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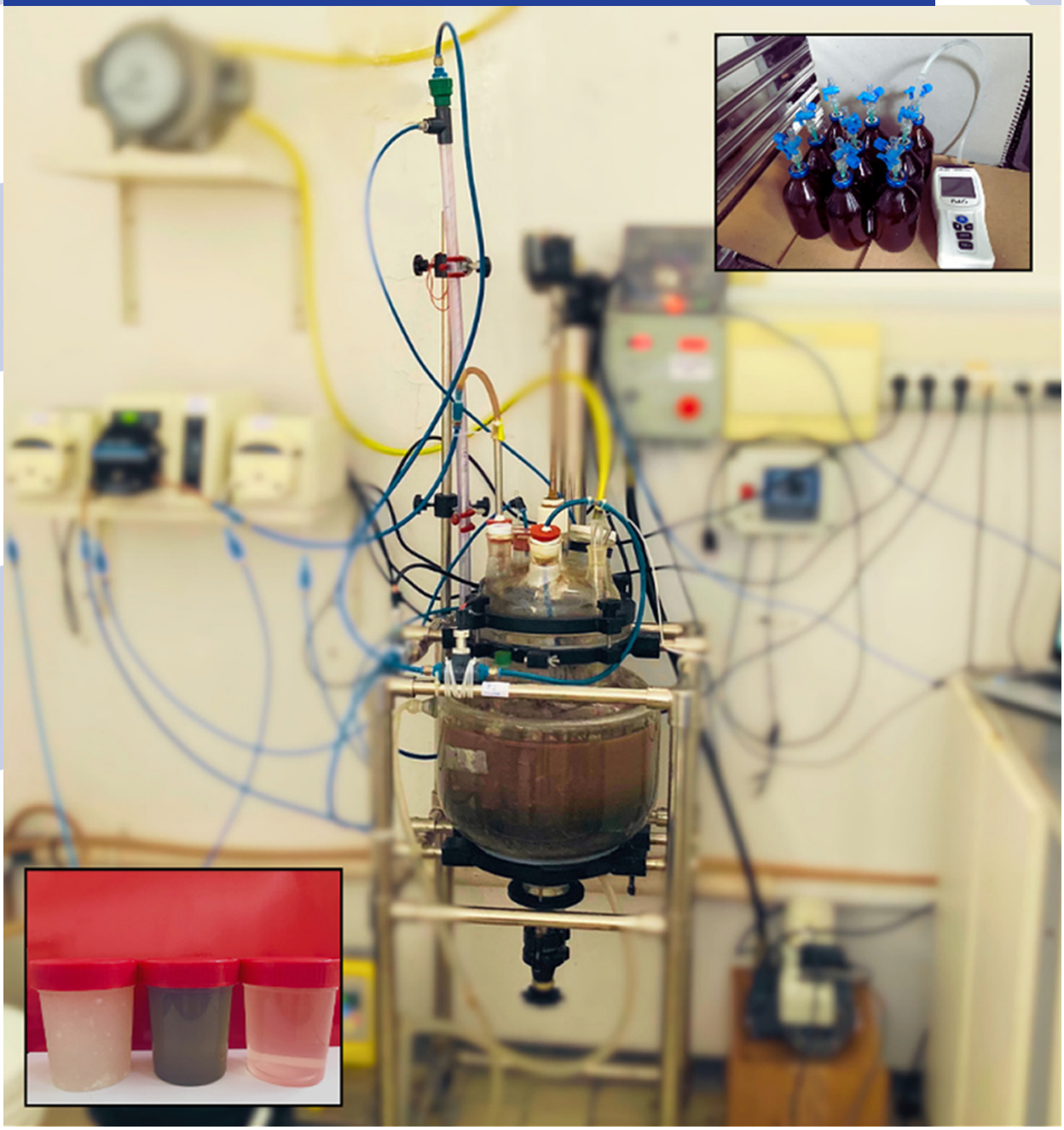

