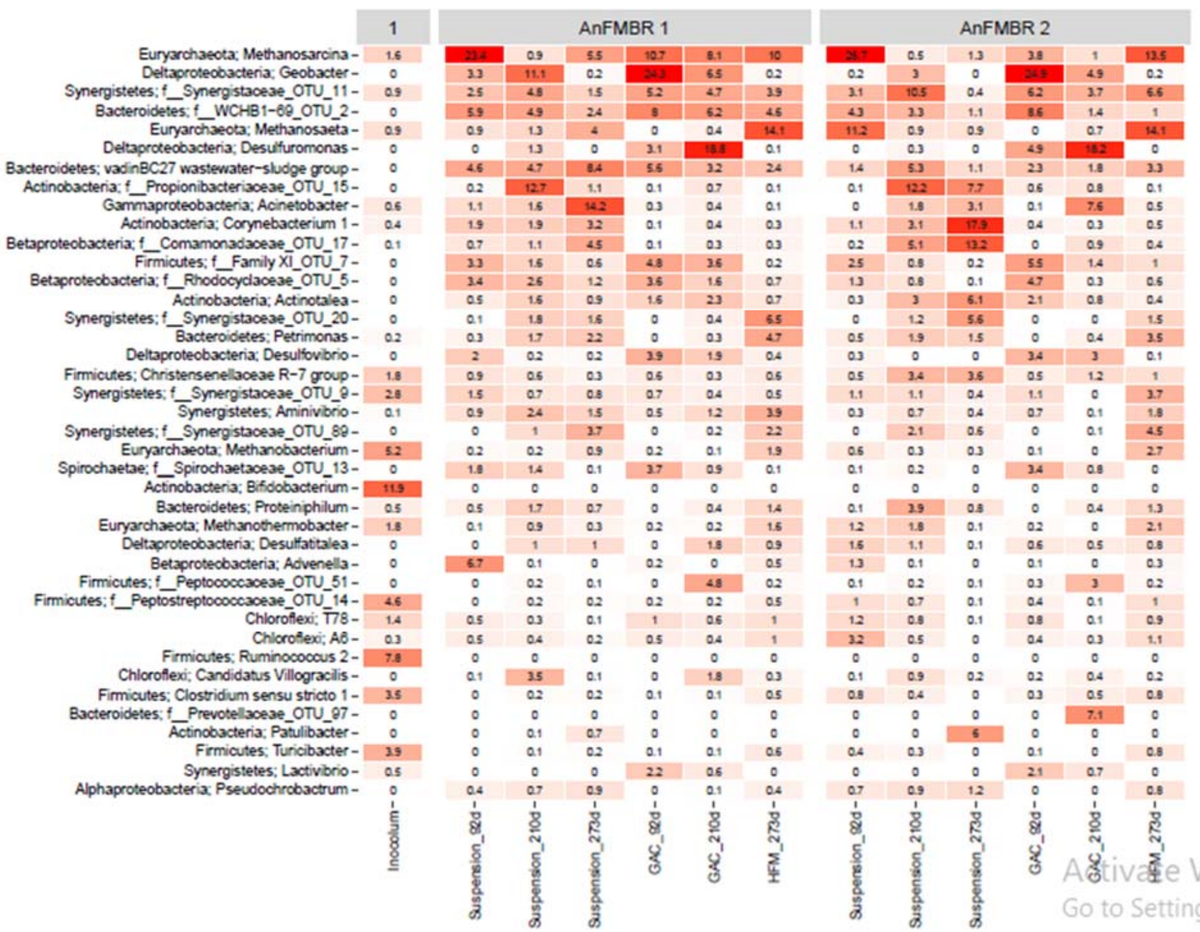

Figure 6: The 40 most abundant genera for both ANFMBRs

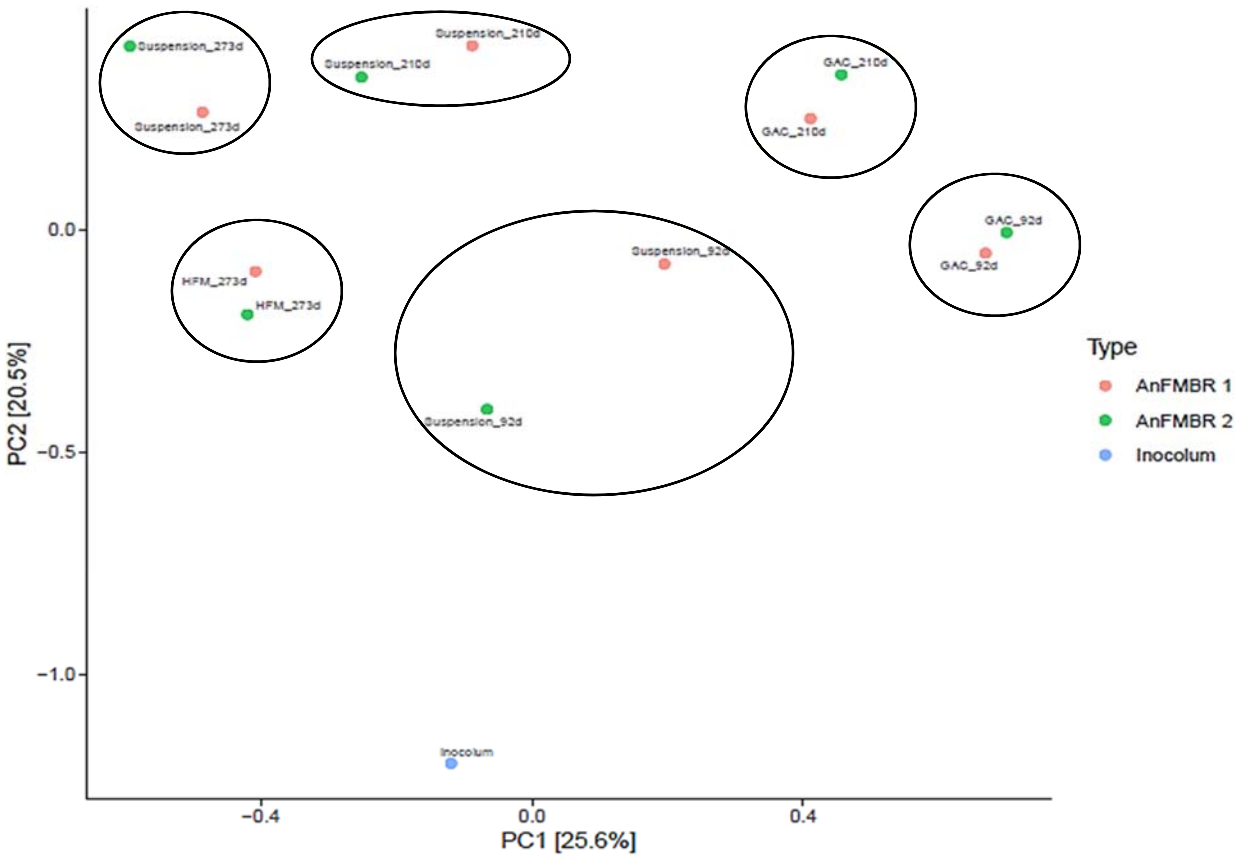

Figure 7: Principle Component Analysis

As for archaea, during this initial period of operation (OLR of 0.43 kg substrate/m^3.day), AnMBR 1 showed that the highest relative abundant species were *Methanosarcina* on GAC and in suspension (10.7% and 23.4%) followed by *Methanosaeta,* which was mainly present in suspension (0.9%). As for AnMBR2, *Methanosarcina* and *Methanosaeta* were equally abundant in suspension (26.7%) but present in a smaller percentage (<5%) on GAC. Although, the inoculum was high in hydrogenotrophic methanogens (5.05% *Methanobacterium*), the anaerobic system developed higher abundance in acetoclastic methanogens mainly due to the acetate fed substrate. According to previous studies, 70% of the methane generated in anaerobic digestion comes from acetoclastic methanogens (Conklin et al., 2006, Anderson et al., 2003). *Methanosarcina* and *Methanosaeta* (known acetoclastic methanogens) have different abilities of transforming the acetate and their predominance in various anaerobic reactors is governed by the acetate concentrations. *Methanosaeta* species has low maximum specific growth rate (µmax) and low half-saturation coefficient (KS), which explains their dominance in a low acetate environment in a conventional mesophilic anaerobic digestion (De Vrieze, 2014, Conklin et al., 2006). As for *Methanosarcina sp.*, which has a high µmax and high KS, it will absorb any increases in acetate efficiently and promotes a more stable methanogenesis (De Vrieze, 2014, Conklin et al., 2006, Yi et al., 2014). Therefore, *Methanosaeta* has an advantage over *Methanosarcina* and other hydrogenotrophic methanogens at low acetate concentrations (not exceeding 100 to 150 mg COD/L), whereas *Methanosarcina* can take over at higher acetate concentration (above 250 to 500 mg COD/L) or at SRT's equal or lower than 10 days (De Vrieze, 2014, Conklin et al., 2006, McHugh et al., 2003, McMahon et al., 2004, Yu et al., 2006, Blume et al., 2010). In this study, the acetate fed to the AnMBRs was initially around 640 mg COD/L which explains the governance of *Methanosarcina sp.* responsible for stability and robustness of the system.

