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Microbial and operational factors affecting energy recovery potential for anaerobic membrane bioreactors treating different wastewater types

By

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Microbial and operational factors affecting energy recovery potential for anaerobic membrane bioreactors treating different wastewater types

Lama Ramadan

ABSTRACT

Water scarcity has become a worldwide issue that is exacerbated in developing countries due to excessive pollution of water resources. In order to allow for sustainable practices that are capable of facilitating wastewater reuse, different types of wastewater treatment systems have been implemented over the years. While wastewater systems are traditionally designed to reduce pollutants and pathogens prior to discharge, a major factor that also requires consideration is energy expenditure and recovery potential during treatment. Recently, anaerobic membrane bioreactors (AnMBRs) have been shown as having certain advantages compared to conventional aerobic systems, as well as other anaerobic treatment methods such as the up-flow anaerobic sludge blanket (UASB). Several factors affect energy recovery from AnMBRs including the influent wastewater strength and type, the operating parameters, and the adaptation of microbial communities. In that context, the overall work of this thesis assessed factors associated with energy use and recovery in an AnMBR and consist of multiple experiments treating different types of wastewaters under varying operating conditions. The first experiment treating synthetic wastewater highlighted the effect of operating three membranes with different permeate fluxes on the energy consumption of the system. The second experiment treating municipal wastewater highlighted the tipping point of energy neutrality of the system due to the low organic content of the influent. The third experiment treating poultry slaughterhouse wastewater (PSW) over three phases with decreasing hydraulic retention times (HRTs) concluded that the microbial adaptation to the high-strength wastewater can directly impact the energy produced from the system. In the PSW experiment, microbial risk-associated elements (such as pathogens and antibiotic resistance genes) were also assessed in effluents. An additional experiment conducted for olive mill wastewater reiterated the AnMBR’s capacity to successfully recover energy from complex agro-industrial wastewaters. It was found that for such high-strength wastewaters, energy expenditure for substrate heating or electrical demand is negligible in comparison to the potential variability in methane production rates achievable.

Keywords: AnMBR, Energy recovery, Wastewater strength, Microbial communities
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<td>AD</td>
<td>anaerobic digestion</td>
</tr>
<tr>
<td>AeMBR</td>
<td>aerobic membrane bioreactor</td>
</tr>
<tr>
<td>AnMBR</td>
<td>anaerobic membrane bioreactor</td>
</tr>
<tr>
<td>ARG</td>
<td>antibiotic resistance gene</td>
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<tr>
<td>CAS</td>
<td>conventional activated sludge</td>
</tr>
<tr>
<td>COD</td>
<td>chemical oxygen demand</td>
</tr>
<tr>
<td>CSTR</td>
<td>continuously stirred tank reactor</td>
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<tr>
<td>DAF</td>
<td>dissolved air flotation unit</td>
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<tr>
<td>EGSB</td>
<td>expanded granular sludge bed reactors</td>
</tr>
<tr>
<td>FBR</td>
<td>fixed bed reactor</td>
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<tr>
<td>HRT</td>
<td>hydraulic retention time</td>
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<td>LRV</td>
<td>log removal value</td>
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<tr>
<td>MBR</td>
<td>membrane bioreactor</td>
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<tr>
<td>MF</td>
<td>microfiltration</td>
</tr>
<tr>
<td>NDS</td>
<td>nitrification/de-nitrification system</td>
</tr>
<tr>
<td>OLR</td>
<td>organic loading rate</td>
</tr>
<tr>
<td>OTU</td>
<td>operational taxonomic unit</td>
</tr>
<tr>
<td>PCoA</td>
<td>principal coordinate analysis</td>
</tr>
<tr>
<td>PVDF</td>
<td>polyvinylidene fluoride</td>
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<tr>
<td>qPCR</td>
<td>quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RDP</td>
<td>ribosomal database project</td>
</tr>
<tr>
<td>SGBR</td>
<td>static granular bed reactor</td>
</tr>
<tr>
<td>SGD</td>
<td>specific gas demand</td>
</tr>
<tr>
<td>SRT</td>
<td>solids retention time</td>
</tr>
<tr>
<td>TF</td>
<td>trickling filter</td>
</tr>
<tr>
<td>TMP</td>
<td>transmembrane pressure</td>
</tr>
<tr>
<td>UASB</td>
<td>up-flow anaerobic sludge blanket</td>
</tr>
<tr>
<td>UF</td>
<td>ultrafiltration</td>
</tr>
<tr>
<td>WWTPs</td>
<td>wastewater treatment plant</td>
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Chapter One

Introduction

1.1. Energy recovery potential from wastewater treatment

In the light of technological advancement and continuous developments in wastewater treatment systems, environmental impacts have become a major concern in terms of water shortage, pollution, and energy deficiency (Cuetos, Gomez, Otero, & Moran, 2008; Maaz et al., 2019). This emerging concern has shifted the focus from the necessity of reliable treatability into improving the sustainability of treatment technologies. Implemented treatment technologies operating at optimal conditions are designed to provide high effluent quality with sufficient organics, metals, and pathogens removal and thus ensuring health safety and allowing for water reuse from the discharged effluents (Seow et al., 2016). Regardless, considerable energy expenditures on operations are generated in order to meet effluent standard limit, highlighting the need to investigate the provision of energy recovery (Awe, Liu, & Zhao, 2016). A net positive energy balance resulting from the wastewater treatment process can allow for energy savings up to 80% (Kong, Wu, et al., 2021). Therefore, the potential for energy recovery is an important parameter that is considered for the basis of selection for a treatment system.

1.1.1. Introduction to energy consumption/production and recovery process, General overview

Generally, any system requires electricity for operations represented as energy consumption, and different systems have different potentials to produce useful energy. The term “energy recovered” indicates the difference between the energy production
and consumption of the system. In anaerobic-based treatment systems, energy production is the result of the sole conversion of organic matter (present in the treated influent) to biogas in the headspace of the reactor. Energy consumption refers to the electricity demand for operations such as aeration, membrane scouring, permeate pumping, mixing and heating process (Martin, Pidou, Soares, Judd, & Jefferson, 2011; Mei, Wang, Miao, & Wu, 2016). Energy produced from the system can be recovered in the form of electricity or heat: methane produced undergoes combustion after which 35% is converted to electricity used as source for operations while the remaining 65% is given off as heat useful as a heating source (McCarty, Bae, & Kim, 2011).

1.1.2. Higher recovery from anaerobic treatment technologies compared to traditional aerobic treatment systems

Biological treatment technologies incorporate two major modes of operations: aerobic and anaerobic systems with each of them possessing different potentials for energy use and recovery. Aerobic treatment systems have either physical or biological process and they operate in the presence of oxygen. They include several traditional methods such as conventional activated sludge (CAS), fixed-bed bioreactors (FBR), trickling filters (TF), dissolved air flotation units (DAF), nitrification/de-nitrification systems (NDS), and aerobic membrane bioreactors (AeMBRs). They exhibit high organics and nutrients (total phosphorous and total nitrogen) removal from the effluent, providing high water quality and potential reusability, and are especially suitable for treatment of low-strength wastewaters (Chan, Chong, Law, & Hassell, 2009). Despite the potential reusability of their effluents, the aeration process constitutes up to 60% of the total energy demand of the system (Martin et al., 2011) without any production of useful energy, which makes them unfavorable (Verrecht, Judd, Guglielmi, Brepols, & Mulder, 2008).
On the other hand, anaerobic systems remove organics in absence of oxygen through biological processes, and include technologies such as anaerobic digesters (ADs), up-flow anaerobic sludge blanket reactors (UASB), expanded granular sludge bed reactors (EGSB), static granular bed reactor (SGBR), and anaerobic membrane bioreactors (AnMBRs) (Shende & Pophali, 2021). Anaerobic treatment systems can achieve sufficient organics and pathogens removal prior to effluent discharge, produce useful biogas, and are preferable for high-strength wastewaters. Unlike aerobic systems, they have lower energy demand and significant methane production, and can thus sustain enough recoverable energy (Aziz et al., 2019). Energy demand for operations of a typical anaerobic digester constitutes less than 30% of the total energy produced from the reactor and thus yields a net positive energy balance (Lubken, Wichern, Schlattmann, Gronauer, & Horn, 2007). Net energy recovery from an anaerobic system was positive equal to 0.6 kWh/m³ and negative equal to -0.1 kWh/m³ from a conventional aerobic system (McCarty et al., 2011). Aerobic systems are therefore not considered sustainable treatment options on the level of energy recovery and can be replaced by advanced anaerobic systems.

1.2. Background: Anaerobic membrane bioreactors (AnMBRs)

Anaerobic membrane bioreactors are advanced anaerobic treatment systems used for high removal of organics and pollutants, especially applicable for treatment of high-strength industrial wastewaters while recovering useful energy. An AnMBR consists of a completely stirred tank reactor (CSTR) coupled with a membrane filtration unit that could either be external or submerged in the tank reactor (Chang, 2013). This system includes both biological treatment through anaerobic digestion in the CSTR as well as physical treatment through the filtration process where solid biofilm is retained on the membrane surface and treated permeate is emitted to the other side of the
membrane sheet. Fouling is controlled when the transmembrane pressure is maintained, through sludge recirculation and biogas sparging that generates turbulence on the membrane surface (Maaz et al., 2019). Submerged membranes don’t require sludge recirculation and are thus less energy consuming, but their presence inside the bioreactor makes the cleaning process more difficult. Compared to other anaerobic treatment systems, the AnMBRs require a smaller footprint, have complete retention of biomass and minimum sludge production, allowing for higher influent loading capacities (Dereli et al., 2012). Membrane-based technologies can achieve up to 99% fat removal and 94% COD removal for high strength wastewater treatment, while maintaining stable conditions (Baker, Mohamed, Al-Gheethi, & Aziz, 2021). The released effluent is also free of solids and pathogens, but is rich in nutrients which could be beneficial for several post-treatment usages (Dvořák, Gómez, Dolina, & Černín, 2016). Compared to other conventional treatment methods, AnMBRs are a sustainable alternative given their capacity to produce effluent of high quality while still having the capacity to produce high recoverable methane volumes.
Chapter Two

Literature review

2.1. Anaerobic systems treating high-strength wastewater: benefits and challenges

High-strength wastewaters refer to industrial wastewaters discharged from all types of industries such as food industry, animal slaughterhouses, breweries, agricultural and pharmaceutical wastes, and chemical and power plants (Vitězová, Kohoutová, Vitěz, Hanišáková, & Kushkevych, 2020). These types of wastewaters possess a wide range of COD values as it could be as low as 500 mg/L for wastes discharged from food industries but can exceed 100 g/L for oil mill wastewater (Abdurahman, Rosli, & Azhari, 2011; C. F. Bustillo-Lecompte & Mehrvar, 2015) implying significant potential for energy recovery.

2.1.1. Anaerobic treatment of industrial wastewaters

Classified as high-strength, industrial wastewaters are composed of complex organic compounds and suspended solids, have high organic strength and energy potential. They are also rich in fat, oil and grease (F, O&G), and proteins (Diez, Ramos, & Cabezas, 2012). The use of anaerobic systems for the treatment of high-rate industrial wastewaters was widely suggested due to their capacity to reduce high organic loads exerted from wastewater application, maintain minimum sludge production, and manage to recover energy (Hamza, Iorhemen, & Tay, 2016; Shende & Pophali, 2021; van Lier, 2008). Food waste discharged from food industries is rich in carbohydrates and proteins and therefore considered an attractive energy source.
Given the low cost and space requirements of anaerobic digesters, they were the most preferred alternative for industrial wastewater treatment of high COD and solids content (Harris & McCabe, 2020). Treating food waste at an OLR of 2.1g-COD/L-d had low VFA concentrations during the startup phase, however, because of prolonged operations, VFAs and soluble COD accumulated caused a 35% drop in methane yield translating into a much lower energy recovery (Tonanzi et al., 2018). The digestion of food waste with an influent COD of up to 4 g/L, reached 90% COD removal and had VFA concentrations as low as 35 mg/L (He, Xu, Li, & Zhang, 2005). Other anaerobic processes such as the UASB were also employed due to their low energy consumption and sludge generation. A UASB treating meat industry wastewater achieved more than 85% COD removal. At increased OLR, the COD removal decreased to 65% and there was a noticeable 17% lower biogas yield (Kwarcia-Kozłowska, Bohdziewicz, Mielczarek, & Krzywicka, 2011). Generally, UASBs achieve more than 60% COD removal but effluent discharged can still fail to meet standard limits.

Given the negative effect of the high loading rate (unavoidable for high-strength wastewater) on the performance of the reactor, AnMBRs were suggested for treatment of industrial wastewater at high loadings (Chan et al., 2009). AnMBRs can operate at very high SRT values which is a factor that reduces sludge production while increasing both methane production and COD removal (Dong, Parker, & Dagnew, 2016; Yurtsever, Calimlioglu, & Sahinkaya, 2017). The treatment of industrial wastewater in an AnMBR had a 98% COD removal and a production of 0.31L of methane/g of COD removed (Dereli et al., 2012). An AnMBR treating palm oil mill wastewater with an influent COD of more than 60 g/L, achieved 96% COD removal at an OLR as high as 11 g-COD/L-d, with a methane yield of 0.45 L/g-COD removed and a 68% methane content (Abdurahman et al., 2011). A submerged AnMBR treating raw snack food
wastewater of high oil and grease content (O&G) achieved 97% COD removal and 100% O&G removal (Diez et al., 2012).

2.1.2. Anaerobic treatment of slaughterhouse wastewaters

Slaughterhouse wastewater (SHW) refers to wastewater discharged from all types of livestock industry. SHW typically has high organic content, is composed of blood, oil, fats, proteins, and sulfate detected at varying levels depending on the wastewater type and source. It can also contain high levels of pathogens, as well as inorganic nitrogen and phosphorus (C. Bustillo-Lecompte & Mehrvar, 2017). Compared to industrial wastewaters, slaughterhouse wastewater exhibits higher levels of lipids and proteins, that can potentially affect the anaerobic biodegradability and methanogenic potential of the biomass (Palatsi, Vinas, Guivernau, Fernandez, & Flotats, 2011). In addition, the high O&G content can affect COD causing accumulations of long chain fatty acids (LCFAs), volatile fatty acids (VFAs) and organic matter (Diez et al., 2012). Due to their low biodegradability, O&G can’t be adsorbed into the biomass but instead accumulate on the surface of the sludge granules and inhibit the performance (Miranda, Henriques, & Monteggia, 2005). Relatively, the presence of VFAs and sulfate can inhibit methanogenesis (Guo et al., 2021; Shende & Pophali, 2021) and excessive biomass growth can affect the removal efficiency (Galib, Elbeshbishy, Reid, Hussain, & Lee, 2016).

The performance of a UASB treating animal SHW with an influent COD of 4500 mg/L was stable at 90% COD removal, with VFAs below limits, and yielding methane of 0.27 L/g-COD operating at OLR of 15g-COD/L-d (Nacheva, Pantoja, & Serrano, 2011). Operated at an HRT of 12 hours, the UASB treating synthetized wastewater representing slaughterhouse wastewater attained 86% COD removal (C. F. Bustillo-Lecompte & Mehrvar, 2015; Chollom, Rathilal, Swalaha, Bakare, & Tetteh, 2018;
Saghir & Hajjar, 2018). Treatment of pig and cattle SHW concluded that while the system achieved a 92% COD removal and 58% O&G removal at relatively low O&G/COD ratio in the influent, one that was higher than 20% caused a biomass washout and led to system failure (Miranda et al., 2005). An anaerobic digester treating cattle SHW, operated at an HRT of 24 hours showed that the system had more than 90% COD removal at an OLR value up to 10g-COD/L-d (Basitere, Rinquiest, Njoya, Sheldon, & Ntwampe, 2017; Musa, Idrus, Harun, Tuan Mohd Marzuki, & Abdul Wahab, 2019). Another treating swine slaughterhouse rich in lipid reached a maximum methane yield equal to 282 mL/d, but this study showed that a high substrate application can drastically reduce the methane production to lower than 125 mL/d, associated with VFAs and LCFAs accumulation (Cuetos et al., 2008; Ning et al., 2018). An anaerobic digester treating SHW had a 10% drop in methane yield when the protein content was increased (Palatsi et al., 2011). Variations in operational parameters of the UASB (OLR & HRT) can develop performance issues and diminished biogas production. The reduction of the HRT from 36 hours to 6 hours caused the COD removal efficiency to drop from 83% to 36% (C. F. Bustillo-Lecompte & Mehrvar, 2015; Chollom et al., 2018; Saghir & Hajjar, 2018). Similarly, COD removal from an anaerobic digester decreased from 90% to 70% when the OLR increased from 10 g-COD/L-d to 16g-COD/L-d (Basitere et al., 2017; Musa et al., 2019), and irreversible membrane fouling was recorded when the OLR exceeded 3g/L-d (Galib et al., 2016).