ANAEROBIC MEMBRANE BIOREACTOR (AnMBR) FOR THE TREATMENT OF LIPID-RICH DAIRY WASTEWATER

Maria Alejandra Szabo Corbacho

ANAEROBIC MEMBRANE BIOREACTOR (AnMBR) FOR THE TREATMENT OF LIPID-RICH DAIRY WASTEWATER

DISSERTATION

for the purpose of obtaining the degree of doctor
at Delft University of Technology
by the authority of the Rector Magnificus prof.dr.ir. T.H.J.J. van der Hagen,
chair of the Board for Doctorates
and
in fulfilment of the requirement of the Rector of IHE Delft
Institute for Water Education, Prof.dr. E.J. Moors,
to be defended in public on
Wednesday, 31 January 2024 at 15:00 hours

by

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To Roberto

SUMMARY

The ongoing growth of the global population has led to increased resource consumption, particularly in the realm of water resources, resulting in potential shortages and environmental concerns. The surge in industrialization has intensified the demand for freshwater, consequently causing significant contamination of global water sources through the discharge of industrial wastewater. This wastewater contains harmful contaminants, such as heavy metals and organic compounds, which pose significant threats to both aquatic ecosystems and human health (Corcoran, 2010). To effectively address this issue, it is imperative to strengthen regulatory measures, promote industry-led initiatives for wastewater reduction and treatment, and foster technological advancements in wastewater management.

Lipids within wastewater systems present both opportunities and challenges. Their high energy content holds promise for bioenergy conversion, yet they can also disrupt anaerobic wastewater treatment processes. Consequently, it is often advisable to extract lipids before commencing biological treatment processes (Alves et al., 2009). Lipids are commonly referred to as fats, oils, and grease (FOG) (Cavaleiro et al., 2008). At the core of FOG composition are triglycerides, formed through the esterification of glycerol with long-chain fatty acids (LCFA) (Alves et al., 2009). Within lipid-rich wastewaters, the prevailing LCFAs identified include palmitic acid (C16:0) and oleic acid (C18:1), as highlighted by Hwu et al. (1996).

Anaerobic digestion (AD) plays a central role in advancing various sustainable development objectives by seamlessly integrating energy and resource recovery from organic residues and wastewater, all while effectively managing pollution. AD's ability to produce renewable gaseous energy, recycle essential nutrients, and minimize excess sludge production, combined with an enhanced understanding of microbiology and ecophysiology, has propelled AD technologies to the forefront. These technologies now serve as environmentally friendly treatment options for a wide range of wastes and wastewaters, as evidenced by their widespread adoption at the global level (van Lier et al., 2020).

Sustainable and efficient conversion of these waste lipids into methane within anaerobic reactors is met with impediments including adsorption, sludge flotation, washout, and inhibition. However, these complications can be circumvented through feeding protocols, optimized mixing, and adept solid separation methods, underpinned by cutting-edge reactor designs and operational methodologies. More recently, developments such as the anaerobic membrane bioreactor (AnMBR) and flotation-based bioreactors have emerged as solutions tailored for lipid-intensive wastewater treatment (Cavaleiro et. al., 2008).

AnMBR, a nexus of anaerobic digestion and membrane filtration, has proven particularly adept for dairy wastewater treatment. It alleviates the challenges tied to gravity-based

separation, yielding effluents devoid of suspended solids and of superior quality (Judd, 201).

The central focus of this research centered on the assessment of solids retention time (SRT) and its critical role in the operational parameters of AnMBR. This was accomplished by studying sludge filterability and membrane filtration performance. Additionally, we investigated how the acclimatization of biomass impacted the transformation of long-chain fatty acids (LCFA) in lipid-rich wastewater.