With decreasing OLR (0.31 kg substrate/m3.day), the bacterial ecology remained quite stable in both reactors, however the *Actinobacteria* phyla dominated in suspension (16.2% in AnMBR1 and 18.3% in AnMBR2). This abundance of *Actinobacteria* was accompanied by a drop in acetoclastic methanogens abundance (in suspension and on GAC) and a decrease in gas generation and methane yield. The change in OLR clearly affected the microbial communities which needed some time for adaptation, and the technical problem that aggravated the situation. The abundant *Actinobacteria* in this case were possibly involved in the fermentation of dead cells. However, *Methanosarcina* archaea survived even after switching to the new OLR of 0.31 kg substrate/m^3.day, suggesting the presence of Direct Interspecies Electron Transfer (DIET). As known Extracellular Electron Transfer (EET) capable bacterium (*Deltaproteobacterea*, *Geobacter* and *Desulfuromonas*) were present in high abundance exclusively on the GAC. Therefore, we predicted that EET organisms and *Methanosarcina* surviving on GAC through DIET as these were found in previous studies to co-exist together and to enhance methane production (Barua et al., 2019, Yin et al., 2016, Zhao et al., 2015).

The HFM sample collected at the end of the operation revealed the presence of *Synergistetes* (17.4% in AnMBR1 and 18.4% in AnMBR2) and *Bacteroidetes* (14.1% in AnMBR1 and 9.3% in AnMBR2) as the most abundant bacteria, while *Methanosaeta* (AnMBR1 and 2: 14.1%) and *Methanosarcina* (AnMBR1: 10 %, AnMBR2: 13.5%) were the most abundant archaea. *Methanobacterium sp.* had relatively low abundance on the HFM, however their presence on every component of the reactor showed that methane production was also occurring from CO_2 reduction through hydrogenetrophic methanogenesis. Therefore, H_2 was available in the reactor due to its production through fermentation of endogenous decay of biomass or forming close associations with syntrophic acetate-oxidizing bacteria (SAOB). The presence of *Synergistaceae* supports the presence of fermenters and the decomposed carbon from dead cells which acts as source for fermentation. Those Heterotrophs/fermenter contributed significantly for HFM biofouling in both reactors due to presence of dead-cell debris/organics accumulated during the filtration process on the HFM surface. Also *Methanosarcina* and *Methanosaeta* were the dominant biofouling communities as they might retain on the surface while filtering the effluent because of their morphological features of (*Methanosarcina* as aggregates and *Methanosaeta* as thread like structure) which favors them to tangle to the membrane surface (Figure 8).