2.1.3. Anaerobic treatment of poultry slaughterhouse wastewater (PSW): a challenging case

The interest in poultry SHW discharged from chicken industry is part of this study. Classified as SHW, PSW can be particularly challenging; it has a notably higher
content of fat and red blood cells which form a stable suspension in liquids that is more difficult to degrade compared to other animals wastewaters (Yordanov, 2010). Anaerobic digestion was proven efficient for treating wastewaters rich in proteins and lipids, and is a potential consideration for poultry SHW treatment (Palatsi et al., 2011). However, high levels in PSW influent can cause VFA and ammonia accumulation over time and induce performance challenges (Guo et al., 2021).

Still, anaerobic treatment of PSW remains of great interest due to its capacity to simultaneously attain sufficient effluent quality and produce recoverable energy in the form of biogas (Vítězová et al., 2020). But previous observations have determined that they can still experience performance drawbacks when operational parameters are modified (Diez et al., 2012). When the anaerobic digester treating PSW was operated at an OLR of 1.7g-COD/L-d, VFA accumulation (acetate, propionate, and butyrate) exceeded 4000 mg/L in the effluent after 30 days compared to 500 mg/L initially. At the same time, biogas yield decreased from 2.4 L/d to less than 0.5 L/d produced with only 45% methane content. When the same setup was first operated at an OLR of 0.9g-COD/L-d to allow for sludge acclimation, the system achieved 61% fat removal at doubled OLR, with only trace levels of VFAs and LCFAs in the effluent and produced up to 4.3L of biogas per day with 65% methane content (Cuetos et al., 2008). Performance issues were resolved through the integration of combined systems: the implementation of membrane technologies as post-treatment was useful to attain more than 97% COD and fat and O&G removal while requiring low operation costs and energy requirements (Meyo, Njoya, Basitere, Ntwampe, & Kaskote, 2021). The addition of an ultrafiltration membrane unit to PSW treatment using SGBR increased the removal of the combined system from 93% and 90% to 98% and 92.4% for COD and FOG removals respectively (Basitere et al., 2017).
2.1.4. Anaerobic treatment of olive mill wastewater: a challenging case

Classified as agro-industrial wastewater, olive mill wastewater (OMW) can cause severe environmental impacts when discharged into the surface water. This requires the need for treatment of OMW to diminish the organic load. OMW is mainly rich in phenols, recalcitrants, pectin, and enzymes that can disturb the system performance. Generally, anaerobic digestion can be sensitive to the high phenolic content of the OMW which can cause a certain level of toxicity/phytotoxicity (Rahmanian, Jafari, & Galanakis, 2014). Previous studies suggested the use of pre-treatment systems or two-phase anaerobic co-digestion to lower phenolic content by up to 78%, and therefore reducing the impact on the digestion process (Fezzani & Cheikh, 2010; Sabbah, Marsook, & Basheer, 2004).

2.1.5. AnMBRs for SHW treatment: efficient removal, energy recovery, and challenges

Traditional anaerobic treatment methods (e.g., SGBRs) can be benefitted from by their combination with membrane separation techniques. Further, given that AnMBRs provide efficient hydrolysis, they have a higher methane production potential compared to other anaerobic treatment systems (Almandoz, Pagliero, Ochoa, & Marchese, 2015; Fatima, Du, & Kommalapati, 2021; Wandera et al., 2018). Since hydrolysis is often found to be a rate limiting step in the performance of the reactor, the use of continuously stirred tank reactor (CSTR) type as AnMBRs could enhance the efficient hydrolysis process. AnMBRs can simultaneously fully retain microbial communities and achieve high particulate and pathogens removal. (Almandoz et al., 2015; Fatima et al., 2021; Wandera et al., 2018).

Previous results of a submerged AnMBR treating SHW showed 97% COD removal, and 65% and 44% of total phosphorous and total nitrogen removal respectively (Aslan,
Ari, Gülşen, Yildiz, & Saatçi, 2013; Gurel & Buyukgungor, 2011; Jensen et al., 2015; Musa et al., 2019). The same system treating cattle SHW had 95% COD removal, more than 90% VFA removal and exceeded 78% removal of nutrients including phosphorous and nitrogen (Aslan et al., 2013; Gurel & Buyukgungor, 2011; Jensen et al., 2015; Musa et al., 2019). An AnMBR treating livestock industrial wastewater with significant O&G content had 95% COD removal, 97% O&G removal, and more than 75% nutrients removal (Aslan et al., 2013). On the other hand, only few studies looked into AnMBRs treating PSW. An AnMBR treating chicken SHW had over 90% COD removal and produced 0.32 L of methane/g-COD removed operating at an OLR up to 8 g/L-d (Fuchs, Binder, Mavrias, & Braun, 2003). Nevertheless, AnMBRs can face performance drawbacks including membrane fouling from organics accumulation on membrane pores (Aslan et al., 2013), and VFA accumulation (A. Saddoud & Sayadi, 2007) that affect the reactor performance as well as the energy balance of the system through increased expenditures and methanogenic inhibition (Awe et al., 2016; Loganath & Senophiyah-Mary, 2020). An AnMBR treating wastewater from a snacks factory maintained a stable performance up to an OLR of 17gCOD/L-d. At an applied OLR of 36 g/L-d and a 20 times higher O&G content, the membrane fouling was 10 times higher, from the accumulation of oily materials especially on the membrane surface (Ramos, Zecchino, Ezquerra, & Diez, 2014).

2.1.5.1. High energy recovery from AnMBRs treating high-strength wastewaters

AnMBRs treating SHW of high organic content possess a high potential for energy recovery and offer a replacement for fossil fuels through methane production and thus reduces carbon dioxide emissions (Salminen & Rintala, 2002).
An anaerobic digester treating food waste with an influent COD of 1.5 g/L, had a net positive energy balance of more than 0.37 kWh/m³, 69% higher than that recovered from a conventional activated sludge treatment system, while an AnMBR treating the same wastewater produced the equivalent of 0.45 kWh/m³ of wastewater (Becker Jr, Yu, Stadler, & Smith, 2017). The AnMBR treating meat processing wastewater with an influent COD of 4.4 g/L operated at varying operating conditions, reached maximum recoverable energy of 5.1 kWh/m³ of treated wastewater at highest OLR of 3.2 g/L-d (Galib et al., 2016).

2.1.5.2. Microbial adaptation to specific wastewater types and membrane fouling are main challenges faced in AnMBRs treating industrial wastewater

The complex composition of the treated industrial wastewater affects the microbial composition of the system (Vítězová et al., 2020). Although limited work has assessed the impact of SHW treatment in AnMBRs on their microbial communities, it is certain that the extended exposure to SHW leads to changes in microbial communities through microbial adaptation to operational changes (Fuchs et al., 2003; Palatsi et al., 2011). Key microbial communities that include methanogens can also undergo functional shifts, which ultimately affect methane production rates (A. Saddoud & Sayadi, 2007). In fact, dominant methanogens shifted from acetoclastic to hydrogenotrophic when the loading rate increased, methanogenic activity was inhibited and accompanied with a 95% drop in bio-methane production (Guo et al., 2021). Existing studies showed that hydrogenotrophic methanogens can be dominant in anaerobic digestion and under certain circumstances while for others, *Methanothrix* (acetoclastic) provided the highest contribution to methane production from anaerobic reactors treating SHW (Senés-Guerrero et al., 2019).
Given the high organic content of SHW, organics are prone to accumulate on the membrane surface, observed through the formation of a biofilm layer on the membrane causing pores blockage (membrane fouling) and leading to a drop in permeate flux (Aslan et al., 2013). In that context, the AnMBR treating industrial wastewater and meat processing wastewater reached irreversible membrane fouling accompanied with a flux decline at high applied OLR (Dereli et al., 2012; Galib et al., 2016; He et al., 2005). Simultaneously, the lower flux increased the power requirements for membrane scouring which reduced the overall energy recovery benefits. However, net energy balance was still achievable when fouling occurred (Loganath & Senophiyah-Mary, 2020), but it still is an undesirable aspect given the increase in operational and maintenance costs of the AnMBR (Hamza et al., 2016).

2.1.5.3. VFA and ammonia accumulation: inhibition of methanogens

Along with membrane fouling, high organic loadings can cause VFA and ammonia accumulation which in turn impact the reactor performance. While the system reached 93.7% COD removal at average applied OLR, the removal decreased to 53% when the OLR was increased, resulting from high VFA accumulations (A. Saddoud & Sayadi, 2007). A submerged AnMBR treating food wastewater with a doubled OLR caused a 39% drop in COD removal resulting from high VFAs accumulation (up to 1000 mg/L), and a low methane yield of 0.136 L/g-COD removed compared to the theoretical 0.385 L/g-COD at 37°C (He et al., 2005).
2.2. **Anaerobic treatment of low-strength wastewater: various available mainstream treatment options**

Low-strength wastewaters could refer to domestic wastewater discharged from households with a COD value of 400-500 mg/L, or to municipal wastewater resulting from the combination of domestic discharge, precipitations and some industrial residues with an overall COD falling in the range of 500-1000 mg/L (Dvořák et al., 2016; Lin, Chen, Wang, Ding, & Hong, 2011). The low organic content of these types of wastewaters reflects the low energy production potential.

2.2.1. **AnMBRs treating low-strength wastewaters: efficient removal and energy recovery**

Low-strength wastewaters generated from households have a common composition in terms of solids content, pollutants, and antibiotic resistant genes. AnMBRs treating low-strength wastewaters can produce a high quality (>85% COD removal), and particle free permeate suitable for non-potable reuses while maintaining minimum sludge production (Ahlem Saddoud, Ellouze, Dhouib, & Sayadi, 2007). AnMBR treating domestic wastewater achieved 99% COD removal, 97% TS removal, and reached a methane yield of 0.5 L/g of COD removed (Cheng et al., 2021). Several studies using membranes with CSTR have achieved COD removal in the range of 83-97% depending on operational HRT values (Smith, Stadler, Love, Skerlos, & Raskin, 2012).

Given its low chemical oxygen demand value and therefore low organic matter content, domestic wastewaters treated using AnMBRs have low energy production potential since the substrate to energy conversion is limited to the small amount of organics available for degradation. In that context, earlier observations have determined that the same AnMBR operating at 35°C and SRT of 360 days had an energy production of
0.42 kWh/m³ of treated domestic wastewater (Mei et al., 2016) compared to 5.1 kWh/m³ of treated industrial wastewater (Galib et al., 2016). A possible mitigation to increase energy production was the implementation of co-management treatment systems. The assessment of co-management of food waste and domestic wastewater using an AnMBR resulted in a positive net energy balance when treating domestic wastewater with a 40% food waste diversion. Further, a 100% food waste content produced a net energy balance of more than 4,000 kWh/day (Becker Jr et al., 2017).

2.2.2. Challenges facing potentials for energy recovery from AnMBRs treating low-strength wastewater

AnMBRs are still prone to several challenges on the level of energy recovery, especially when the wastewater treated is of low-strength. Common issues that form barriers against using AnMBRs as sustainable wastewater reuse technology include membrane fouling, increased energy consumption, and effluent methane emissions (Wu & Kim, 2020). It has been determined that anaerobic membrane bioreactors today consume more energy and have a higher global warming potential compared to aerobic MBRs and conventional activated sludge treatment systems (Smith et al., 2014) (Figure 1). Nonetheless, AnMBRs can provide high recoverable methane volumes, favoring them in terms of energy recovery. The long-term objective is to develop sustainable AnMBRs in the future that can provide positive energy recovery as represented on the x-axis.
Figure 1 Global warming potential and energy recovery from different treatment systems (Smith et al., 2014).

2.2.3. Dissolved methane and effluent methane oversaturation: potential causes and environmental implications

A main issue faced in AnMBR systems is the effluent methane released into the atmosphere. In fact, methane emissions from US wastewater treatment plants accounted for 0.3% of the overall GHG emissions in 2016 (S. Chen, Harb, Sinha, & Smith, 2018) and methane losses contributed to 81% of the overall global warming effect of the AnMBR (Crone, Garland, Sorial, & Vane, 2016). AnMBRs, particularly when treating low-strength wastewaters, have high methane losses in the effluent that could reach 43% (Gimenez, Marti, Ferrer, & Seco, 2012). Classified as a Green House Gas (GHG), methane released constitutes a negative environmental impact which requires more sustainable alternatives. Also, the global warming potential of an AnMBR treating domestic wastewater could generate energy losses of up to 26,000 kWh/day. (Becker Jr et al., 2017).
Methane dissolved in the effluent of the AnMBR is expected to be at least at equilibrium indicating a saturation ratio of 1 where dissolved methane in the effluent is equal to the expected calculated based on Henry’s law (Joanna Cookney et al., 2016). Henry’s law is a constant depending on the solubility of the component in its liquid phase and its partial pressure in the enclosed reactor (Crittenden, Trussell, Hand, Howe, & Tchobanoglous, 2012). Solubility depends on sludge temperature while partial pressure is a function of the methanogenic potential of the system (Bandara et al., 2011). Methane losses in the effluent translate into energy losses from the system in the form of dissolved methane released with the effluent.

2.2.4. Decline in membrane flux increases energy demand

The main challenge faced in the operations of AnMBRs is the membrane fouling control which directly decreases the transmembrane flux. Fouling develops once organic or inorganic substances accumulate on the membrane pores and cause a higher TMP (Smith et al., 2012). This results in a sharp decrease in permeate flux associated with higher energy requirement for membrane scouring, and therefore increasing the operational costs of the system (Maaz et al., 2019). Based on these observations, the only way to provide positive or even neutral energy recovery from systems with limited energy production potential would be through minimizing the energy demands for fouling control.
2.3. Potential for energy recovery from AnMBRs

Selection of the most convenient anaerobic treatment unit depends on both effluent quality and energy recovery potential. Adequately, the system is expected to meet standard limits for the permeate in terms of organic, metals, nutrients, and pathogens removal, as well as yield enough methane to allow for positive energy recovery (Aydin, Ince, & Ince, 2016). Beside successful organic and pollutants removal through biodegradation process, AnMBRs can convert 98% of influent COD to biogas with 80-90% methane content (Skouteris, Hermosilla, Lopez, Negro, & Blanco, 2012).

2.3.1. AnMBRs treating different types of wastewaters

The potential for energy recovery depends on the available strength in the treated wastewater known as the chemical oxygen demand (COD) which converts organic matter into energy available for recovery as electricity or heat (Awe et al., 2016). Potential for energy recovery from high-strength wastewater depends on the composition of the treated wastewater which differs between the different discharge sources affecting the system performance and impacting the energy production potential (Dereli et al., 2012). Low-strength wastewater generated from different sources all possess same influent characteristics and components, but the treatment system used directly impacts the energy demand for operations.

2.3.2. Components of energy recovery

Energy recovery is the deficit between the energy produced and that consumed for operation requirements. Energy production itself depends only on the recoverable methane volume from the reactor headspace (Mei et al., 2016). On the other hand, energy consumption depends on several components including energy required for membrane scouring, incorporating biogas sparging and sludge recirculation, energy
for effluent pumping, CSTR mixer, heat required for substrate heating and heat losses into the atmosphere (Shin & Bae, 2018). Theoretically, it was determined that biogas sparging for membrane scouring constitutes 70% of the total energy demand of the system but is necessary to prevent membrane fouling (Cheng et al., 2021; Martin et al., 2011). Sludge recirculation is the component with second highest energy demand and is an important operational aspect that helps stabilize the performance of the reactor (Dohdoh, 2019). Even though the energy demand for membrane scouring is relatively high, it only equates less than 10% of the energy required for the aeration process (Martin et al., 2011). Energy consumed for effluent pumping and mixing is minimal compared to membrane scouring (Cheng et al., 2021; Mei et al., 2016). Energy required for heating the substrate is the highest between all components at mesophilic or thermophilic conditions. Also, heat losses to the atmosphere are certain due to external exposure of the reactor (Lübken, Wichern, Schlattmann, Gronauer, & Horn, 2007).

2.3.3. Major parameters affecting energy consumption

As previously mentioned, several components collectively contribute to the total energy consumption, and are directly associated with performance parameters such as membrane fouling, operating thermophilic conditions, and substrate accumulations. AnMBRs treating high-strength wastewater have shown organics accumulation on membrane surface that can lead to membrane fouling, and subsequent decline in flux (Aslan et al., 2013), causing a sharp increase in the energy expenditures for membrane scouring (Loganath & Senophiyah-Mary, 2020). Also, operations under conditions that require heating to above room temperature, drastically increase the heat demand for substrate heating. The higher difference between the ambient and operating temperatures also increases the heat losses from the system into the atmosphere and
increases the energy demand for heating (Lübken et al., 2007). The heat requirement for an AnMBR treating domestic wastewater and operated at 35°C with an ambient temperature of 20°C was equal to 17.4 kWh/m³, 3 times higher than that required if the system was operated at 25°C (Mei et al., 2016).

Energy production potential raises a concern in AnMBRs treating low-strength wastewaters with limited organics available for conversion to biogas, and where energy produced is insufficient to compensate for the energy demand of the system. Methanogenic activity of the microbial communities can also affect methane production potential of the biomass: high-strength wastewaters are prone to face VFA and ammonia accumulations that inhibit methanogenic activity (Awe et al., 2016; A. Saddoud & Sayadi, 2007). For low-strength wastewaters, and even with a submerged membrane and no energy demand for recirculation, the system could not achieve a net energy recovery of more than 0.2 kWh/m³ (Kong, Li, et al., 2021).

2.4. Microbial communities affect treatment efficiency and energy recovery rates

Beside their impact on methane production potential, microbial communities can directly affect the treatment efficiency of the AnMBR represented by the COD removal, VFA concentrations, and biomass growth.

2.4.1. Communities in AnMBRs treating high-strength wastewater

Microbial communities of the biomass and the membrane biofilm are key to an efficient treatment. Influent substrate composition which dictates the microbial composition can indirectly impact the filtration performance of the membrane while the loading rate applied on the anaerobic digester has the potential to decrease the microbial diversity of the biomass and therefore reducing the reactor performance
At the same time, microbial communities undergo functional changes to adapt to the operational changes of the system while maintaining a stable reactor performance. Particularly, methanogens in the biomass or the membrane biofilm can shift between hydrogenotrophic (consume hydrogen or carbon dioxide to produce methane) and acetogenic (convert acetate to methane) based on types of substrates available for degradation (Palatsi et al., 2011). Acetogens are expected to develop when the OLR is increased and VFAs are prone to start accumulation, in order to assist their removal (Wijekoon, Visvanathan, & Abeynayaka, 2011). Given that PSW is rich in lipids and proteins, bacterial genus *Clostridium* responsible for their degradation dominates the anaerobic digester (Guo et al., 2021).

### 2.4.2. Communities in AnMBRs treating low-strength wastewater

Undoubtedly, microbial communities undergo functional modifications when the operating conditions of the system are varied (Rizzo et al., 2013). Unlike high-strength wastewaters, the relative abundance of specific microbial groups is a function of the system configuration, independent of the wastewater composition.

Following domestic wastewater introduction, impacted groups include methanogens and other groups. Earlier observations have determined the dominance of hydrogenotrophic methanogens such as archaeal genus *Methanobacterium* in the reactor sludge with an average relative abundance greater than 1% with low levels of *Methanosaeta* and *Methanospirillum*. *Syntrophs* presence in the sludge biomass usually grow in co-culture with hydrogen-consuming bacteria (Moustapha Harb, Wei, Wang, Amy, & Hong, 2016). In addition, communities falling under the *Syntrophaceae* family are expected to be the most abundant genera in the AnMBR samples (Zhang et al., 2019)
2.5. **Microbial safety of AnMBRs effluent for potential reuse and discharge**

2.5.1. **Pathogens and ARGs in AnMBRs treating high-strength wastewater**

Slaughterhouse wastewaters, known to be rich in both pathogens and antibiotic resistant genes (ARGs), pose unique safety concerns on the level of public health and environmental impact (Tang, Liang, Li, Zhao, & An, 2021). This requires the usage of advanced treatment systems to ensure effluent within safety limits to be discharged into free water bodies for water reuse such as agricultural irrigation, or urban and industrial purposes (Fatima et al., 2021).

It has been determined that systems treating high-strength industrial wastewater have the capacity to ensure high ARGs removal from the effluent (Fiorentino et al., 2019). Genes of interest correlated with poultry slaughterhouse wastewater include sulfonamides, *intI1*, and tetracycline resistance genes, and the 16S rRNA gene. A treatment plant equipped with advanced membrane processing systems and treating swine slaughterhouse wastewater, achieved high ARGs removals from the effluent with a total of log removal values (LRVs) falling in the range of 5-9.5 LRV for the different detected genes (Lan, Kong, Sun, Li, & Liu, 2019). The use of membrane filtration also allowed for pathogens removal from the effluent prior to discharge and reached a value of 90% removal (Liang et al., 2021), and therefore reducing the health risks from free effluent discharge.

Given that anaerobic-based membrane treatment lacks sufficient nutrients removal from its effluent (Aslan et al., 2013), it has been suggested to use the effluents of anaerobic systems, rich in nitrogen and phosphorous, as agricultural fertilizers for benefits instead of industrial manufacturing (McCarty et al., 2011). However, for the
consideration of such reuse practices to be appropriate, effluents need to be addressed from a microbial safety perspective.

Limited work has been completed on the level of assessing the proliferation of ARGs and development of pathogens in the effluents of AnMBRs treating PSW specifically under varying operating conditions.

2.5.2. Pathogens and ARGs in AnMBR treating low-strength wastewater

Antibiotic resistant bacteria (ARB) and antibiotic resistance gene (ARG) release from wastewater treatment plants results from the functional microbial communities present within the plants (Rizzo et al., 2013). ARGs detected in the influent wastewater determine the composition of resistance profiles of the treatment systems, while the type of the individual unit treatment process (i.e., aerobic, anaerobic, anoxic) impacts the nature of dominant ARGs (Tong et al., 2019). Based on these observations, it is important to understand how emerging treatment technologies, such as the AnMBR, behave with respect to antibiotic resistance element dissemination through their effluents.

Only few recent studies have addressed the antibiotic resistance profiles in AnMBRs treating real municipal wastewater sources (Kappell et al., 2018; Lou, Harb, Smith, & Stadler, 2020). Studies have provided results of high LRVs of ARGs across AnMBR effluents regardless of the presence or absence of ARGs in real wastewater influent. Previous work showed that membrane-based technologies are more efficient than conventional anaerobic treatment systems in providing safe effluents with log removal values (LRVs) of ARGs of over 3 and LRVs of pathogens of over 5 when treating municipal wastewaters (M. Harb & Hong, 2017; Kappell et al., 2018), and therefore allowing for direct water reuse for irrigation purposes. In that context, detected ARGs
were often correlated to specific pathogens present in the effluent (Zarei-Baygi, Harb, Wang, Stadler, & Smith, 2020) and should be addressed for specific wastewater influent and treatment system combinations as part of the evaluation of effluent safety.

In addition, microbial diversity can affect ARG presence in the effluents of AnMBRs treating domestic wastewater; lower diversity increases the susceptibility of the system to inhibition, which enhances ARG proliferations in the effluent (Zarei-Baygi et al., 2020).
Chapter Three

Aim and objectives

3.1. Research Aim

The aim of this work was to present a comparison of the energy recovery potential from a single operating lab-scale AnMBR treating different types of wastewaters while still providing high effluent quality. The study also aimed to highlight the main parameters of the experimental setup, as well as the key microbial communities that can impact the production potential of the reactor. In addition, it was intended to analyze the microbial safety of treated AnMBR effluents, especially for PSW which presents extreme treatment challenges in terms of safe effluent discharge.

3.2. Research objectives

1. Evaluate overall reactor performance
   1.1. Evaluate effluent quality for pollutants and pathogens removal
   1.2. Evaluate headspace methane and effluent methane production

2. Determine the impact of different types of wastewaters on the energy production potential

3. Assess the different operational and design parameters that can affect the energy production potential
   3.1. Methane content and effluent saturation affects energy production
   3.2. Microbial communities responsible for energy production
   3.3. Increased energy consumption from the system

4. Determine optimal conditions for maximum energy recovery
   4.1. Proposed experimental mitigations for improved methane production
4.2. Operational modifications for reduced energy consumption

3.3. Scope of work

The study was conducted using an AnMBR operated at 35°C and was connected to external membrane filtration units of different numbers and pore sizes during three experimental setups. The first experiment treated synthetic wastewater over 2 phases with three external microfiltration units, with the first stage consisting of the membranes operated at different permeate fluxes. The variation in operating flux allowed for a variation in transmembrane pressures between the membranes, used as basis of comparison for potential of energy recovery from the reactor. The second stage with same operating fluxes between membranes had close TMP values and also showed similar potentials for energy recovery.

The second experiment treated low-strength municipal wastewater at 35°C over 4 phases. Phase 1 operated with 2 microfiltration membrane units, Phases 2 and 3 also had 2 membranes with one microfiltration and one ultrafiltration unit, while Phase 4 operated with 2 microfiltration membrane units and one ultrafiltration unit. Temperature during Phase 4 was reduced to 30°C to reflect the overall change in the potential for energy recovery. The consecutive phases highlighted the low energy recovery from low-strength wastewater treatment with a constantly negative heat recovery potential.

The third experiment treated PSW at 35°C over 3 phases with different HRT values, and connected to a single microfiltration membrane unit. This experiment highlighted the effect of prolonged poultry exposure on the overall performance of the system, as well as its potential for energy recovery at different operating conditions.
Throughout the experiment, transmembrane pressure (TMP) and sludge pH were monitored, and effluent volumes and biogas production were measured on a daily basis. COD, VFA’s and methane content were measured at least twice a week, depending on reactor’s performance and sludge biomass was tested once a week for solids concentrations.

Olive mill wastewater (OMW) was also treated using the same AnMBR, to determine the effect of this type of wastewater on the reactor performance. The reactor achieved good effluent quality even at increased OMW COD contribution. The experiment consisted of 4 phases with different contribution of the OMW and had 2 external ultrafiltration membrane units.
Chapter Four

Methodology

4.1. AnMBR configuration and operations

A lab-scale treatment system was used for all three experiments. The operated AnMBR consisted of continuously stirred tank reactor (CSTR), with varying working volume 3-3.5 L (Chemglass Life Science, USA) between the different experiments. The reactor undergoes continuous mixing at 200 rpm using an internal impeller with curved blades, and operates at run under mesophilic conditions with temperature maintained at 35 °C inside the water-jacket at all times. The reactor was connected to external cross-flow membrane units, characteristics and number of membranes varied between the experiments. Sludge retention time (SRT) was 360 days, and sludge pH was maintained at 7. Membrane fouling was prevented through membrane scouring by continuous biogas sparging at 290 rpm across the membrane surface and continuous sludge recirculation at 690 rpm. The membrane was relaxed for 60 seconds every 59 minutes and backwashed for 20 minutes daily.

4.1.1. AnMBR treating synthetic wastewater

The operated AnMBR was connected to three external cross-flow microfiltration membrane units (MFs) connected in series, flat sheet polyvinylidene difluoride (PVDF) membranes of 0.2 µm pore size (Microdyn Nadir, Germany) and having a 57cm² effective area. The sludge used for seeding the reactor was obtained from an anaerobic digestor in Lebanon, and the reactor was then fed with synthetic wastewater with an influent COD of 1.5 g/L, at an organic loading rate between 0.9 and 1.3 g/L-d over two phases. Phase 1 consisted of operating the three membranes under different
fluxes controlled by setting different effluent pumping rates (0.5 mL/min, 0.8 mL/min, and 1.5 mL/min), and ran for 19 days. Phase 2 had the membranes operated at the same permeate flux of 0.5 mL/min for a total of 8 days. This variation will allow to analyze the difference in the performance between the membranes, between different phases as well as compare the energy potential from the system under the different conditions.

4.1.2. AnMBR treating real wastewater

The same AnMBR setup was connected to two external cross-flow microfiltration membrane units for treating real wastewater. The system was operated over 4 consecutive phases treating real wastewater: wastewater samples were collected from different sources around Lebanon and stored at 4°C prior to feeding into the reactor. Phase 1 operated with 2 microfiltration membrane units, was 41 days long and had an organic loading rate of 0.31±0.03 g/L-d. Phase 2 operated with one microfiltration and another ultrafiltration membrane unit, was 51 days long and had an OLR of 0.56±0.18 g/L-d. Similar to operations of Phase 2, Phase 3 was ran for 43 days and had an OLR of 0.53±0.15 g/L-d. Unlike previous phases, Phase 4 was operated at 30°C with three external membrane units: 2 microfiltration units and a single ultrafiltration unit connected in series. This phase was ran for 46 days and had an OLR of 0.4±0.3 g/L-d.

4.1.3. AnMBR treating PSW

The reactor was connected to a single external cross-flow microfiltration membrane unit. The operational stage consisted of 3 successive phases of PSW with gradually decreased HRT values and corresponding fluxes (Table 3). The AnMBR treated PSW collected from Tanmia development in the Beqaa District in Lebanon; 60 L of collected PSW samples, consisting of both liquid and solid portions, were stored at -20°C prior
to treatment. Raw PSW included primary settled liquid PSW and settleable solid particles. Daily desired volumes were later unfrozen according to the required influent rate and then screened for separation of solids from the liquid wastewater. The treatment process comprised anaerobic treatment of the two portions: liquid wastewater portion was fed into the reactor during the 3 operating phases for wastewater treatment using AnMBR, and the screened solids were fed into anaerobic digesters using batch reactors for assessment of potential for energy recovery from raw PSW influent. Feed was supplied to the reactor intermittently based on desired hydraulic retention time (HRT) and corresponding influent rate. Phase 1 was operated for 16 days with an OLR of 1.3±0.18 g/L-d, Phase 2 for 14 days at an OLR of 1.34±0.07 g/L-d, and Phase 3 over 9 days at an OLR of 1.86±0.22 g/L-d.

Screened solids are diluted in 10-20mL solution of a PBS (phosphate-buffer saline) buffer solution of a 7.4 pH and injected into the 500mL reactor using a syringe. Operation was over 2 phases with varying OLR values according to the amount of solids added. Phase 1 was operated for 19 days at an OLR of 1.27 g/L-d, and Phase 2 was operated for 10 days at an OLR of 2.54 g/L-d. Over the entire duration, pH and biogas production were measured on a daily basis. Soluble COD, VFA’s and methane content were measured at twice a week.

4.1.4. AnMBR treating OMW

The reactor was connected to two external cross-flow ultrafiltration membrane units. The system was operated for 4 phases with different contribution of the OMW to the overall influent COD, resulting in increased COD loadings on the reactor (Table 4). The AnMBR treated OMW collected from different olive oil extraction sites in Lebanon and stored at room temperature prior to feeding. Phase 1 was operated for 16
days with an OLR of 4.24±0.41 g/L-d with 26% contribution of the OMW to the total influent COD, Phase 2 for 9 days at an OLR of 3.7±0.12 g/L-d with 35% contribution to COD, Phase 3 over 7 days at an OLR of 4.55±0.05 g/L-d and 50% COD contribution, and Phase 4 was ran for 7 days with an OLR of 5.24±0.06 g/L-d and 70% COD contribution.

4.2. **Effluent water quality testing**

Water quality of the effluent was regularly monitored by testing chemical oxygen demand (COD) and volatile fatty acids (VFAs). COD was tested according to Reactor Digestion Method then measured on a Hach DR3900 Spectrophotometer by colorimetric determination. For batch reactors, a 5mL sludge sample was centrifuged at 12000 rpm for 10 mins, the supernatant was then collected and diluted by a factor of 1/10. Soluble COD is measured following filtration of the diluted supernatant using 0.2 µm Nylon syringe filters. VFAs (acetate, propionate, and butyrate) were determined using an ion chromatograph (882 Compact IC Plus) with a conductivity detector coupled with 858 Professional Sample Processor (Metrohm AG, Switzerland). Collected effluent samples were filtered using 0.2 µm Nylon syringe filters before being tested on a Metrosep Organic Acids - 250/7.8 (6.1005.200) column at flow 0.5 mL/min. Other compounds were tested on a Metrosep A Supp 5 - 250/4.0 (6.1006.530) column at flow 0.7 mL/min. Total suspended solids (TSS) and volatile suspended solids (VSS) were tested following APHA Standard Method 2540 (Baird, Eaton, & Rice, 2005).