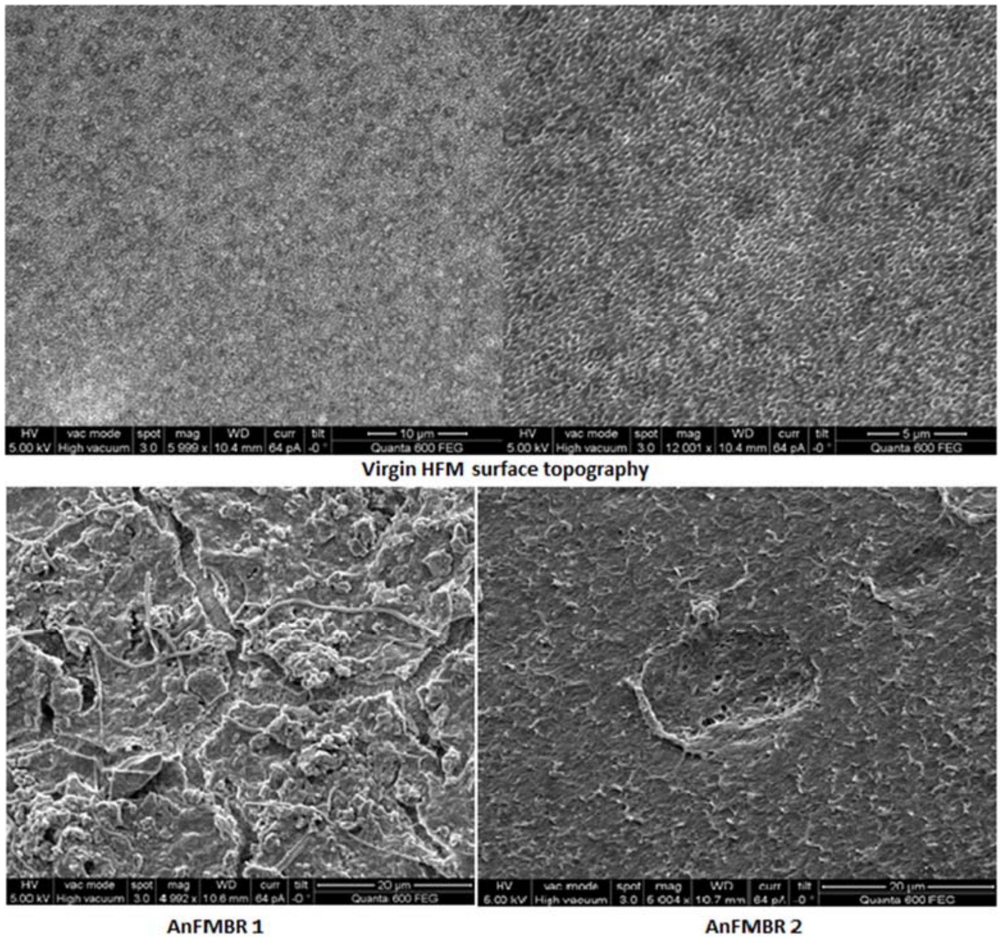

Figure 8: Virgin HFM vs. Biofouled HFM surface

CHAPTER IV
CONCLUSION

This study indicated that wastewater treatment using the AnMBR continues to be promising in terms of treatment efficiency and energy recovery.The overall COD removal was 90% for AnFMBR1 and 89% for AnFMBR2, and the average methane yield for both reactors was 0.26 and 0.13 L/g.COD removed at an OLR of 0.43 and 0.31 Kg substrate/m3.day respectively. The new system configuration proved to be **efficient in controlling fouling** since a long period operation was achieved before reaching 30 KPa TMP (196 days for AnFMBR1 and 188 days for AnFMBR2) and without subjecting the membrane to any type of cleaning. The dominant bacteria classes were Proteobacteria, *Bacteroidetes*, *Firmicutes*, *Synergistetes*, and *Actinobacteria.* For archaea, acetoclastic methanogens dominated over hydrogenotophic methanogens with relatively same abundance of methanosaeta and methanosarcina in AnMBR2 and a small greater dominance of methanosarcina in AnFMBR1. Methanosarcina dominance was also associated with relative abundance of geaobacter genus in both reactors, specifically on the GAC, ensuring that electron transfer between those species is behind the enhanced methane production of the system. The AnFMBR required a net energy of 0.06 kWh/m^3 for a lab-scale operation offering a potential energy advantage over other aerobic and anaerobic systems. The similar results obtained in AnFMBR1 and 2 **confirmed** the main target of this study which is **the reproducibility of the AnFMBR system**. However, one of the main disadvantages of the application of the AnMBR is the long start-up time compared to aerobic system as methanogens have slow growth rates. Hence, future studies should target decreasing the acclimation period by testing out different inocula or adjusting the system's configuration.

REFERENCES

AKRAM, A. & STUCKEY, D. C. 2008. Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Process Biochemistry,* 43**,** 93-102.

ALBERTSEN, M., KARST, S. M., ZIEGLER, A. S., KIRKEGAARD, R. H. & NIELSEN, P. H. 2015. Back to basics–the influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. *PloS one,* 10**,** e0132783.

AN, Y., WANG, Z., WU, Z., YANG, D. & ZHOU, Q. 2009. Characterization of membrane foulants in an anaerobic non-woven fabric membrane bioreactor for municipal wastewater treatment. *Chemical Engineering Journal,* 155**,** 709-715.

ANDERSON, K., SALLIS, P. & UYANIK, S. 2003. 24 - Anaerobic treatment processes. *In:* MARA, D. & HORAN, N. (eds.) *Handbook of Water and Wastewater Microbiology.* London: Academic Press.

ARIESYADY, H. D., ITO, T. & OKABE, S. 2007. Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. *Water Research,* 41**,** 1554-1568.

ASLAM, M., CHARFI, A. & KIM, J. 2019. Membrane scouring to control fouling under fluidization of non-adsorbing media for wastewater treatment. *Environmental Science and Pollution Research,* 26**,** 1061-1071.

BARUA, S., ZAKARIA, B. S., LIN, L. & DHAR, B. R. 2019. Shaping microbial communities with conductive carbon fibers to enhance methane productivity and kinetics. *Bioresource Technology Reports,* 5**,** 20-27.

BLUME, F., BERGMANN, I., NETTMANN, E., SCHELLE, H., REHDE, G., MUNDT, K. & KLOCKE, M. 2010. Methanogenic population dynamics during semi-continuous biogas fermentation and acidification by overloading. *Journal of applied microbiology,* 109**,** 441-450.

BOLGER, A. M., LOHSE, M. & USADEL, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* 30**,** 2114-2120.

BUER, T. & CUMIN, J. 2010. MBR module design and operation. *Desalination,* 250**,** 1073-1077.

CAPORASO, J. G., KUCZYNSKI, J., STOMBAUGH, J., BITTINGER, K., BUSHMAN, F. D., COSTELLO, E. K., FIERER, N., PENA, A. G., GOODRICH, J. K. & GORDON, J. I.

2010. QIIME allows analysis of high-throughput community sequencing data. *Nature methods,* 7**,** 335.

CHANG, S. 2013. Anaerobic membrane bioreactors (AnMBR) for wastewater treatment. *Advances in Chemical Engineering and Science,* 2014.

CHANG, S. 2014. Anaerobic Membrane Bioreactors (AnMBR) for Wastewater Treatment. *Advances in Chemical Engineering and Science,* 04**,** 56-61.

CHEN, C., GUO, W., NGO, H. H., LEE, D.-J., TUNG, K.-L., JIN, P., WANG, J. & WU, Y. 2016. Challenges in biogas production from anaerobic membrane bioreactors. *Renewable Energy,* 98**,** 120-134.

CHOUARI, R., LE PASLIER, D., DAEGELEN, P., GINESTET, P., WEISSENBACH, J. & SGHIR, A. 2005. Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. *Environmental Microbiology,* 7**,** 1104-1115.

CONKLIN, A., STENSEL, H. D. & FERGUSON, J. 2006. Growth Kinetics and Competition Between Methanosarcina and Methanosaeta in Mesophilic Anaerobic Digestion. *Water Environment Research,* 78**,** 486-496.

DE VRIEZE, J. 2014. *Methanosaeta vs. Methanosarcina in anaerobic digestion: the quest for enhanced biogas production.* Ghent University.

DENG, Y., ZHANG, Y., GAO, Y., LI, D., LIU, R., LIU, M., ZHANG, H., HU, B., YU, T. & YANG, M. 2012. Microbial community compositional analysis for series reactors treating high level antibiotic wastewater. *Environmental Science and Technology,* 46**,** 795-801.

EDGAR, R. C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature methods,* 10**,** 996.

GUO, X., WANG, C., SUN, F., ZHU, W. & WU, W. 2014. A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings. *Bioresource Technology,* 152**,** 420-428.

HASHISHO, J. & EL-FADEL, M. 2016. Membrane bioreactor technology for leachate treatment at solid waste landfills. *Reviews in Environmental Science and Bio/Technology,* 15**,** 441-463.

JUDD, S. 2008. The status of membrane bioreactor technology. *Trends in Biotechnology,* 26**,** 109-116.

KATURI, K. P., KAVANAGH, P., RENGARAJ, S. & LEECH, D. 2010. Geobacter sulfurreducens biofilms developed under different growth conditions on glassy carbon electrodes: insights using cyclic voltammetry. *Chemical communications (Cambridge, England),* 46**,** 4758.

KATURI, K. P., WERNER, C. M., JIMENEZ-SANDOVAL, R. J., CHEN, W., JEON, S., LOGAN, B. E., LAI, Z., AMY, G. L. & SAIKALY, P. E. 2014. A novel anaerobic electrochemical membrane bioreactor (AnEMBR) with conductive hollow-fiber membrane for treatment of low-organic strength solutions. *Environmental science & technology,* 48**,** 12833-12841.

KIM, J., KIM, K., YE, H., LEE, E., SHIN, C., MCCARTY, P. L. & BAE, J. 2011. Anaerobic Fluidized Bed Membrane Bioreactor for Wastewater Treatment. *Environmental Science & Technology,* 45**,** 576-581.

KOLA, A., YE, Y., HO, A., LE-CLECH, P. & CHEN, V. 2012. Application of low frequency transverse vibration on fouling limitation in submerged hollow fibre membranes. *Journal of Membrane Science,* 409-410**,** 54-65.

LEW, B., TARRE, S., BELIAVSKI, M., DOSORETZ, C. & GREEN, M. 2009. Anaerobic membrane bioreactor (AnMBR) for domestic wastewater treatment. *Desalination,* 243**,** 251-257.

LI, K., ZHU, H., ZHANG, Y. & ZHANG, H. 2017. Characterization of the Microbial Communities in Rumen Fluid Inoculated Reactors for the Biogas Digestion of Wheat Straw. *Sustainability,* 9**,** 243.

LI, Y., HU, Q. & GAO, D.-W. 2018. Dynamics of archaeal and bacterial communities in response to variations of hydraulic retention time in an integrated anaerobic fluidized-bed membrane bioreactor treating benzothiazole wastewater. *Archaea,* 2018**,** 9210534-11.

LIAO, B.-Q., KRAEMER, J. T. & BAGLEY, D. M. 2006. Anaerobic membrane bioreactors: applications and research directions. *Critical Reviews in Environmental Science and Technology,* 36**,** 489-530.

LIN, H., PENG, W., ZHANG, M., CHEN, J., HONG, H. & ZHANG, Y. 2013. A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. *Desalination,* 314**,** 169-188.

MAGOČ, T. & SALZBERG, S. L. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics,* 27**,** 2957-2963.

MALAEB, L., KATURI, K. P., LOGAN, B. E., MAAB, H., NUNES, S. P. & SAIKALY, P. E. 2013. A hybrid microbial fuel cell membrane bioreactor with a conductive ultrafiltration membrane biocathode for wastewater treatment. *Environmental Science and Technology,* 47**,** 11821-11828.