4.3. **Headspace biogas and effluent methane testing**

A 300 mL sample biogas volume was taken from the reactor headspace twice a week and stored in biogas bags. To measure methane content, a 60 mL sample volume was
injected from the previously stored bag into an Agilent 7890B gas chromatograph with thermal conductivity detection (GC-TCD), with the oven set at 90 °C, and front detector temperature at 250 °C. Effluent dissolved methane was also measured on the GC-TCD after performing the headspace technique: a 30 mL effluent sample was directly collected into a syringe placed flat at the end of the permeate line of each membrane, then injected into a 155 mL flask previously sparged with nitrogen, with a gas bag connected to the cap. After injection, the flask was shaken, and the syringe was used to mix the gas content of the flask which was then heated for 10 minutes. Once the temperature of the liquid content increased, the flask was shaken again briefly to strip the dissolved methane into the gas phase and allow for excess methane volume to be collected in the gas bag used for sampling.

GC results will provide the percent of methane content in the headspace of the flask containing the effluent sample. The procedure for calculation of the effluent dissolved methane volume is listed in Equation 1 and Equation 2 in Appendix A below.

### 4.4. Estimation of energy recovery potential

Energy recovery potential was calculated from the difference between the energy produced and the energy consumed for operations.

**Energy production potential:**

\[ E_0 \ (\text{kWh/m}^3) = V \times \text{methane conversion potential} \times \frac{Q}{q} \]  \hspace{1cm} (Equation 4)

- Electricity produced from biogas: \( E_p = 0.35 \times E_0 \)
- Heat recovered from biogas: \( E_r = 0.65 \times E_0 \)

**Energy consumption:**

1. Membrane scouring:
   - Energy for biogas sparging (Verrecht et al., 2008):
     \[ E_g = \frac{p^y \times \left( \frac{Q_A}{Q_p} \right) \times \left( \frac{10^4y + p}{p} \right)^{1-y}}{2.73 \times 10^5} \]  \hspace{1cm} (Equation 5)
   - Energy for sludge recirculation (Shin & Bae, 2018):
\[ E_S = \frac{\rho g Q H}{1000 \times ex \times A} \times 10^{-3} \]  
*Equation 6*

2. Effluent pump energy consumption (Mei et al., 2016):

\[ E_p = \frac{q y h}{1000 qn} \]  
*Equation 7*

3. Energy for heating requirement:
   - Heat required for substrate heating (Mei et al., 2016):

\[ E_{H0} (\text{kWh/m}^2) = \frac{c_p \times m \times \Delta t}{q} \]  
*Equation 8*

Assuming 50% of the heat needed for the substrate heating was ensured through heat released from the effluent, then total energy needed to heat substrate is:

\[ \rightarrow E_{H1} = 0.5 \times E_{H0} \]

- Heat lost from system (including connection tubes and reactor jacket):
  - \[ K = U \cdot A \cdot \Delta t \]  
   *Equation 9*

  U=1 kWh/h.m². °C for the tubes
  
  U=0.08 kWh/h.m². °C for the reactor jacket: Consider that the reactor jacket was to be insulated using polyurethane foam. Typical thermal conductivity of polyurethane foams is in the range of 0.022-0.035KW/m·K. Assuming a thermal conductivity equal to 0.022 KW/m. °C with an exposed height of 27.5 cm, heat transfer coefficient=\[ \frac{0.022}{0.275} \] 0.08 kWh/h.m². °C

  The reactor jacket had an inner diameter of 13 cm, an outer diameter of 17.5 cm, and a total exposed height of 27.5 cm \[ \rightarrow A = 0.039 \text{ m}^2 \]

  U=0.08 kWh/h.m². °C for the connecting tubes

  The tubes used for connecting the water bath to the reactor have an inner diameter of 0.7 cm, an outer diameter of 1.1 cm \[ \rightarrow A = 0.0038 \text{ m}^2 \]

  \[ T(t) = T_s + (T_0 - T_s)e^{-Kt} \]  
   *Equation 10*

  Assuming a 1 °C drop in system temperature, with ambient temperature of 25 °C:

  \[ 34 = 25 + (35-25)e^{-Kt} \]

  \[ e^{-Kt} = \frac{9}{10} = 0.9 \]

  \[ -Kt = \ln(0.9) = -0.105 \]

  \[ t = \frac{0.105}{K} \] (time for system temperature to drop by 1 degree)

  \[ \rightarrow t_0 = \frac{24}{t} \] number of times to heat the system per day
\[ E_{H2} \left( \frac{kWh}{m^3} \right) = \left( \frac{C_p \times t_0 \times m \times \Delta t}{Q} \right) \]  
\textit{(Equation 11)}

\[ m = V \times t_0 \]

for connecting tubes: assuming the length of the connection could be ultimately dropped down to a total of 30cm: 
\[ V = 0.3 \times \frac{\pi}{4} \times 0.007 \times 0.007 = 0.0000115 \text{ m}^3 \]

for reactor jacket: 
\[ V = 0.275 \times \frac{\pi}{4} \times (0.175^2 - 0.13^2) = 0.003 \text{ m}^3 \]

\( E_{H2} \): energy from heat lost due to exposure of tubes (kWh/m\(^3\))

\( E_{H3} \): energy from heat lost due to exposure of reactor (kWh/m\(^3\))

4. Energy for mixing (Mei et al., 2016):

\[ E_B = \frac{N_{hp}}{1000q} \left( \frac{\pi}{60} \right)^3 \left( \frac{D}{1000} \right)^5 \]  
\textit{(Equation 12)}

The total energy recovered as electricity from the AnMBR would be calculated as follows:

\[ E_t = E_p - E_s - E_p - E_B \]  
\textit{(Equation 13)}

The total heat recovered from the AnMBR would be equal to:

\[ E_{Hr} = E_r - E_{H1} - E_{H2} - E_{H3} \]  
\textit{(Equation 14)}

Beside energy expenditures from the operating AnMBR system, the batch reactors required additional energy for substrate heating.

Methane production potential was calculated using the formula of \textit{Equation 3}, with influent feed rate calculated from the solids density:

\[ E_0 = \frac{E}{(m \times \rho)} \]

Solids mass is fixed for each phase (g/d)

Solids density was calculated experimentally based on the amount of solids present in a full liter of PSW: \( \rho = 27.5 \text{ g/L} \)

Heat lost for substrate heating was determined based on the volume of solution used to dilute the injected solids sample, using formula of \textit{Equation 7}. Additional heat was lost into the atmosphere due to external exposure of the reactor.

\textbf{4.5. Biomass sampling and microbial community characterization}
Samples of interest for microbial communities characterization include samples of effluent, biomass sludge, and membranes taken at the end of each phase. Experiment of interest in terms of microbial characterization in relevance to energy balance of the system is limited to the AnMBR treating PSW. Collected samples also include a single poultry influent sample stored for testing after screening the solids.

Samples were collected for DNA extraction and stored at –20 °C. Influent and effluent samples were filtered on 0.45 µm mixed cellulose ester (MCE) circular membrane filters (Millipore, USA). A 2 mL volume of sludge was taken from the reactor and centrifuged at 12,000 rpm for 10 minutes for pellet retention and stored for suspended biomass sampling. At the end of each phase when harvesting the membranes, equal cut sections of 4.75 cm x 3 cm were suspended in RNAprotect Reagent (Qiagen, USA) and stored for membrane sampling. Following the manufacturer’s protocol of DNeasy PowerSoil Kit (Qiagen, USA), DNA was extracted for all samples; concentrations and DNA qualities were measured on a Nanodrop ND 1000 spectrophotometer Version 3.3.0.

In order to characterize archaeal microbial and bacterial communities, universal primers 515F and 806R were used to amplify the targeted V4 region of the 16S rRNA genes. Amplicons were then multiplexed and sequenced on Illumina NovaSeq 6000 platform along with read lengths of paired-end 250 bp by Novogene Genomics (Singapore). Based on the Schloss Miseq SOP (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013), generated FASTQ files were analyzed on Mothur bioinformatics platform (Schloss et al., 2009). Sequences aligned by the SILVA reference database (Quast et al., 2012) were filtered using the UCHIME algorithm. Operational taxonomic units were clustered based on a 0.03 cutoff limit for average neighbor algorithm. Taxonomical classification on the genus level (Wang, Garrity, Tiedje, & Cole, 2007)
was completed by the Ribosomal Database Project (RDP) classifier database assisted with the 16S rRNA gene Training Set (Version 18).

4.6. **Antibiotic resistance gene quantification**

All DNA samples extracted from influent, effluent, suspended biomass, and membrane samples were used to establish their associated ARG profiles by quantitative PCR (qPCR). Targeted ARGs are specified to represent genes commonly found in slaughterhouse wastewater and that are resistance to multiple antibiotic classes; those genes include tetracyclines (*tet*), sulfonamides (*sul*), and class 1 integron-integrase gene (*intI1*) (Pereira, Paranhos, de Aquino, & Silva, 2021). qPCR standards were prepared using the amplification of the targeted genes after extracting DNA from several poultry influent samples and extracting gel bands after electrophoresis. qPCR was performed on a CFX Connect Real-Time PCR Detection System (BioRad, USA) with Forget-Me-Not qPCR Master Mix (Biotium, USA). Amplicon specificity was determined based on the melt curve analysis; achieved by increasing temperatures from 65 to 95 °C at 0.5 °C increments. Details on applied qPCR procedure, primer sequences, thermocycling conditions and amplicon sizes used for qPCR quantification are shown in **Appendix A**.
Chapter Five

Results

5.1. Overall reactor performance
5.1.1. AnMBR treating synthetic wastewater

The same AnMBR system was used for treatment of different types of wastewaters (synthetic, domestic, and industrial). In all three studies, the reactor achieved a stable overall performance while providing high effluent quality and pollutants removal.

Table 1. Operating parameters and performance results for synthetic wastewater treatment. The three MF membranes used are noted as A, B, and C referring to membranes of Phase 1 with effluent pumping rates of 0.4, 0.8, and 1.5 mL/min respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (d)</td>
<td>1.32±0.28</td>
<td>1.41±0.2</td>
</tr>
<tr>
<td>OLR (g-COD/L-d)</td>
<td>1.2±0.15</td>
<td>1.16±0.22</td>
</tr>
<tr>
<td>COD loading (g/d)</td>
<td>3.32</td>
<td>2.74</td>
</tr>
<tr>
<td>% COD removal</td>
<td>97±1.4%</td>
<td>98.7±0.6%</td>
</tr>
<tr>
<td>VFAs (mg/L)</td>
<td>8-14</td>
<td>13-15</td>
</tr>
<tr>
<td>VSS (g/L)</td>
<td>5.83±0.6</td>
<td>6.3±0.7</td>
</tr>
<tr>
<td>Flux (LMH)</td>
<td>4.04</td>
<td>5.25</td>
</tr>
<tr>
<td></td>
<td>6.83</td>
<td>4.78</td>
</tr>
<tr>
<td></td>
<td>6.87</td>
<td>5.06</td>
</tr>
<tr>
<td>TMP (inHg)</td>
<td>0.053</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.34</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>14.6</td>
<td>0.56</td>
</tr>
</tbody>
</table>

The first experiment treated synthetic wastewater of 1.5 g/L influent COD, using an AnMBR with three external membrane units operated over 2 phases. Overall, temperature was kept at 35°C and pH had an average of 7±0.14 over the 2 phases. In Phase 1, the three membranes were operated at different fluxes and developed various transmembrane pressure values while the same operating flux was maintained for all three during Phase 2. Based on the operating conditions, the membrane with the highest flux had the highest recorded average transmembrane pressure of more than 14 inHg. The reactor maintained stable performance during both phases, with a COD removal
greater than 97%, and VFA concentrations (acetate, propionate, butyrate) lower than 15 mg/L for all three membranes. There was a considerable increase in the concentration of VSS: from 5.8 g/L in Phase 1 to 6.3 g/L in Phase 2, represented in a 9% higher TSS to VSS ratio.

5.1.2. AnMBR treating real wastewater

Table 2. Operating parameters and performance results for real wastewater experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment 1 (Selaata/Jounieh)</th>
<th>Treatment 2 (Chekka)</th>
<th>Treatment 3 (Selaata)</th>
<th>Treatment 4 (Chekka)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (d)</td>
<td>1.57±0.14</td>
<td>1.56±0.42</td>
<td>1.25±0.18</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>OLR (g-COD/L-d)</td>
<td>0.31±0.03</td>
<td>0.56±0.18</td>
<td>0.53±0.15</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>COD loading (g/d)</td>
<td>0.92</td>
<td>1.03</td>
<td>1.5</td>
<td>2.15</td>
</tr>
<tr>
<td>% COD removal</td>
<td>85.9±3%</td>
<td>88±4%</td>
<td>86.2±6%</td>
<td>86±4%</td>
</tr>
<tr>
<td>VFAs (mg/L)</td>
<td>&lt;60</td>
<td>-</td>
<td>&lt;20</td>
<td>&lt;10</td>
</tr>
<tr>
<td>VSS (g/L)</td>
<td>5±0.7</td>
<td>4.1±0.4</td>
<td>4.5±0.5</td>
<td>7.2±0.6</td>
</tr>
<tr>
<td>Flux (LMH)</td>
<td>6.83</td>
<td>6.68</td>
<td>6.36</td>
<td>5.52</td>
</tr>
<tr>
<td>TMP (inHg)</td>
<td>2.57</td>
<td>4.07</td>
<td>0.24</td>
<td>3.36</td>
</tr>
</tbody>
</table>

After reaching stable conditions under synthetic feeding, the reactor was fed with real wastewater over 4 phases with varying operating conditions. The temperature and pH of the system were kept at 35°C and 7.1±0.2 respectively during the first 3 phases, the temperature was dropped to 30°C in Phase 4. Summarized performance parameters are shown in Table 2. TMP values did not exceed 6 inHg during the entire experiment, and the reactor managed to maintain high effluent quality. The system reached stable COD removal of more than 86% over the 4 phases, with no significant levels of VFAs in the effluent except in Phase 1, attributed to the introduction of real wastewater. VSS concentrations had the same trend with lowest concentration in Phase 2, and highest
of 4.7 g/L in Phase 3, correlated to a gradual increase in VSS to TSS ratio between the 3 Phases, and therefore indicating biomass growth.

5.1.3. AnMBR treating poultry SHW

Table 3. Operating parameters and performance results for PSW experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (d)</td>
<td>6.00</td>
<td>5.00</td>
<td>3.00</td>
</tr>
<tr>
<td>OLR (g-COD/L-d)</td>
<td>1.3 ± 0.18</td>
<td>1.34 ± 0.07</td>
<td>1.86 ± 0.22</td>
</tr>
<tr>
<td>COD loading (g/d)</td>
<td>4.63</td>
<td>4.3</td>
<td>6</td>
</tr>
<tr>
<td>% COD removal</td>
<td>95.1 ± 0.6%</td>
<td>93.6 ± 2.6%</td>
<td>96.0 ± 0.2%</td>
</tr>
<tr>
<td>VFAs (mg/L)</td>
<td>80-230</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>VSS (g/L)</td>
<td>4.55 ± 0.20</td>
<td>4.10 ± 0.30</td>
<td>4.73 ± 0.02</td>
</tr>
<tr>
<td>Flux (LMH)</td>
<td>3.34 ± 1</td>
<td>4.86 ± 0.48</td>
<td>7.24 ± 0.54</td>
</tr>
<tr>
<td>TMP (inHg)</td>
<td>0.04</td>
<td>0.86</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids mass (g/d)</td>
<td>0.4</td>
<td>0.93</td>
</tr>
<tr>
<td>COD mass* (g/d)</td>
<td>0.54</td>
<td>1.27</td>
</tr>
<tr>
<td>OLR (g-COD/L-d)</td>
<td>1.27</td>
<td>2.54</td>
</tr>
<tr>
<td>VSS (g/L)</td>
<td>3.8</td>
<td>4</td>
</tr>
<tr>
<td>Soluble COD (mg/L)</td>
<td>540</td>
<td>1188</td>
</tr>
</tbody>
</table>

*: refer to Appendix A for detailed calculations

The pre-experimental phase of synthetic wastewater feeding achieved stable conditions. The reactor was then fed with PSW over 3 phases of gradually decreasing HRT values. The temperature and pH of the reactor were stable at 35°C and 7.1 ± 0.2 respectively at all times. The influent PSW includes only the liquid portion of the previously collected and frozen poultry samples. Summarized operation and performance parameters are shown in Table 3. TMP values did not exceed an average of 0.9inHg during the entire experiment, and PSW did not impact the overall performance. The system had stable COD removal of up to 96%. Although
slaughterhouse wastewater has the potential to cause VFA accumulations and inhibit anaerobic digestion (Jensen et al., 2015), there were no significant levels of VFAs detected in the reactor effluent except in Phase 1 of poultry treatment. The peak concentration of 230 mg/L is associated with the shock of poultry introduced, having a high acetate concentration of 376 mg/L, then managing to attain sufficient removal and decreasing to 31 mg/L at the end of Phase 3, corresponding to more than 90% removal. VSS concentrations decreased from 4.5 to 4.1 g/L in Phase 1, then increased to 4.7 g/L in Phase 3, correlated to biomass growth.

On the other hand, screened solids were fed to a separate batch reactor over 2 phases of increasing OLR values and COD loadings. Operating conditions and performance results, including solids feeding and COD concentrations are shown in Table 3. Phase 2 had constant biomass concentrations, but soluble COD exhibited accumulations at the end of the phase.
5.1.4. AnMBR treating olive mill wastewater

Table 4. Operating parameters and performance results for olive mill wastewater experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT (d)</td>
<td>3.99±0.29</td>
<td>4.8±0.3</td>
<td>4±1.6</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td>OLR (g-COD/L-d)</td>
<td>4.24±0.41</td>
<td>3.7±0.1</td>
<td>4.5±0.1</td>
<td>5.2±0.1</td>
</tr>
<tr>
<td>%COD contribution</td>
<td>26%</td>
<td>35%</td>
<td>50%</td>
<td>70%</td>
</tr>
<tr>
<td>COD loading (g/d)</td>
<td>14.96</td>
<td>14.2</td>
<td>18.1</td>
<td>20</td>
</tr>
<tr>
<td>% COD removal VSS (g/L)</td>
<td>85.7±3.9</td>
<td>92.8±0.8</td>
<td>91.8±0.1</td>
<td>93.1±0</td>
</tr>
<tr>
<td>UF/M</td>
<td>5.2±0.63</td>
<td>7±0</td>
<td>9.43±0</td>
<td>12.9±0</td>
</tr>
<tr>
<td>UF/T</td>
<td>28±4</td>
<td>89±111</td>
<td>27±4</td>
<td>28±3</td>
</tr>
<tr>
<td>UF/M</td>
<td>22±2</td>
<td>24±3</td>
<td>22±0</td>
<td>25±0</td>
</tr>
<tr>
<td>UF/T</td>
<td>22±0</td>
<td>25±0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetate (mg/L)</td>
<td>59±36</td>
<td>122±98</td>
<td>24±3</td>
<td>30±7</td>
</tr>
<tr>
<td>Propionate (mg/L)</td>
<td>30±0</td>
<td>61±32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Butyrate (mg/L)</td>
<td>3.64</td>
<td>3.75</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Flux (LMH)</td>
<td>3.64</td>
<td>3.3</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>TMP (inHg)</td>
<td>0.37</td>
<td>0.33</td>
<td>0.44</td>
<td>0</td>
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</tbody>
</table>

Although OMW is expected to cause toxicity for the system from high phenolic content, the results of our experiment showed constant performance even at increased COD loading. At highest COD loading of 20g/d, the COD removal was maintained at 93%, and VFA concentrations were low in the effluent of both membranes. Biomass growth was gradually increasing over the phases with the increased OLR.
5.2. Methane content and biogas production:

5.2.1. AnMBR treating low-strength wastewater

Table 5. Methane production from AnMBR treating synthetic wastewater. The three MF membranes used are noted as A, B, and C referring to membranes of Phase 1 with effluent pumping rates of 0.4, 0.8, and 1.5 mL/min respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane volume (mL/d)</td>
<td>1153±221</td>
<td>988±143</td>
</tr>
<tr>
<td>Expected methane (mL/d)</td>
<td>1290±97</td>
<td>1101±152</td>
</tr>
<tr>
<td>% Methane</td>
<td>76.9%</td>
<td>78.5%</td>
</tr>
<tr>
<td>Methane yield (L/g-COD)</td>
<td>0.34±0.06</td>
<td>0.36±0.12</td>
</tr>
<tr>
<td>Effluent methane (mL/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.8</td>
<td>4.5</td>
</tr>
<tr>
<td>B</td>
<td>5.7</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>13.3</td>
<td>6</td>
</tr>
<tr>
<td>Saturation ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.71</td>
<td>0.65</td>
</tr>
<tr>
<td>B</td>
<td>0.84</td>
<td>0.78</td>
</tr>
<tr>
<td>C</td>
<td>1.9</td>
<td>0.88</td>
</tr>
</tbody>
</table>

During the synthetic wastewater treatment, the headspace methane volume produced in Phase 1 was 1.1±0.2 L/d. Methane content in the headspace was equal to 76.9% and average methane recovered was 344±61 mL/g-COD removed. Saturation ratio was close to 1 for membranes A and B operating at relatively lower flux, while membrane C had a ratio of 2 attributed to the higher operating flux. During Phase 2, actual methane volume produced was 0.99±0.14 L/day, slightly lower than that produced in Phase 1 due to the lower COD loading. Methane content in the headspace was 78.5% and average methane recovered was equal to 360±120 mL/g-COD removed. During both phases, the methane yield was close to the theoretical value of 382 mL/g-COD. Saturation ratio for all membranes was lower than equilibrium concentrations with an overall average of 0.77±0.1. Comparing both phases, the biomass growth of Phase 2 (Table 1) didn’t affect the methane yield of the system.
Table 6. Methane production from AnMBR treating real wastewater.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment 1 (Selaata/Jounich)</th>
<th>Treatment 2 (Chekka)</th>
<th>Treatment 3 (Selaata)</th>
<th>Treatment 4 (Chekka)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane volume (mL/d)</td>
<td>289±121</td>
<td>348±130</td>
<td>261±60</td>
<td>590±160</td>
</tr>
<tr>
<td>Expected methane (mL/d)</td>
<td>297±39</td>
<td>439±109</td>
<td>467±148</td>
<td>654±134</td>
</tr>
<tr>
<td>Methane yield (L/g-COD)</td>
<td>0.38±0.08</td>
<td>0.29±0.06</td>
<td>0.24±0.07</td>
<td>0.33±0.08</td>
</tr>
<tr>
<td>% Methane</td>
<td>78.5±4%</td>
<td>84±5%</td>
<td>83±3%</td>
<td>88±4%</td>
</tr>
<tr>
<td>Effluent methane (mL/L)</td>
<td>MF 20.5 22.2</td>
<td>MF 19.1 21.5</td>
<td>MF 15.8 23.6</td>
<td>MF 21.6 15.9 22.1</td>
</tr>
<tr>
<td>Saturation ratio</td>
<td>1.5 1.5</td>
<td>1.6 1.7</td>
<td>2 3</td>
<td>1.15 0.91 1.29</td>
</tr>
</tbody>
</table>

Based on the different COD loadings, methane headspace volumes were varying between the phases. Although Phase 3 had a higher COD loading, methane volume was 10% lower than that of Phase 1 with higher COD, and also 44% lower than expected methane production. There was a gradual increase in methane content over the duration of the experiment where it increased from 78% in Phase 1 to 89% at the end of Phase 4. After initial exposure to real wastewater, methane yield was constant at 0.38 L/g-COD, but decreased over the duration of the experiment to reach a minimum of 0.24 L/g-COD in Phase 3, then reached an average of 0.33±0.08 in Phase 4. For the AnMBR treating real wastewater, the effluent methane volume was higher compared to the synthetic wastewater even at a relatively lower saturation ratio of 1.5.
5.2.2. AnMBR treating high-strength wastewater

Table 7. Methane production from AnMBR treating PSW.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane volume (mL/d)</td>
<td>1726±353</td>
<td>1810±274</td>
<td>1739±232</td>
</tr>
<tr>
<td>Expected methane (mL/d)</td>
<td>1650±212</td>
<td>1590±20</td>
<td>2240±23</td>
</tr>
<tr>
<td>Methane yield (L/g-COD)</td>
<td>0.37±0.07</td>
<td>0.38±0.07</td>
<td>0.28±0.04</td>
</tr>
<tr>
<td>% Methane</td>
<td>72.40±6.6</td>
<td>72.40±2.1</td>
<td>74.55±6.1</td>
</tr>
<tr>
<td>Effluent methane (mL/L)</td>
<td>11.2</td>
<td>13.0</td>
<td>10.4</td>
</tr>
<tr>
<td>Saturation ratio</td>
<td>0.74</td>
<td>0.72</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Methane production was near stable and in the range of 1.7-1.8 L/d, and methane content was higher than 72% during the three phases of the experiment. On the other hand, there were some noticeable changes in methane yield at the end of Phase 3. Earlier observations concluded that poultry treatment could potentially inhibit the methanogenic activity of the biomass (C. Chen et al., 2016; Jensen et al., 2015) and therefore disturb methane yield. Our results showed that after initial exposure to poultry (Phase 1), methane yield on the basis of per gram of COD removed was unchanged compared to the startup phase but reached a 38% drop by the end of Phase 3 with a minimum of 0.28 L/g-COD. Simultaneously, a contrary trend was recorded in the sludge biomass concentrations. There was a 10% depletion in volatile solids concentrations between Phases 1 and 2, followed by a 15% growth in Phase 3, also attributed with the highest difference between expected and produced methane volumes where the produced was equal to 77% of the expected volume. Saturation ratios were near equilibrium during the three phases and effluent methane volumes
were around 50% lower than those recorded from the effluents of the AnMBR treating low-strength wastewater.

As for the batch reactors used for solids digestion, the methane yield was 15% lower when the loading was doubled in Phase 2, while Phase 1 had the highest methane yield of 233 mL/g-COD. The significant decrease in methane production in this case can be attributed to the accumulation of soluble COD (result of possible high ammonia concentrations) which indicates the lower degradation of organic matter.

**Table 8. Methane production from AnMBR treating olive mill wastewater.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane volume (mL/d)</td>
<td>4770±430</td>
<td>3310±0</td>
<td>5360±0</td>
<td>5548±150</td>
</tr>
<tr>
<td>Expected methane (mL/d)</td>
<td>4300±680</td>
<td>5050±106</td>
<td>6353±80</td>
<td>7631±0</td>
</tr>
<tr>
<td>Methane yield (L/g-COD)</td>
<td>0.37±0.08</td>
<td>0.25±0.06</td>
<td>0.32±0.07</td>
<td>0.3±0.08</td>
</tr>
<tr>
<td>% Methane</td>
<td>65.2±0.7</td>
<td>57.5±0</td>
<td>65.4±0</td>
<td>63.9±1.2</td>
</tr>
</tbody>
</table>

During the 4 phases of olive mill wastewater feeding, major variations were observed in the methane volumes produced from the reactor headspace especially in Phase 2 compared to other phases. Methane yield decreased from 0.37 L/g-COD in Phase 1 to 0.25 L/g-COD in Phase 2, 35% lower than methane yield expected at 25°C. Simultaneously, methane in the headspace reached a minimum of 57% in Phase 2, and a maximum of 65% in Phases 1 and 3. It was also noticeable that the difference between methane produced and expected was highest during Phase 2, represented in the graph of Figure 2.
Figure 2. Methane headspace volumes and expected methane volume in the AnMBR treating olive mill wastewater.

The figure above shows the difference between the methane headspace volume and the expected methane volume based on the measured %COD removal. Produced methane was 35% lower than the expected volume in Phase 2, while the values were closer for Phases 1 and 3. As for Phase 4, the increased contribution of the olive mill wastewater to the influent COD and the relatively higher COD loading caused a slight decrease in the methane volume produced from the headspace of the reactor.

5.3. Dissolved methane and effluent saturation of AnMBR treating low-strength wastewater

The concern in methane dissolved in the effluent rises when the AnMBR is treating low-strength wastewaters in which case the % of methane losses in the effluent out of the total produced significantly increases compared to systems treating high-strength wastewaters. The high methane losses in the effluent have contribution to greenhouse gas emissions and can also cause energy losses from the system.

Effluent methane volumes depend on several performance factors such as the transmembrane pressure, percentage and volume of methane in the reactor headspace.
Transmembrane pressure was one of the major factors contributing to methane super saturation in the effluent. Saturation ratios are calculated using equation 3 shown in Appendix A. During Phase 1 of synthetic feeding, membrane C developed the highest TMP with average of 14.6 inHg and had the higher oversaturation ratio of 2 (Figure 3(A)) compared to the lower values of 0.7 and 0.8 of membranes A and B. On the other hand, saturation ratios were in the range of 0.7-1 during Phase 2 for all three membranes when they were operated at similar lower flux values.
At higher saturation ratios measured in Phase 1, the total methane volumes released with the effluent reached an average of 21.7 mL/L, corresponding to methane losses of up to 4%. Phase 2 operating at lower fluxes had effluent methane volumes of 15 mL/L and corresponded to methane losses of less than 3%.

![Figure 4](image)

**Figure 4.** Effect of saturation ratio on effluent and headspace methane volumes for the real wastewater experiment.

Effluent methane volumes increase throughout the phases of the experiment and were measured in the range of 29-82 mL/day on average with daily losses reaching a maximum of 14% during Phase 4. While TMP affects effluent methane volumes, the released volume can also impact the methane produced into the headspace of the reactor. **Figure 4** above shows that the higher saturation ratio of 2 measured in Phase 3 of the experiment indicated an increase in methane volumes in the effluent from 31±8 to 41±11 mL/d and was simultaneously accompanied with a decrease in the headspace methane volume from 348 to 261 mL/d. The high effluent methane and low
headspace methane caused the system to have a total methane loss equivalent to more than 13±3.3%.

**Figure 5.** Actual-Expected methane production versus saturation ratio for real wastewater treatment.

Saturation ratio has also been shown to affect the methane production potential of the reactor. In fact, when the ratio increases, the difference between the expected and total production also increases. In Phases 1 and 4, when the ratio was equal to 1.1, production was equal to the expectation. At a ratio of 1.4, the difference was 21% and at a ratio of 2, the difference increased to more than 44% (Figure 5).
5.4. Energy recovery from AnMBRs

5.4.1. Energy recovery from AnMBR treating low-strength wastewater

Figure 6. Total electricity demand for operations of AnMBR treating synthetic wastewater. The three MF membranes used are noted as A, B, and C referring to membranes of Phase 1 with effluent pumping rates of 0.4, 0.8, and 1.5 mL/min respectively.

The figure above represents the energy expenditures for each component of the system. It is clear that the highest difference is between the membranes during Phase 1. In this case, the main component affecting energy expenditures was the permeate flux and the transmembrane pressure. Membrane A with lowest operating flux (Table 1) had an electricity demand for sparging and sludge recirculation that is 74% higher than that of membranes B and C. At the same time, membrane C with highest TMP of 14 inHg (Table 1) had the highest electricity demand for effluent pumping, still no more than 0.025 kWh/m$^3$. Insignificant differences are observed in the energy expenditures of the membranes of Phase 2.
Table 9. Energy balance for synthetic wastewater treatment based on actual methane production. The three MF membranes used are noted as A, B, and C referring to membranes of Phase 1 with effluent pumping rates of 0.4, 0.8, and 1.5 mL/min respectively.

<table>
<thead>
<tr>
<th>kWh/m³</th>
<th>Membrane A</th>
<th>Membrane B</th>
<th>Membrane C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase 1</td>
<td>Phase 2</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Electricity demand</td>
<td>0.78</td>
<td>0.59</td>
<td>0.45</td>
</tr>
<tr>
<td>Potential electricity generated</td>
<td>1.97</td>
<td>1.95</td>
<td>1.92</td>
</tr>
<tr>
<td>NEB</td>
<td>1.19</td>
<td>1.36</td>
<td>1.47</td>
</tr>
<tr>
<td>Heat Consumed</td>
<td>6.65</td>
<td>6.80</td>
<td>6.65</td>
</tr>
<tr>
<td>NEB</td>
<td>-2.99</td>
<td>-3.18</td>
<td>-3.09</td>
</tr>
</tbody>
</table>

Figure 7. Energy balance for synthetic wastewater treatment based on actual methane production. The three MF membranes used are noted as A, B, and C referring to membranes of Phase 1 with effluent pumping rates of 0.4, 0.8, and 1.5 mL/min respectively.