MARTINEZ-SOSA, D., HELMREICH, B., NETTER, T., PARIS, S., BISCHOF, F. & HORN, H. 2011. Anaerobic submerged membrane bioreactor (AnSMBR) for municipal wastewater treatment under mesophilic and psychrophilic temperature conditions. *Bioresource Technology,* 102**,** 10377-10385.

MCHUGH, S., CARTON, M., MAHONY, T. & O'FLAHERTY, V. 2003. Methanogenic population structure in a variety of anaerobic bioreactors. *FEMS microbiology letters,* 219**,** 297-304.

MCMAHON, K. D., ZHENG, D., STAMS, A. J., MACKIE, R. I. & RASKIN, L. 2004. Microbial population dynamics during start-up and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. *Biotechnology and bioengineering,* 87**,** 823-834.

OZGUN, H., DERELI, R. K., ERSAHIN, M. E., KINACI, C., SPANJERS, H. & VAN LIER, J. B. 2013. A review of anaerobic membrane bioreactors for municipal wastewater treatment: Integration options, limitations and expectations. *Separation and Purification Technology,* 118**,** 89-104.

QUAST, C., PRUESSE, E., YILMAZ, P., GERKEN, J., SCHWEER, T., YARZA, P., PEPLIES, J. & GLÖCKNER, F. O. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research,* 41**,** D590-D596.

RAGSDALE, S. W., EL KASMI, A., EL KASMI, A., RAJASEKHARAN, S., RAJASEKHARAN, S., RAGSDALE, S. W. & RAGSDALE, S. W. 1994. Anaerobic pathway for conversion of the methyl group of aromatic methyl ethers to acetic acid by clostridium thermoaceticum. *Biochemistry,* 33**,** 11217-11224.

RIVIÈRE, D., DESVIGNES, V., PELLETIER, E., CHAUSSONNERIE, S., GUERMAZI, S., WEISSENBACH, J., LI, T., CAMACHO, P. & SGHIR, A. 2009. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *The ISME Journal,* 3**,** 700-714.

SANTOS, A., MA, W. & JUDD, S. J. 2011. Membrane bioreactors: Two decades of research and implementation. *Desalination,* 273**,** 148-154.

SUI, P., WEN, X. & HUANG, X. 2008. Feasibility of employing ultrasound for on-line membrane fouling control in an anaerobic membrane bioreactor. *Desalination,* 219**,** 203-213.

TRZCINSKI, A. P. & STUCKEY, D. C. 2016. Effect of sparging rate on permeate quality in a submerged anaerobic membrane bioreactor (SAMBR) treating leachate from the organic fraction of municipal solid waste (OFMSW). *Journal of Environmental Management,* 168**,** 67-73.

VAN LIER, J. B. 2008. High-rate anaerobic wastewater treatment: diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. *Water Science and Technology,* 57**,** 1137-1148.

WANG, Y.-K., SHENG, G.-P., SHI, B.-J., LI, W.-W. & YU, H.-Q. 2013. *A Novel Electrochemical Membrane Bioreactor as a Potential Net Energy Producer for Sustainable Wastewater Treatment*.

WINTER, J., POPOFF, M. R., GRIMONT, P. & BOKKENHEUSER, V. D. 1991. Clostridium orbiscindens sp. nov., a human intestinal bacterium capable of cleaving the flavonoid C-ring. *International Journal of Systematic Bacteriology,* 41**,** 355-357.

WISZNIOWSKI, J., ROBERT, D., SURMACZ-GORSKA, J., MIKSCH, K. & WEBER, J. V. 2006. Landfill leachate treatment methods: A review. *Environmental Chemistry Letters,* 4**,** 51-61.

YANG, Y., QIAO, S., JIN, R., ZHOU, J. & QUAN, X. 2019. Novel Anaerobic Electrochemical Membrane Bioreactor with a CNTs Hollow Fiber Membrane Cathode to Mitigate Membrane Fouling and Enhance Energy Recovery. *Environmental Science & Technology,* 53**,** 1014-1021.

YE, Y., LABARGE, N., KASHIMA, H., KIM, K.-Y., HONG, P.-Y., SAIKALY, P. E. & LOGAN, B. E. 2016. An aerated and fluidized bed membrane bioreactor for effective wastewater treatment with low membrane fouling. *Environmental Science: Water Research & Technology,* 2**,** 994-1003.

YI, J., DONG, B., JIN, J. & DAI, X. 2014. Effect of Increasing Total Solids Contents on Anaerobic Digestion of Food Waste under Mesophilic Conditions: Performance and Microbial Characteristics Analysis. *PloS one,* 9**,** e102548.

YIN, Q., ZHU, X., ZHAN, G., BO, T., YANG, Y., TAO, Y., HE, X., LI, D. & YAN, Z. 2016. Enhanced methane production in an anaerobic digestion and microbial electrolysis cell coupled system with co-cultivation of Geobacter and Methanosarcina. *Journal of environmental sciences (China),* 42**,** 210-214.

YOO, R., KIM, J., MCCARTY, P. L. & BAE, J. 2012. Anaerobic treatment of municipal wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR) system. *Bioresource Technology,* 120**,** 133-139.

YU, Y., KIM, J. & HWANG, S. 2006. Use of real-time PCR for group-specific quantification of aceticlastic methanogens in anaerobic processes: population dynamics and community structures. *Biotechnology and bioengineering,* 93**,** 424-433.

ZHANG, S., CHANG, J., LIU, W., PAN, Y., CUI, K., CHEN, X., LIANG, P., ZHANG, X., WU, Q., QIU, Y. & HUANG, X. 2018. A novel bioaugmentation strategy to accelerate methanogenesis via adding Geobacter sulfurreducens PCA in anaerobic digestion system. *Science of The Total Environment,* 642**,** 322-326.

ZHANG, Y., XIE, J., LIU, M., TIAN, Z., HE, Z., VAN NOSTRAND, J. D., REN, L., ZHOU, J. & YANG, M. 2013. Microbial community functional structure in response to antibiotics in pharmaceutical wastewater treatment systems. *Water Research,* 47**,** 6298-6308.

ZHAO, Z., ZHANG, Y., WANG, L.-Y. & QUAN, X. 2015. Potential for direct interspecies electron transfer in an electric-anaerobic system to increase methane production from sludge digestion. *Scientific Reports,* 5**,** 11094.

APPENDIXES

Table 4: Pressure Data in KPA for the AnFMBR reactors

Days	Presure(KPa)	
	AnFMBR1	AnFMBR 2
88	6.213649186	4.1234051
89	6.248378366	4.1572576
90	6.337967989	4.1432129
91	6.448945319	4.276922
92	6.394050349	4.2682908
93	6.567712913	4.4341503
94	6.610363999	4.5110248
95	6.710986285	4.7096964
96	7.544697456	5.5641684
97	6.907867027	4.9432495
98	7.043601465	5.1226995
99	7.142616845	5.2168846
100	7.164204869	5.2711149
101	7.253215557	5.4029606
102	7.350082375	5.5704436
103	7.44185149	5.7260302
104	7.516167827	5.8220668
105	7.580386806	5.770316
106	7.989685979	5.9935745
107	8.097649165	6.0673792
108	7.423177939	5.5084855
109	7.653665484	5.199361
110	7.791197688	5.2358676
111	8.008190223	5.3073589
112	8.239201223	5.358382
113	8.36747684	5.4511471
114	8.41517326	5.4410307
115	8.587190138	5.5608098
116	8.51414074	5.5128412
117	8.459202307	5.4905226
118	8.608470509	5.5620768
119	8.876103351	5.7908378
120	8.941060651	5.8856078
121	8.898432792	5.8807144

Days	Presure(KPa)	
	AnFMBR1	AnFMBR 2
122	9.0342043	6.011019008
123	9.1699759	6.141323642
124	9.1683151	6.145477178
125	9.3119523	6.198970975
126	9.3911788	6.236982761
127	9.2521053	6.171907224
128	9.4357362	6.244109844
129	9.4795746	6.101996057
130	9.8152412	6.328874193
131	9.876968	6.46289528
132	9.288134	6.507790136
133	9.1226059	6.703478131
134	10.487407	6.69006022
135	10.435272	6.567675897
136	10.201155	6.155708904
137	9.9816177	6.288300308
138	9.4661015	6.245707923
139	9.8841682	6.29598381
140	10.360053	6.283141995
141	9.757227	6.085235064
142	10.1004	6.305026901
143	9.3791208	5.95722081
144	10.507305	6.378863502
145	10.697736	6.422345496
146	11.013102	6.478433464
147	11.439834	6.841300948
148	10.920332	6.569975826
149	11.039521	6.499996224
150	10.917731	6.399560337
151	11.657549	6.690527405
152	11.899522	6.830185213
153	12.212079	6.952661383
154	12.811087	7.096661474
155	12.843781	7.151350897

Days	Presure(KPa)	
	AnFMBR1	AnFMBR 2
156	13.32433272	7.6052056
157	13.50176906	7.9001888
158	13.64566262	7.470299
159	13.88669294	7.4563065
160	13.90067864	7.5457097
161	10.84059503	6.4178624
162	9.437332359	7.5799668
163	9.923520697	8.9354997
164	10.13944131	9.4369228
165	9.990712371	9.6399971
166	10.07015819	10.120855
167	10.24272076	10.638592
168	10.52012943	11.252785
169	10.53331589	11.697253
170	10.82872581	12.47528
171	11.19880155	13.667217
172	11.31553157	14.116063
173	11.70171205	15.115347
174	11.90020342	16.105359
175	12.10537684	17.107883
176	11.79177462	17.167676
177	12.01752409	18.312319
178	12.77657963	18.469886
179	12.9532012	18.944208
180	13.3683175	20.151659
181	13.91721397	21.32998
182	14.52348403	22.523767
183	15.38396318	23.720222
184	15.68239492	24.34154
185	15.81647635	24.558553
186	16.47135274	25.928463
187	17.79009084	28.374942
188	19.19028448	30.561264
189	21.34422218	33.394787
190	22.80339341	35.125377
191	23.41012715	35.938063
192	24.19826666	36.821137
193	25.26657036	37.845487
194	26.99145424	39.037123

Days	Presure(KPa)	
	AnFMBR1	AnFMBR 2
195	27.52855	38.97498518
196	30.200929	40.0607459
197	31.952865	41.04686486
198	33.299389	41.64572524
199	35.954222	43.10638274
200	36.801611	43.34387362
201	38.404085	43.8290462
202	39.353225	44.70388415
203	41.941325	46.75628546
204	44.43692	48.74899602
205	46.236063	49.05022141
206	49.328467	51.03222667
207	51.93252	52.42288065
208	52.40217	52.41741192
209	53.017041	52.27403157
210	48.233622	53.94677889
211	46.390592	55.33865451
212	41.443893	57.7067237
213	45.186244	58.65467291
214	49.542497	60.23967012
215	51.851453	61.12393773
216	53.121651	62.06860752
217	55.464807	63.49305959
218	56.062245	63.93252021
219	56.068608	65.27119429
220	56.90953	66.52351689
221	54.324281	65.39059192
222	57.23967	68.06224463
223	58.321764	70.01486985
224	59.338655	71.12165106
225	59.342345	71.30897039
226	58.858633	72.03505389
227	55.748251	70.97870653
228	59.445274	73.68421065
229	60.078928	74.33967448
230	61.289186	75.30897039
231	59.062245	74.13691165
232	54.156203	72.67160208
233	60.271023	74.42443207