The overall energy balance of the system was near stable during the 2 phases and even between the different membranes. Energy produced was enough to cover the consumptions of the system and net positive electricity generation was achieved in the range 1.2-1.5 kWh/m³. The membrane with the relatively higher energy recovery was that operated at highest flux having lowest energy for membrane scouring. Regardless,
heat recovery was negative around 3.2 kWh/m³ due to the high heat requirement for substrate heating prior to feeding.

Figure 8. Electricity demand for AnMBR treating real wastewater.

In general, AnMBRs treating low-strength wastewater require around 70% of their energy for sludge recirculation. Energy expenditure was highest in Phase 2 of the experiment associated with the significant drop in flux from 6.76 LMH in Phase 1 to 5.94 LMH in Phase 2. Phase 4 of the experiment had a 34% lower energy demand compared to Phase 2 given the higher operating flux.

Table 10. Energy balance for Real wastewater experiment based on actual methane production. Values are tabulated in kWh/m³.

<table>
<thead>
<tr>
<th>kWh/m³</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electricity demand</td>
<td>0.48</td>
<td>0.54</td>
<td>0.43</td>
<td>0.32</td>
</tr>
<tr>
<td>Potential electricity generated</td>
<td>0.65</td>
<td>0.83</td>
<td>0.50</td>
<td>0.60</td>
</tr>
<tr>
<td>NEB</td>
<td>0.17</td>
<td>0.29</td>
<td>0.07</td>
<td>0.28</td>
</tr>
<tr>
<td>Heat Consumed</td>
<td>6.24</td>
<td>6.27</td>
<td>6.18</td>
<td>3.00</td>
</tr>
<tr>
<td>Heat Produced</td>
<td>1.20</td>
<td>1.54</td>
<td>0.92</td>
<td>1.11</td>
</tr>
<tr>
<td>NEB</td>
<td>-5.04</td>
<td>-4.73</td>
<td>-5.26</td>
<td>-1.89</td>
</tr>
</tbody>
</table>
Figure 9. Energy balance for Real wastewater experiment based on actual methane production.

The drop in energy production in Phase 3 is associated with the drastic decrease in methane yield to from 0.38 L/g-COD in Phase 1 to 0.24 L/g-COD in Phase 3, indicating a 37% drop in energy production. On the other hand, the significantly lower heat consumption during Phase 4 is attributed to the operations at 30 °C which compensated to 50% of the heat required in the previous phases. Phase 3 had the lowest overall energy balance where the system could only recover 0.07 kWh of energy/m³ of real wastewater.

The main issue faced with low-strength wastewaters treatment is the low energy production compared to the relatively high consumption, especially when substrate heating is required. For both cases of synthetic and real wastewater feeding, the system was able to achieve a positive electricity generation based on 35% of total energy produced from methane, but heat recovery was still in deficit even at half the heat consumption.
5.4.2. Energy recovery from AnMBR treating high-strength wastewater

Figure 10. Electricity demand for AnMBR treating PSW.

As previously mentioned, membrane flux and TMP are factors affecting the energy expenditures of the system. In the case of the PSW treatment, there was no pressure build up throughout the duration of the experiment and therefore the flux was the only dictating parameter. When the flux of Phase 3 doubled compared to that of Phase 1, the energy consumption decreased by half. Compared to the low-strength wastewater treatment systems, the split is even between both dominating components with sparging consuming 49% and recirculation consuming 50% of the total energy expenditures during all three phases of operations.

Table 11. Energy balance for Poultry experiment based on actual methane production. Values are tabulated in kWh/m³.

<table>
<thead>
<tr>
<th>kWh/m³</th>
<th>AnMBR Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Combined treatment Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electricity demand</strong></td>
<td>1.26</td>
<td>0.87</td>
<td>0.58</td>
<td>-1.26</td>
<td>-0.87</td>
<td>-0.58</td>
</tr>
<tr>
<td><strong>Potential electricity generated</strong></td>
<td>12.38</td>
<td>10.47</td>
<td>7.70</td>
<td>47.98</td>
<td>46.07</td>
<td>43.30</td>
</tr>
<tr>
<td><strong>NEB</strong></td>
<td>11.11</td>
<td>9.60</td>
<td>7.12</td>
<td>46.71</td>
<td>45.20</td>
<td>42.72</td>
</tr>
<tr>
<td><strong>Heat Consumed</strong></td>
<td>10.45</td>
<td>9.52</td>
<td>8.47</td>
<td>14.88</td>
<td>13.95</td>
<td>12.90</td>
</tr>
<tr>
<td><strong>Heat Produced</strong></td>
<td>22.99</td>
<td>19.45</td>
<td>14.30</td>
<td>89.10</td>
<td>85.57</td>
<td>80.42</td>
</tr>
<tr>
<td><strong>NEB</strong></td>
<td>12.54</td>
<td>9.92</td>
<td>5.83</td>
<td>74.23</td>
<td>116.82</td>
<td>110.24</td>
</tr>
</tbody>
</table>
Figure 11. Energy balance for PSW experiment based on actual methane production. Based on Table 7, Phase 3 with a 26% lower methane yield compared to Phase 2, had the lowest total energy produced (22 kWh/m³) and therefore the lowest energy recovered. Unlike low-strength wastewaters, the AnMBR treating PSW didn’t face energy recovery issues where both electricity generation and heat recovery were positive.

In order to assess the full potential for energy recovery from raw PSW treatment, screened solids were fed to batch reactors over 2 phases. It was determined that Phase 1 of batch feeding, although having the lowest solids loading, allowed for highest energy production from the system, equal to 101.7 kWh/m³ (attributed to the lower methane yield in Phase 2) (Table 7). The plot of Figure 11 shows that if the raw PSW influent was to be treated without solids separation, then the treatment process would allow for an energy recovery that is 5 times higher than that available from the AnMBR, particularly in Phase 1 (120.9 kWh/m³ from the combination compared to 23.6 kWh/m³).
Figure 12. Energy balance for AnMBR treating olive mill wastewater based on actual methane volume produced.

In accordance with the drop in methane yield in Phase 2 of the experiment, energy recovered was also the lowest compared to the other 3 phases. The system with highest COD loading had the highest energy production potential relative to the higher organic content. Compared to PSW treatment, the AnMBR treating OMW was able to achieve an overall energy recovery that is 2.5 times higher.
5.5. Microbial community adaptation to PSW influent

Figure 13. Principal coordinates analysis (PCoA) plot of microbial abundance similarity for AnMBR samples using a Theta-YC similarity distance matrix for genus-level sequence clustering, taken from samples of suspended biomass, membrane biofilm and effluents at the end of each experimental phase with a single poultry influent sample tested at the beginning of the experiment.

In order to determine similarities and differences between different tested samples, we developed the principal coordinates analysis (PCoA) theta-YC plot (Figure 13). Referring to the plot, we see that the biomass samples appear to be affected by the introduction of PSW for treatment. Samples show high differences between each other and especially the ones of PSW treatment compared to the startup phase sample.
5.5.1. Biomass and membrane microbial communities

![Relative abundance (%) of microbial community operational taxonomic units (OTUs) in suspended biomass and membrane samples taken at the end of each phase of the experiment, having a relative abundance greater than 5%.

Different microbial groups dominated each phase (Figure 14); in the startup phase, Bacteroidetes, Candidatus Cloacamonas, Azonexaceae, Fervidobacterium, and Zoogloea were dominant (relative abundance greater than 5%). At the end of Phases 1 and 2, the relative abundance of Syntrophaceae, Unclassified Bacteria, Methanocorpusculum, and Anaerolineaceae increased to more than 5% while that of Azonexaceae and Zoogloea decreased to less than 1%. Major observations over extended exposure to PSW (Phase 3) include variation in Pseudomonas detection, with relative abundance dropping from an average of 2.5% for the previous phases to 0.9% in Phase 3. While Halomonas was only detected in Phase 1 following PSW introduction, other groups such as Methanocorpusculum, Candidatus Cloacamonas, Synthrophaceae, and Anaerolineaceae peaked after PSW introduction with relative abundances more than double that of the startup phase.
Certain communities are detected based on the specific substrates present for degradation. Given its composition and richness in lipids and proteins, detected communities are expected to have the capacity to degrade these substrates. While previous studies have proven the dominance of *Clostridium* in systems treating slaughterhouse wastewater (Ning et al., 2018), that was not the case in our study, where other microbial groups of the same function were dominant instead: Phase 2 has highest relative abundance of *Fervidobacterium* and *Bacteroidetes* which are bacteria also responsible for proteins and lipids degradation (Guo et al., 2021). In the same context, it was determined that lower abundance of genus *Clostridium* was associated with a higher methane production. This in fact, was applicable to the results of our study where methane yield decreased by 26% in Phase 3 when the relative abundance was highest equal to 0.55% compared to 0.02% in other phases.

On the other hand, the membrane samples represented on the principal coordinates analysis thetayc plot (*Figure 13*) appear to be more clustered than the biomass samples are, signifying that the microbial communities are more similar to each other between different phases and possibly having similar dominating microbial communities. Some common dominant groups over the 3 phases include *Fervidobacterium*, *Candidatus Cloacamonas*, *Bacteroidetes*, and *Azonexaceae*. At decreasing HRT, richness and evenness of membrane samples were increasing and thus indicating microbial adaptation to operational changes with poultry treatment. This shows that the introduction of PSW for treatment had a lower effect on membrane biofilm than on the suspended biomass samples, in terms of composition as well as abundance.
5.5.2. Microbial shift of the biomass

Figure 15 Relative abundance (%) of microbial community operational taxonomic units (OTUs) for suspended biomass samples from all phases of the experiment. Figure (A) represents groups with decreasing relative abundance as the OLR was increased. Figure (B) represents groups with increasing relative abundance as the OLR was increasing.

The plots of Figure 15 refer to the observed shift in microbial communities in the suspended biomass of the reactor as the HRT was decreasing. There was a significant decrease in the abundance of some groups while others have peaked at the end of Phase 3, indicating a replacement process. This transition in communities could possibly have a negative effect on the performance of the reactor: at the end of Phase 3 and after the shift in microbial communities, %COD removal and methane content in the
headspace were still maintained at 95% and 75% respectively but methane production per g of COD removed dropped by 38%. In fact, although the total abundance of these groups did not vary between the two categories from first existing until being replaced (total in Phase 1 in (A) is 39% and 46% in Phase 3 of (B)). Some microbial communities (Figure 12 (A)) were replaced by others (Figure 12 (B)): *Sulfuricurvum* was replaced by *Anaerohalosphaera* both being sulfur reducing bacteria. Also, *Rectinema* and *Aquabacterium* having a combined abundance of 6.8%, are amino acids consuming bacteria and were replaced by *Clostridiales, Romboutsia* and *Leuconostoc* that have a total abundance of 5.5%. The function of existing communities was maintained through phases but the transition caused a disruption in the performance of the reactor especially on the level of biogas production most observed in Phase 3.

5.5.3. Methanogens

![Diagram showing relative abundance of Methanogens](image-url)
Figure 16. Relative abundance (%) of microbial community operational taxonomic units (OTUs) representing the different methanogens identified in the suspended biomass (A) and on the membrane biofilm (B) from samples collected at the end of each phase including the startup.

Methanogens responsible for production of methane in both the headspace and effluent of the reactor can be either hydrogenotrophic or acetogenic, depending on the type of substrate digested. The addition of PSW and the corresponding prolonged treatment can lead to shifts in dominant methanogens in the anaerobic digester. Hydrogenotrophic methanogens generally dominate the performance of an anaerobic digester (Tong et al., 2019), but can be modified based on substrate availability. Following poultry addition, total methanogens abundance peaked at 11% in the suspended biomass and 3.2% in the membrane biofilm. Specifically, Methanocorpusculum, classified as a hydrogenotrophs, dominated Phase 1 of PSW treatment with a RA of 9.5% and 2.3% in the biomass and membrane respectively. Methanocorpusculum was gradually replaced by Methanothrix throughout the duration until the end of Phase 3 where the RA of the former was less than 0.2%.

Classified as acetate consuming methanogens, the higher presence of Methanothrix was directly associated with VFA concentrations. The high RA of Methanothrix of 5.8% in Phase 3 consumed the acetate present in the system and contributed to the
decrease in its concentration between Phase 1 and Phase 3. At the same time, the high presence of methanogens on the membrane didn’t affect the effluent supersaturation ratios, all maintained at around 1.

5.5.4.  Microbial communities in the effluent

Referring to the principal coordinates analysis thetayc plot (Figure 14), microbial communities of the effluent samples are more clustered and relatively close between the 3 phases of PSW treatment as well as the synthetic feeding startup phase. Statistically, the t-test performed on the effluent samples of PSW treatment, as two-tailed and considering a two-sample unequal variance, have shown values greater than 0.84 for all three comparisons, indicating high similarities between the phases. Effluent samples, and especially those of poultry treatment phases, seem to be the least affected by PSW introduction compared to the biomass and membrane samples which showed more variations.

Also showing the similarities between the 3 phases, the evenness value based on the Shannon index was equal to 0.57 during the startup phase, Phase 2 and Phase 3 of PSW. Phase 1 had a slightly lower evenness of 0.53, attributed to the introduction of PSW considered as a transition phase for the system which could have possible affected the groups present at the time.
Figure 17. Relative abundance (%) of microbial community operational taxonomic units (OTUs) representing the microbial communities detected in the effluent of the AnMBR at the end of each phase.

Some groups peaked in the effluent samples after the introduction of PSW, including *Candidatus cloacamonas*, *Pseudomonadaceae*, and *Anaerolineaceae*, while other communities such as *Pseudomonas*, *Azonexaceae*, Bacteroidetes, *Macellibacteroides*, and *Comamonadaceae* dominated all 3 phases of the experiment.

5.5.5. **Microbial diversity**

Table 12. Diversity indices of microbial communities.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Richness</th>
<th>Shannon Index</th>
<th>Evenness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry Influent</td>
<td>942</td>
<td>3.38</td>
<td>0.54</td>
</tr>
<tr>
<td>Biomass-Startup phase</td>
<td>816</td>
<td>3.46</td>
<td>0.56</td>
</tr>
<tr>
<td>Biomass-Phase 1</td>
<td>802</td>
<td>3.21</td>
<td>0.52</td>
</tr>
<tr>
<td>Biomass-Phase 2</td>
<td>1072</td>
<td>3.51</td>
<td>0.56</td>
</tr>
</tbody>
</table>
AnMBRs treating slaughterhouse wastewaters could impact microbial composition of the biomass, causing changes in diversity indices, accompanied with a shift in some microbial groups. Biomass sample from end of Phase 2 has the highest diversity and richness of microbial communities, directly associated with the fact that highest methane production was also recorded in this phase. The presence of more diverse microbial groups compared to Phase 1, helped in the adaptation of the system and allowed for higher biogas production. Phase 1 was a transition phase between synthetic and poultry and caused a modification in the performance of the system, while Phase 3 of lower diversity had the lowest methane yield. Calculated Shannon index showed similar values between the startup phase and Phase 2 of poultry treatment, followed by a noticeable reduction in the diversity of communities from 3.51 in Phase 2 to 3.27 at end of Phase 3. Highest diversity was associated with highest methane yield from the reactor and vice versa. The effect of PSW introduction on microbial diversity is represented by the lowest Shannon index calculated in Phase 1 for all three compounds on biomass, effluent, and membranes. The subsequent adaptation of communities to poultry is reflected by the gradual increase in diversity indices and evenness values until the end of Phase 3 where similar values are recorded compared to the startup phase: the effluent of Phase 3 has a Shannon index of 3.57, close to the value of 3.54 of the startup phase.
5.6. Microbial safety of AnMBRs effluents

5.6.1. ARGs removals from effluents of AnMBR treating high-strength wastewater

As previously mentioned, microbial communities in the anaerobic digester are expected to adapt to the PSW in order to maintain the stable reactor performance. Following acclimation, more antibiotic resistant genes (ARGs) are prone to develop and the capacity of the reactor to biodegrade antibiotics may be affected (Noor et al., 2021).

![Figure 18](image)

**Figure 18.** Antibiotic resistance gene (ARG) and intI1 gene copy abundances detected in the poultry influent wastewater and AnMBR effluent samples collected at the end of each phase of the experiment including the startup phase.