Days	Presure(KPa)	
	AnFMBR1	AnFMBR 2
234	65.71500726	77.78005
235	65.57807371	76.850443
236	64.32737029	75.53703
237	65.42249826	76.424416
238	66.33922201	77.294252
239	69.42443207	78.798787
240	68.13691165	77.919847
241	66.8614213	77.646186
242	69.73480283	78.255459
243	70.29180782	79.197064
244	72.73992394	80.740796
245	73.21984662	80.323059
246	74.35693745	80.271023
247	75.48441615	81.578074
248	77.86264646	82.171601
249	76.08370279	82.422498
250	75.42441615	82.681236
251	74.43983425	81.841301
252	73.98526077	81.901157
253	75.50986142	82.734803
254	74.91773106	82.39956
255	73.07636308	81.153651
256	73.89952214	81.830185
257	74.25545878	82.310241
258	74.81108743	83.096661
259	75.75604767	83.173612
260	76.08171547	83.3773
261	76.50176906	82.900189
262	75.23946478	82.810027
263	75.88669294	83.456307
264	76.67420271	83.7458
265	77.84059503	83.917862
266	78.43347579	84.191014
267	77.49638883	83.78942
268	77.13944131	83.636923
269	76.73972755	83.60515

Table 5: Start-up gas data (volume and percentage by mass)

Day	Volume(ml)		Percentage by mass (%)		Volume(ml)		Percentage by mass (%)	
	AnFMBR1				AnFMBR2			
	CH4	CO2	CH4	CO2	CH4	CO2	CH4	CO2
4	4.660366	3.6435391	18.86801	81.1319929	0	4.927067	0	100
5	5.243498	6.1938231	13.339	86.6609991	0	5.652372	0	100
8	9.446399	5.734598	23.04748	76.9525194	2.420731	7.06489	5.864512	94.13548796
22	3.206026	3.8234255	13.22898	86.77101879	9.083573	3.522291	31.92127	68.0787282
24	8.428627	5.0429572	23.3061	76.69389638	4.184632	5.224666	12.7114	87.28859571
26	7.238801	7.3221776	15.23612	84.76387633	7.854602	7.863383	15.37008	84.62992406
33	3.149999	4.3967543	11.52489	88.47511304	17.40509	8.403445	27.35616	72.64384452
36	3.98408	4.2226822	14.6426	85.35740163	18.57613	7.521364	30.98933	69.01066695
40	7.756874	5.2589571	21.14676	78.85323596	12.19176	6.542609	23.46997	76.53002723
43	4.702776	4.9497936	14.72994	85.27005814	5.807392	5.563853	15.95061	84.04938751
44	12.8141	8.5956977	21.32469	78.67530868	16.12864	12.45412	19.05866	80.94134199
45	13.92171	5.4232677	31.82129	68.17871124	11.7073	6.64745	24.25465	75.74534927
48	13.9612	6.9169839	26.84608	73.15392394	22.1599	7.525613	34.8696	65.13040257
50	23.7992	8.5207256	33.67977	66.32023416	26.12229	6.432614	42.47412	57.52587844
54	25.94142	8.3559356	36.08033	63.9196684	24.08491	11.84851	26.98538	73.01462077
62	29.07133	8.5387657	38.23437	61.76562969	28.18063	9.797729	34.33809	65.66191241
64	27.13212	8.7405882	36.07737	63.92262578	29.69189	7.663737	41.32918	58.67082095
65	26.14761	8.7293498	35.25884	64.74116334	28.7731	8.265681	38.75982	61.24018028
62	99.08608	38.410414	31.92789	68.07211102	90.23916	37.32146	30.53705	69.46295403
65	57.30689	14.629981	41.59552	58.40448067	79.46011	24.45915	37.13342	62.86657993

Table 6: Gas yield data (operation phase)

Time			Gas yield (LCH4/g.COD removed)	
Weeks of operation	First day of week	End day of week	AnFMBR1	AnFMBR2
1	89	91	0.31	0.19
2	92	98	0.28	0.24
3	99	105	0.17	0.20
4	106	112	0.19	0.27
5	113	119	0.30	0.16
6	120	126	0.22	0.10
7	127	133	0.25	0.17
8	134	140	0.19	0.18
9	141	147	0.09	0.10
10	148	154	0.09	0.13
12	155	161	0.07	0.06
13	162	168	0.08	0.06
14	169	175	0.10	0.07
15	176	182	0.11	0.06
16	183	189	0.06	0.03
17	190	196	0.04	0.04
18	197	203	0.04	0.06
19	204	210	0.05	0.04
20	211	217	0.04	0.06
21	218	224	0.14	0.08
22	225	231	0.14	0.12
23	232	238	0.12	0.12
24	239	245	0.04	0.16
25	246	252	0.08	0.08
26	253	259	0.12	0.12
27	260	266	0.12	0.15

Table 7: COD removal data (operation phase)

Time			COD removal (%)	
Weeks of operation	First day of week	End day of week	AnFMBR1	AnFMBR2
1	89	91	95.06	98.92
2	92	98	96.11	98.70
3	99	105	99.42	98.56
4	106	112	73.65	74.95
5	113	119	28.90	75.38
6	120	126	60.69	68.90
7	127	133	44.71	41.83
8	134	140	8.86	56.80
9	141	147	92.66	89.20
10	148	154	91.94	89.20
12	155	161	93.95	79.48
13	162	168	98.39	77.54
14	169	175	88.48	92.87
15	176	182	89.98	89.34
16	183	189	64.15	79.27
17	190	196	92.01	85.75
18	197	203	82.94	74.08
19	204	210	80.35	89.63
20	211	217	91.14	91.79
21	218	224	90.28	89.42
22	225	231	93.30	93.74
23	232	238	85.75	90.50
24	239	245	96.33	86.61
25	246	252	95.68	90.28
26	253	259	97.19	91.36