Influent PSW is rich in antibiotics and its addition to AnMBRs impacts the chemical composition of the effluent discharged (Tang et al., 2021). Results of ARG’s showed lowest concentration in the effluent of the startup phase (**Figure 18**), followed by a peak concentration in Phase 1 after poultry introduction, for all genes detected. It’s important to note that gene *tetQ* was only detected in poultry effluents and not synthetic. Throughout the phases of poultry treatment, ARG removal gradually increased for all genes detected and reached a maximum LRV of 3 for gene *ampC*. 

66
ARG concentrations corresponding with genes \textit{tetQ} and \textit{blaTEM} were higher at the end of Phase 3, unlike the trend observed by other genes.

Microbial communities and ARGs can be correlated through microbial diversity where Phase 1 with lowest calculated diversity index had the highest proliferation of ARGs. Some microbial communities serve as potential hosts for specific genes (Zarei-Baygi et al., 2020). The lower abundance of \textit{Sulfuricurvum}, \textit{Acinetobacter}, and Bacteroidetes in Phase 3 corresponds with the lower abundance of genes \textit{sul1}, \textit{sul2}, and \textit{intl1} respectively compared to Phase 1.

Overall, poultry introduction along with the low microbial diversity increase the susceptibility of the system to face inhibition, which highly contributes to the proliferation of ARGs in the effluent of the AnMBR.

\textbf{5.6.2. Pathogens in effluents of AnMBR treating high-strength wastewater}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{pathogens.pdf}
\caption{Relative abundance (\%) of microbial community operational taxonomic units (OTUs) representing pathogens detected in the effluent of the AnMBR at the end of each phase.}
\end{figure}
Poultry SHW contains high levels of pathogens which could impact the effluent water 
quality and pollutants levels. Previous studies concluded that AnMBRs have efficient 
pathogens and pollutants removal allowing for potential water reuse applications. After 
starting poultry treatment, abundance of total potential pathogens in Phase 1 had a sudden 
drop (18%) compared to that of the synthetic startup phase, with only *Acinetobacter* 
dominating, and equating a total reduction in the relative abundance of the potential 
pathogens by up to 69%. At the end of Phase 3, the total relative abundance of all potential 
pathogens detected was highest between all three phases of poultry treatment, equal to 
32%, but was still similar to that of the startup phase (Figure 19). In Phase 3, 
*Pseudomonas* were the only dominating group with higher than 5% RA. Bacteroides, 
*Zoogloea, Macellibacteroides, Cloacibacterium, and Aeromonas* had a RA higher than 
1% while they were undetected in Phase 1. We also noticed that the potential pathogenic 
groups detected in Phase 3 are close to those present in the poultry influent, in terms of 
relative abundance of different groups. This is also represented by the evenness value of 
the two samples calculated and shown in Table 12. Poultry SHW contains high levels of 
pathogens which could impact the effluent water quality and pollutant levels. After 
starting PSW treatment, *Acinetobacter* was the only dominating group, with high RA of 
*Pseudomonas* and *Aliarcobacter*. At the end of Phase 3, the total relative abundance of 
all potential pathogens detected was highest, equal to 32%, with high RA of different 
groups including *Pseudomonas, Acinetobacter, Bacteroides, Aliarcobacter,* and 
*Zoogloea* (Figure 18). In accordance with the previous observation from the PCoA, we 
can also determine from the plot of potential pathogens that the sample of Phase 3 and 
the influent sample have common dominating groups including: *Pseudomonas,* 
*Acinetobacter,* Bacteroides, and *Aliarcobacter.*
Chapter Six

Discussion and analysis

6.1 Strategies for improving energy balance of the AnMBR

6.1.1 Elimination of sludge recirculation by having a submerged membrane

Membrane scouring is an important factor to consider when operating AnMBRs as it mostly helps in reducing membrane fouling. Regardless, sludge recirculation constituted around 70% of the total energy demand for the operation of the AnMBR treating low-strength wastewaters. Given the relatively high electricity consumption compared to the low production potential, and especially for systems treating real wastewaters with recovery as low as 0.07 kWh/m³, it is necessary to find alternatives for higher recovery. A possible operational mitigation to help increase the energy recovery would be the elimination of the sludge recirculation parameter, and therefore decreasing the energy demand by more than 70% in cases of low permeate flux. In fact, the use of a submerged membrane instead of an external one is a viable alternative but its main drawback is the associated high operational cost generated from the difficult filtration and membrane fouling mitigation processes (Dvořák et al., 2016), as well as the difficulty in cleaning and accessing the membrane units: such operations would require opening the reactor and thus exposing the anaerobic sludge to the ambient (Hoque, Kimura, Miyoshi, Yamato, & Watanabe, 2012). This concern is not an issue faced with external membranes that undergo easy backwashing.
Table 13. Energy recovery for submerged membrane. Values are tabulated in kWh/m³.

<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electricity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>demand</td>
<td>0.16</td>
<td>0.18</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Electricity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>produced</td>
<td>0.65</td>
<td>0.83</td>
<td>0.50</td>
<td>0.60</td>
</tr>
<tr>
<td>NEB</td>
<td>0.49</td>
<td>0.65</td>
<td>0.36</td>
<td>0.51</td>
</tr>
<tr>
<td>Increase</td>
<td>2.9</td>
<td>2.24</td>
<td>5.1</td>
<td>1.8</td>
</tr>
</tbody>
</table>

For real wastewater treatment and compared to our experimental results, a submerged membrane allows for an energy recovery that could be as high as 0.65 kWh/m³ compared to the maximum of 0.29 kWh/m³ available from the external membrane. If the membrane was considered to be internal with no recirculation, the net energy recovery of the system would increase by a factor of 2-5 depending on the membrane flux during the different phases of operation.

### 6.1.2. Reduction of energy required for substrate heating

Another parameter affecting the energy balance of the system is the heat requirement for substrate heating which dominates heat lost through the reactor jacket and tubing. Despite its high contribution to the system expenditures, heating is a necessary requirement for the system; operations at mesophilic and even thermophilic temperatures are recommended for optimal bacterial activity and destruction of pathogens (Salminen & Rintala, 2002). For the AnMBR treating real wastewater, highest heat recovery was during Phase 2 with highest methane production and therefore highest heat generation potential. Given the targeted 30-35°C temperature for bacterial activity, the substrate heating by 10°C was necessary, which adds up to 88% of the total heat consumed by the system. Our work has showed that even at operational temperatures of 30°C with ambient of 25°C, the system could not achieve a neutral heat recovery. In that context, positive heat recovery would only be attained when substrate
heating is not required, meaning ambient temperature is relatively high or mesophilic conditions are not targeted.

Table 14. Heat recovery from AnMBR treating real wastewater assuming no heat required. Values are tabulated in kWh/m³.

<table>
<thead>
<tr>
<th></th>
<th>Phase1</th>
<th>Phase2</th>
<th>Phase3</th>
<th>Phase4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat Consumed</td>
<td>0.41</td>
<td>0.43</td>
<td>0.35</td>
<td>0.09</td>
</tr>
<tr>
<td>Heat produced</td>
<td>1.20</td>
<td>1.54</td>
<td>0.92</td>
<td>1.11</td>
</tr>
<tr>
<td>NEB-Initial</td>
<td>5.04</td>
<td>4.73</td>
<td>5.26</td>
<td>1.89</td>
</tr>
<tr>
<td>NEB-Heat</td>
<td>0.80</td>
<td>1.11</td>
<td>0.58</td>
<td>1.02</td>
</tr>
</tbody>
</table>

In the case where the reactor is operating at room temperature with no heating requirement, the heat recovery can be positive: Phase 3 having the highest negative recovery when the substrate was heated by 10 °C, achieved a net positive recovery of 0.58 kWh/m³ when no heating was performed.

Ultimately, a heating temperature could be specified as the baseline for neutral heat recovery. Considering Phase 3 as the most critical one in terms of heat consumption, the maximum temperature that we could use for operations while still recovering a positive value was determined to be equal to only 1°C (Table 14). This difference in temperature was the cutoff point indicating the maximum temperature to elevate that of the substrate. Given the results, we can clearly state that operating such systems in relatively warm climates with minimal heating required, is most appropriate for beneficial resource recovery.
Table 15. Neutral heat recovery from AnMBR treating real wastewater. Values are tabulated in kWh/m³.

<table>
<thead>
<tr>
<th></th>
<th>Phase1</th>
<th>Phase2</th>
<th>Phase3</th>
<th>Phase4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat Consumed</td>
<td>0.99</td>
<td>1.02</td>
<td>0.93</td>
<td>0.67</td>
</tr>
<tr>
<td>Heat produced</td>
<td>1.20</td>
<td>1.54</td>
<td>0.92</td>
<td>1.11</td>
</tr>
<tr>
<td>NEB-Initial</td>
<td>5.04</td>
<td>4.73</td>
<td>5.26</td>
<td>1.89</td>
</tr>
<tr>
<td>NEB-Heat</td>
<td>0.21</td>
<td>0.52</td>
<td>0.01</td>
<td>0.44</td>
</tr>
</tbody>
</table>

6.1.3. Decrease biogas sparging rate to critical rate for lower energy consumption

A main factor affecting the overall energy demand of the system is the specific gas demand (SGD = \( \frac{\text{biogas sparging rate}}{\text{membrane flux}} \)), which is a necessary component for turbulence on membrane surface and performance. Biogas sparging can however be ultimately reduced to lower the electricity consumption of the system, especially when treating low-strength wastewater. In our study, the biogas recirculation rate was set at 210 mL/min, yielding an electricity demand in the range of 0.09-0.17 kWh/m³ of real wastewater, depending on the operating flux. Although the energy recovered from the AnMBR was slightly positive, it can be further increased when the sparging rate is reduced to a critical margin that can be determined experimentally. In fact, a 30% lower biogas sparging rate will increase the net energy balance of the system treating real wastewater by an average of 28.5±17.7% with highest value of 57% recorded for Phase 3 with very low energy production.

For the AnMBR treating high-strength wastewater, the biogas sparging constituted around 50% of the total electricity consumption, and its reduction can largely impact the recovery potential. Nevertheless, given that the total electricity consumption from this system doesn’t exceed more than 10% of the energy produced and therefore the
reduction of the sparging rate will have little effect on the total electricity demand especially when the flux is higher; a 50% lower sparging rate would only increase the overall recovery by less than 2%.

6.1.4. Implementation of post-treatment systems for effluent methane recovery from effluent of low-strength wastewaters

Even though AnMBRs have high energy production potential, they still remain questionable alternatives in terms of sustainable water reuse given the high methane volumes dissolved in the effluents. Regardless of cogeneration from biogas production, AnMBRs treating low-strength domestic wastewaters have a negative environmental impact represented by the effluent methane emissions which constitute 81% of the total contribution of the system to global warming (Becker Jr et al., 2017). Beside the fact that higher methane released with the effluent increases greenhouse gas emissions, it also causes additional losses to the system on the level of useful recoverable energy. In that context, several post-treatment systems have been suggested for adaptation in order to benefit from excess methane present in the effluent. Post-treatment methods include different recovery techniques such as down-flow hanging sponge system (DHS), de-nitrifying anaerobic methane oxidation (DAMO), and microbial fuel cells (MFCs) as biological processes (S. Chen et al., 2018; J Cookney, Cartmell, Jefferson, & McAdam, 2012). Other implemented physio-chemical post-treatment processes include different types of membrane contactors such as hollow fiber membranes, degassing membranes, sweep gas, and vacuum driven membranes. Their differences lay in the operation process where each has different energy consumption requirement and corresponding production potential depending on recovery efficiency. Based on previous studies, poly-di-methyl-siloxane membrane contactors have been determined to be the most suitable for recovery of methane from effluents of AnMBRs treating
low-strength wastewaters. The process for methane recovery from this system requires an energy demand for operations of 0.04 kWh/m³ and the membrane contactor has a methane recovery efficiency up to 80% even at transmembrane pressures higher than 20 inHg (Sanchis-Perucho, Robles, Durán, Ferrer, & Seco, 2020). At the same time, these membranes have the potential to reduce GHG emissions from an initial value in the range of 0.3-2.8 kg CO₂/m³ to less than 0.2 kg CO₂/m³ of treated domestic wastewater, while individually producing a net electricity of more than 0.043 kWh/m³ from the recovery process (S. Chen et al., 2018; J Cookney et al., 2012).

The results of our study showed methane losses in the effluent of the AnMBR treating real wastewater reaching 14% of the total methane produced. In case of post-treatment systems implementation, then additional methane could be recovered from the effluent instead of being emitted into the atmosphere as useless gas. Assuming the theoretical 80% recovery efficiency of dissolved methane, and the required energy demand of 0.043 kWh/m³, then the net electricity generation would increase by 0.02 kWh/m³. The recovery process would at the same time allow for additional energy recovery potential and yield lower greenhouse gas emissions from the AnMBR.

Table 16. Energy recovery potential with effluent methane recovery. Values are tabulated in kWh/m³.

<table>
<thead>
<tr>
<th></th>
<th>Phase1</th>
<th>Phase2</th>
<th>Phase3</th>
<th>Phase4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial electricity generated</td>
<td>0.65</td>
<td>0.83</td>
<td>0.50</td>
<td>0.60</td>
</tr>
<tr>
<td>Initial consumption</td>
<td>0.48</td>
<td>0.54</td>
<td>0.43</td>
<td>0.32</td>
</tr>
<tr>
<td>Initial NEB</td>
<td>0.17</td>
<td>0.29</td>
<td>0.07</td>
<td>0.28</td>
</tr>
<tr>
<td>Electricity generated with recovery</td>
<td>0.71</td>
<td>0.89</td>
<td>0.56</td>
<td>0.67</td>
</tr>
<tr>
<td>Additional consumption for recovery</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>NEB from effluent recovery</td>
<td>0.19</td>
<td>0.31</td>
<td>0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>% Increase</td>
<td>12%</td>
<td>7%</td>
<td>29%</td>
<td>11%</td>
</tr>
</tbody>
</table>
Initially, energy produced from the AnMBR would increase by 13% in Phase 3 of real wastewater treatment, translating into a higher energy recovery given the higher recoverable methane volume. The interpretation of the results of recovery potential directly showed that effluent methane recovery process can help increase the amount of energy recovered from the system. Energy recovery would increase by 10% to 30% depending on the initial amount of methane lost in the effluent and the correspondingly relatively different saturation ratios. Since saturation ratios highly impact effluent methane volumes, then Phase 3 had the highest total additional energy recovered with post-treatment and was around 30% higher than that recovered from methane headspace only. Compared to other phases, Phase 3 had the highest super saturation ratio of 2 and was therefore the most affected when effluent recovery method was implemented.
Chapter Seven

Conclusions

7.1. Summary and conclusion

The AnMBR has been proven to be an efficient technology for treating different types of wastewaters in terms of providing high effluent quality, emerging contaminant removal and high potential to produce energy. Several studies have previously determined that various operational and design factors can impact the overall energy recovery of the system. The results of this thesis showed the direct effect of the performance of the reactor on methane recovery potential for a range of wastewater types. The work also focused on determining the parameters affecting the overall energy recovery and the role of microbial adaptation in efficient methane production. High effluent quality was generally maintained during treatment of different wastewater strengths by AnMBRs, but the capacity to reduce abundance of pathogens is crucial when the treated wastewater is complex and has potentially resistant pathogens in its source. On the other hand, energy recovery can be impacted by the organic content, gas sparging rate, permeate flux, and temperature conditions. The potential for methane capture was limited when the reactor was treating domestic wastewater of low-strength, and energy consumption was critical when transmembrane pressure built for the membrane with high operating flux. The low HRT in Phase 3 of the poultry slaughterhouse wastewater experiment caused a 38% drop in overall energy produced due to the excess biomass growth. These observations highlight the importance of designing operating conditions to ensure maximum energy recovery while reducing system consumption and also ensuring sufficient pollutant removal to provide useful effluent for water reuse.
7.2. **Future work**

Given the need to maximize energy recovery from AnMBRs, the implementation of post-treatment systems for methane recovery is suggested based on a calculated increase using theoretical values of recovery efficiency and energy demand for the operation process. In that context, future works should include determining experimental values of the energy consumption and its actual recovery efficiency for accurate additional recovery potential. In addition, proposed solutions for decreased energy consumption include lowering the biogas sparging rate, and therefore it would be useful to determine the critical biogas sparging rate below which critical flux would be reached and membrane fouling will start developing.
References


Granular Sludge Bed Reactor (EGSB), and a Membrane Bioreactor (MBR). *Membranes (Basel)*, 11(5). doi:10.3390/membranes11050345


membrane bioreactor and conventional treatment systems with anaerobic digestion. Environmental science & technology, 48(10), 5972-5981.


# Appendix A

## Synthetic wastewater composition

Table S17. Characteristics of 1.5 g/L influent synthetic wastewater.

<table>
<thead>
<tr>
<th>Concentrate solution</th>
<th>Dilution water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagent</strong></td>
<td><strong>Conc. (mg/L)</strong></td>
</tr>
<tr>
<td>Ammonium Chloride</td>
<td>17.5</td>
</tr>
<tr>
<td>Calcium Chloride</td>
<td>18.1</td>
</tr>
<tr>
<td>Iron Sulfate</td>
<td>9.1</td>
</tr>
<tr>
<td>Sodium Sulfate</td>
<td>18.1</td>
</tr>
<tr>
<td>Sodium Acetate</td>
<td>481.8</td>
</tr>
<tr>
<td>Peptone</td>
<td>63.8</td>
</tr>
<tr>
<td>Yeast</td>
<td>191.1</td>
</tr>
<tr>
<td>Milk Powder</td>
<td>283.4</td>
</tr>
<tr>
<td>Starch</td>
<td>446.5</td>
</tr>
<tr>
<td>Copper Chloride</td>
<td>6.25</td>
</tr>
<tr>
<td>Manganese Sulfate</td>
<td>1.3</td>
</tr>
<tr>
<td>Lead Chloride</td>
<td>5</td>
</tr>
</tbody>
</table>

## Theoretical COD calculations

Table S18. Composition of real wastewater.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (mg/L)</td>
<td>423.63</td>
<td>166.36</td>
<td>239.1</td>
<td>612</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>343.63</td>
<td>147.27</td>
<td>180.9</td>
<td>-</td>
</tr>
<tr>
<td>Phosphate (mg/L)</td>
<td>6.42</td>
<td>4.52</td>
<td>4.5</td>
<td>10.07</td>
</tr>
<tr>
<td>Sulfate (mg/L)</td>
<td>11.03</td>
<td>15.84</td>
<td>46.45</td>
<td>29.02</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>57</td>
<td>56.3</td>
<td>47.7</td>
<td>44.5</td>
</tr>
<tr>
<td>NH3-N (mg/L)</td>
<td>71</td>
<td>70</td>
<td>67</td>
<td>56</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>0.08</td>
<td>0.07</td>
<td>-</td>
<td>4.14</td>
</tr>
<tr>
<td>Nitrate (mg/L)</td>
<td>-</td>
<td>1.82</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TKN (mg/L)</td>
<td>70.92</td>
<td>68.11</td>
<td>67</td>
<td>51.86</td>
</tr>
<tr>
<td>Organic nitrogen (mg/L)</td>
<td>13.92</td>
<td>11.81</td>
<td>19.3</td>
<td>7.36</td>
</tr>
</tbody>
</table>

## PSW composition

Table S19. Composition of the PSW influent.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mgCOD/L)</td>
<td>7200</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Ammonia (mgNH₃-N/L)</td>
<td>34.6</td>
</tr>
<tr>
<td>Acetic Acid (mg/L)</td>
<td>376</td>
</tr>
<tr>
<td>Propionic Acid (mg/L)</td>
<td>25</td>
</tr>
<tr>
<td>Butyric acid (mg/L)</td>
<td>0</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>16.1</td>
</tr>
<tr>
<td>pH</td>
<td>6.57</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>6.24%TS</td>
</tr>
<tr>
<td>Proteins</td>
<td>25.91%TS</td>
</tr>
<tr>
<td>Lipids</td>
<td>63.64%TS</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>1484</td>
</tr>
<tr>
<td>g COD/g VS</td>
<td>2.13</td>
</tr>
</tbody>
</table>
Expected methane production

\[ 0.25 \text{ g CH}_4/\text{g COD} = \frac{0.25 \times 0.0821 \times 298}{16 \times 1} = 0.382 \text{ L CH}_4/\text{g COD} \]

Expected volume (L/d) = % COD removal \times COD_{influent} \times Q \times 0.382

Methane yield (L CH\textsubscript{4}/g COD\textsubscript{removed}) = \frac{\text{daily methane volume}}{\% \text{ COD removal} \times COD\textsubscript{influent}}

**Effluent methane and supersaturation ratio calculation:**

Volume of effluent sample collected in flask = 32mL

Flask volume = 155mL

Volume of headspace biogas (flask headspace + biogas bag volume) = 50 mL

- For sample calculation from the poultry experiment:

  % methane in headspace (measured on GC) = 0.226%

Methane volume in the headspace:

\[ V = \text{total headspace volume} \times \% \text{ methane in headspace} \]
\[ = (155 + 50 - 32) \times (0.226) \times 0.01 = 0.39 \text{ mL} \]

Concentration of methane in the effluent:

\[ C = \frac{V}{\text{effluent volume}} \]
\[ = \frac{0.39}{0.032} = 12.2 \text{ mL/L of effluent} \]

- Effluent methane volume: \( V = 12.2 \times V_{\text{out}} = 12.2 \times 0.962 = 11.7 \text{ mL/d} \)

- Supersaturation ratio = \( \frac{\text{Measured effluent concentration}}{\text{Expected effluent concentration}} \) (Equation 3)

Measured effluent concentration = 12.2 mL/L

Expected effluent concentration = % methane in reactor headspace \times methane solubility

% methane in reactor headspace measured using the GC = 39%

Methane solubility = 16.5 mg/L (At 35°C)

Methane volume from solubility = \( \frac{16.5 \times 308 \times 0.0821}{16 \times 1} = 26.1 \text{ mL/L} \)

Expected effluent concentration = 10.2 mL/L

Saturation ratio = 1.2
COD mass balance
\[ \text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O} \]

16 g CH\textsubscript{4}/64 g O\textsubscript{2}=0.25 g CH\textsubscript{4}/ g COD

\[ \text{COD}_{\text{removed}} = \text{COD}_{\text{headspace methane}} + \text{COD}_{\text{biomass}} + \text{COD}_{\text{effluent}} \]

For poultry experiment sample calculation:

- \[ \text{COD}_{\text{removed}} = 0.96 \times 6,538 = 6,277 \text{ mg COD/L} = 6,277 \times 0.93 = 5.833 \text{ g COD/day} \]
- \[ \text{COD}_{\text{headspace methane}} = 1,850 \text{ mL CH}_4/\text{d} \]

\[ PV = \frac{m}{M}RT \text{ (ideal gas law)}: \]

- P: atmospheric pressure= 1 atm
- V: gas volume (L)
- m: mass of gas (g)
- M: molar mass of gas (g/mol)
- R: gas law constant (atm.L/mol.K)

\[ m = \frac{P \times V \times M}{R \times T} = 1 \times 1.85 \times 16 \times 0.0821 \times 298 = 1.21 \text{ g CH}_4/\text{day} = 4.84 \text{ g COD/L} \]

- \[ \text{COD}_{\text{effluent}} = 266 \text{ mg COD/L} = 247 \text{ mg COD/d} \]
- Biomass growth between days 42 and 48 = 4.73-3.75 = 0.98 g/L/6 days

\[ \text{Biomass growth} = 0.16 \text{ g VSS/L/d} = 3.2L \times 0.16 = 0.51 \text{ g VSS/d} \]

Assuming a standard concentration of 1.42 g COD/g VSS:

COD consumed for biomass growth = 0.72 g COD/d
COD content of solids

VS concentration was experimentally tested using APHA Standard Method 2540. Values were determined to have an average of 0.51815g VS/g dry solids.

COD content of volatile solids was determined from the individual COD content of lipids, proteins, and carbs.

COD content of lipids = 2.875 g COD/g lipids
COD content of proteins = 2.108 g COD/g proteins
COD content of carbs = 0.987 g COD/g carbs

\[ \text{Total COD content: } (0.3746 \text{ g lipids/g solid} \times 2.875 \text{g COD/g lipids}) + (0.1337 \text{g proteins/g solids} \times 2.108 \text{ g COD/g proteins}) + (0.011 \text{g carbs/g solids} \times 0.987 \text{ g COD/g carbs}) = 1.37 \text{ g COD/g solids for Phase 1} \]

\[ \text{Daily COD loading} = 1.37 \text{ g COD/g solids} \times 0.396 \text{g solids/day} = 0.54 \text{ g COD/d} \]
Energy recovery components
From the AnMBR

1. \( E_0 = \frac{V \times \text{methane conversion potential}}{Q} \)  
   \[ \text{(Equation 4)} \]
   
   \( E_0 \): energy produced (kWh/m³)

   \( V \): daily methane volume produced (mL/d)

   Methane conversion potential = 11 kWh/m³ (Mei et al., 2016)

   \( Q \) (m³/d): influent feed rate

   • Electricity produced from biogas: \( E_p = 0.35 \times E_0 \)

   • Heat recovered from biogas: \( E_r = 0.65 \times E_0 \)

2. \( E_G = \frac{P\gamma}{2.73 \times 10^5 \epsilon (p-1) \times \frac{Q_A}{Q_p} \left(\frac{10^4 y_p}{p}\right)^{1-\frac{1}{y}} } \)  
   \[ \text{(Equation 5)} \]

   \( E_G \): energy consumed for biogas sparging (kWh/m³)

   \( P \): blower inlet pressure (bar)=1.05 bar

   \( T \): air temperature (in reactor headspace)=303K

   \( \epsilon \): blower efficiency=60%

   \( y \): heat capacity ratio=1.3 for biogas

   \( y \): membrane tank depth=0.25 m

   \( Q_A \): biogas sparging rate (210 mL/min)=210×60×10^{-6} =0.0126 m³/h

   \( Q_p \): effluent pumping (m³/h)

3. \( E_S = \frac{\rho \cdot g \cdot Q \cdot H}{1000 \times \epsilon \cdot J \times A} \times 10^{-3} \)  
   \[ \text{(Equation 6)} \]

   \( E_S \): energy consumed for sludge recirculation (kWh/m³)

   \( \rho \): sludge density (kg/m)=970 kg/ m³

   \( g \): gravity (N/kg)=9.81N/kg

   \( Q \): sludge recirculation ratio (500 mL/min)=\frac{500 \times 10^{-6}}{60} =8.33 \times 10^{-6} m³/s

   \( h \): head difference (m)=0.1725m

   \( \epsilon \): pump efficiency=65%

   \( J \): flux (L/m².h) (for each membrane)

   \( A \): membrane surface area (m²) =57×10^{-4} m²
4. \[ E_P = \frac{Qvh}{1000qn} \]  
\( E_P \): energy consumed for effluent pumping (kWh/m³)  
\( Q \): effluent pumping flow rate (m³/s)  
\( v \): 9800 N/m³  
\( h \): hydraulic pressure head (m)=2.07 m  
\( q \): pump feed rate (m³/h)  
\( n \): pump efficiency (%) = 84%  

5. \[ E_{H0} = \left( \frac{C_p \times m \times \Delta t}{Q} \right) \]  
\( E_{H0} \): energy consumed for substrate heating (kWh/m³)  
\( C_p \) specific heat capacity at constant pressure=4.2 KJ/kg.K  
\( m \) quantity of heated water (kg) (equivalent of feed)  
\( \Delta t \) temperature difference (K) =10K (5K for Phase 4 of real wastewater experiment)  

\[ K = U \times A \times \Delta t \]  
\( K \) is the efficiency of heat transfer  
\( U \): heat transfer coefficient dependent on the material (kWh/h.m². °C);  
\( \Delta t \): temperature difference (°C)  
\( A \): surface area of the tubes (0.0038 m²) or reactor jacket (0.039 m²)  

\[ T(t) = T_s + (T_0 - T_s) e^{-Kt} \]  
\( T(t) \) is the temperature at time t  
\( T_s \) is the temperature of the surroundings (25°C in this case)  
\( T_0 \) is the initial temperature of water (35°C in this case, except for Phase 4 of the real wastewater treatment, \( T_0 = 30 ^\circ C \))  
\( t \) is the time after which the system would lose a temperature value equal to \( T_0 - T_s \)  

Calculate the value of \( K \) using \textbf{Equation 9}  

\[ \text{For 1 degree drop in water's temperature, it will be re-heated: assuming T at time} \]  
\( t=34 ^\circ C(1 \text{ degree lower than its initial temperature)} \)  
\[ 34 = 25 + (35-25)e^{K\times t} \]  
\[ e^{K\times t} = \frac{9}{10} = 0.9 \]
\(-K \times t = \ln(0.9) = -0.105\)

\(t = \frac{0.105}{K} \) (time to drop 1 degree) \(\Rightarrow t_0 = \frac{24}{t} \) (number of times to heat the system per day)

\(E_{H2} = \frac{C_p \times t_0 \times V \times \Delta T}{Q} \) (kWh/m³) \(\text{(Equation 11)}\)

\(m\): mass of water to be re-heated: \(m = V \times t_0\)

for connecting tubes: assuming the length of the connection can be ultimately dropped down to a total of 30 cm: \(V = 0.3 \times \frac{\pi}{4} \times 0.007 \times 0.007 = 0.0000115 \text{ m}^3\)

for reactor jacket: \(V = 0.275 \times \frac{\pi}{4} \times (0.175^2 - 0.13^2) = 0.003 \text{ m}^3\)

6. \(E_B = \frac{N_h \rho}{1000q} \left(\frac{n}{60}\right)^3 \left(\frac{D}{1000}\right)^5\) \(\text{(Equation 12)}\)

\(E_B\): energy consumed for mixing (kWh/m³)

\(\rho\)=density of sludge=970 kg/ m³

\(\mu\)=viscosity of sludge=0.0035 Pa.s

\(n\) rotating speed= 200 rpm

\(D\) diameter of mechanical mixer=16 mm

\(N_p\)= power number (based on Reynolds’ number and agitator generic curves)

\(\text{Re} = \frac{\rho \cdot n \cdot D^2}{\mu} = 2320 \Rightarrow N_p = 1.2\)

\(q\) feed rate (m³/h)

The total energy recovered as electricity from the AnMBR would be calculated as follows:

\(E_t = E_p - E_G - E_S - E_P - E_B\) \(\text{(Equation 13)}\)

The total heat recovered from the AnMBR would be equal to:

\(E_{Hr} = E_r - E_{H1} - E_{H2} - E_{H3}\) \(\text{(Equation 14)}\)

From the batch reactor

Sample calculation of Phase 1 operated over 19 days (All values are reported as average per day)

Solid fraction added=0.396 g/d

Total PBS volume injected=6.8 mL/d
Methane volume produced from batch reactors=133 mL/d

1. **Methane production from solids:**

\[
E = \frac{\text{recovery potential} \times \text{methane volume}}{\text{volume of solids}}
\]  
(Equation 15)

Density of solids determined from the measured solids masses in poultry samples (Table 4)

**Table S20.** Dry Masses of screened solids.

<table>
<thead>
<tr>
<th>Batch Sample</th>
<th>Dry Mass (g solids/L of WW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.02</td>
</tr>
<tr>
<td>2</td>
<td>27.67</td>
</tr>
<tr>
<td>3</td>
<td>28.91</td>
</tr>
<tr>
<td></td>
<td>27.2</td>
</tr>
</tbody>
</table>

Density=27.2g of dry solids mass in 1 L of mixed wastewater

Total production=\(\frac{11 \times 133 \times 0.001}{27.2}\) = 101.6 kWh/ m³ of poultry WW

\(\Rightarrow\) Electricity generated=0.35×101.6=35.6 kWh/ m³

\(\Rightarrow\) Heat produced=0.65×101.6=66 kWh/ m³

2. **Heat loss for substrate heating:**

Assuming the substrate fed into the batch reactors will have to be heated by 20K and the volume required to be heated is that of the PBS solution added.

\[
E_H = C_P \times m \times \Delta t = 4.2\text{KJ/Kg/K} \times 20\text{K} \times \frac{1\text{kWh}}{3600\text{kJ}} \times \frac{0.396\text{g/d}}{0.0068\text{d}} = 1.36 \text{ kWh/ m³}
\]

Heat losses from external exposure to the atmosphere are also calculated using the formula of Equation 10 and using the following parameters:

\(U=0.08 \text{ KWh/h.m².°C}\)

Exposed volume of liquid=sludge volume=500mL

Surface area of the reactor=0.004 m²

Atmospheric temperature=25°C

\(\Rightarrow E_{H2} = \left(\frac{4.2 \times t_0 \times V \times 10}{Q}\right)\)