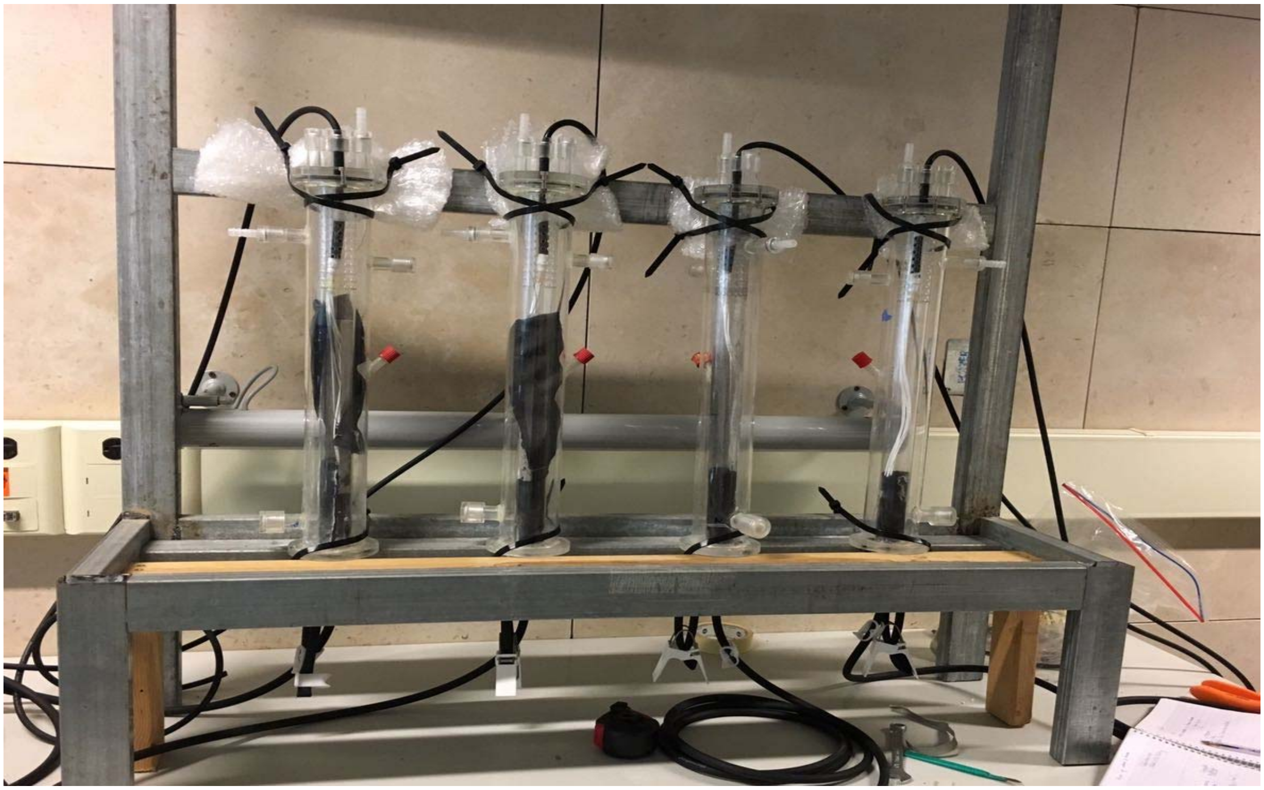

Figure 9: Experimental set-up during installation

Figure 10: Experimental set-up during operation

Figure 11: AnFMBR reactor during operation

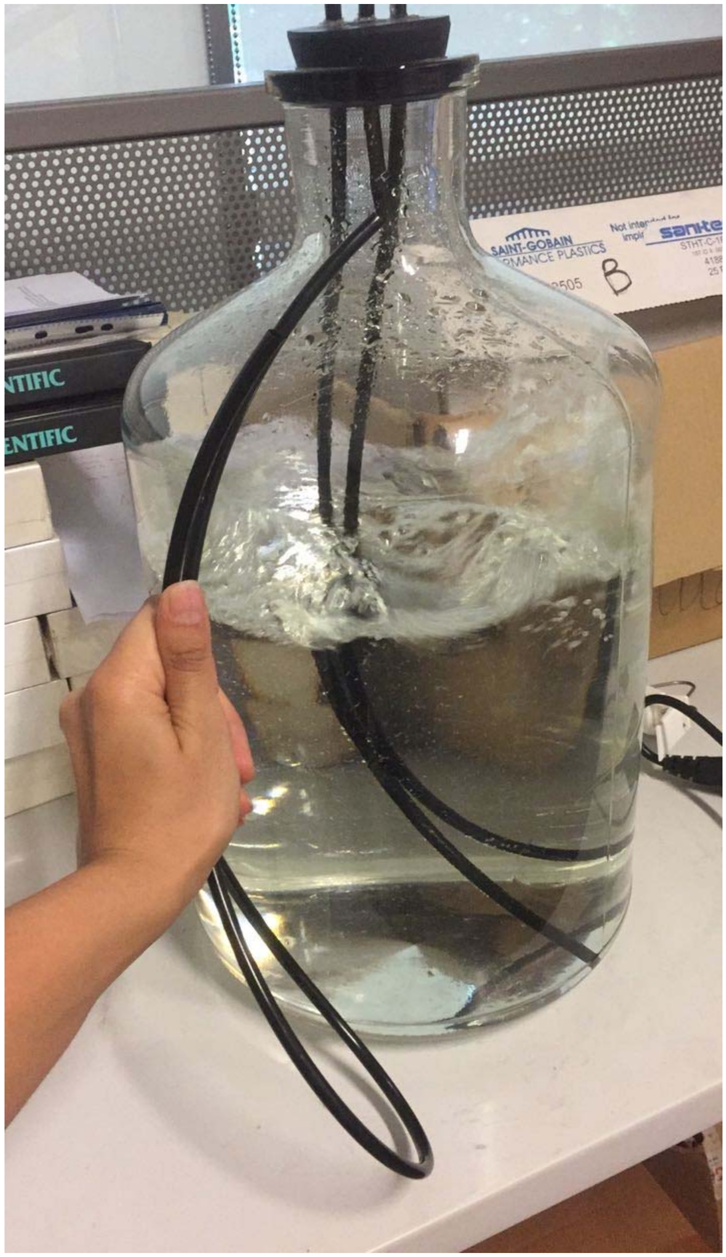

Figure 12: Feed tank while sparging