Initial evaluations emphasized the role of SRT on AnMBR efficiency during the treatment of synthetic dairy wastewater laden with lipids. Employing two distinct AnMBR configurations with SRTs of 20 and 40 days, both systems manifested approximately 99% efficiency in waste removal at an organic loading rate of 4.7 g COD L^{-1} d^{-1}. Significantly, lipid sedimentation was absent, facilitating their continued anaerobic degradation. LCFA accumulation was minimal in both systems, with the 40-day SRT configuration showing slightly enhanced biological conversion and stability.

Subsequently, the study delved into the effects of SRT on the filtration efficacy of AnMBR using lipid-rich synthetic dairy wastewater. When confronted with 40-day SRT, the system encountered elevated pressures and resistances, presumably due to escalated contaminant levels, including fats, oils, and LCFAs. While both systems showcased analogous filterability, the 20-day configuration exhibited superior membrane performance, suggesting potential membrane operational refinements for the 40-day SRT.

Lastly, the influence of LCFA on anaerobic sludge processes was investigated. Trialing three distinct sludge samples—two lipid-acclimated and one non-acclimated—they were exposed to varying oleic and palmitic acid concentrations, ranging between 50 to 600 mg COD/L. Oleic acid showed superior degradation capabilities compared to palmitic acid across all samples, with heightened methane production. Lipid-acclimated sludges demonstrated augmented LCFA degradation potential. However, upon reaching LCFA concentrations beyond 400 mg/L, degradation of both acids into intermediate products was inhibited, albeit without affecting methane production. Intriguingly, specific bacterial taxonomies associated with LCFA degradation were identified in lipid-acclimated sludge samples, underscoring the potential of sludge adaptation strategies in enhancing anaerobic treatment of lipid-rich effluents.

In this doctoral research, we elucidated the prospects and challenges associated with the utilization of AnMBR for treating lipid-rich dairy wastewater. We highlighted the critical importance of Solid Retention Time (SRT), a key operational parameter that exerts a profound influence on both the biological and membrane aspects of the system. Furthermore, our study underscored the paramount role played by the two most prevalent Long-Chain Fatty Acids (LCFAs), namely oleic and palmitic acid, within the domain of anaerobic digestion.

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SAMENVATTING

De aanhoudende groei van de wereldbevolking heeft geleid tot een toegenomen consumptie van hulpbronnen, vooral op het gebied van watervoorraden, wat heeft geleid tot potentiële tekorten en zorgen over het milieu. De sterke stijging van de industrialisatie heeft de vraag naar zoetwater geïntensiveerd, waardoor de mondiale waterbronnen aanzienlijk zijn vervuild door de lozing van industrieel afvalwater. Dit afvalwater bevat schadelijke verontreinigende stoffen, zoals zware metalen en organische verbindingen, die een aanzienlijke bedreiging vormen voor zowel aquatische ecosystemen als de menselijke gezondheid (Corcoran, 2010). Om dit probleem effectief aan te pakken, is het absoluut noodzakelijk om de regelgevingsmaatregelen te versterken, door de industrie geleide initiatieven voor de vermindering en behandeling van afvalwater te bevorderen en de technologische vooruitgang op het gebied van afvalwaterbeheer te bevorderen.

Lipiden in afvalwatersystemen bieden zowel kansen als uitdagingen. Hun hoge energie-inhoud is veelbelovend voor de omzetting van bio-energie, maar ze kunnen ook anaerobe afvalwaterzuiveringsprocessen verstoren. Daarom is het vaak raadzaam om lipiden te extraheren voordat biologische zuiveringsprocessen worden gestart (Alves et al., 2009). Lipiden worden gewoonlijk vetten, oliën en vetten (FOG) genoemd (Cavaleiro et al., 2008). De kern van de FOG-samenstelling zijn triglyceriden, gevormd door de verestering van glycerol met vetzuren met lange keten (LCFA) (Alves et al., 2009). Binnen lipiderijke afvalwaters omvatten de belangrijkste geïdentificeerde LCFA's palmitinezuur (C16:0) en oliezuur (C18:1), zoals benadrukt door Hwu et al. (1996).

Anaerobe vergisting (AD) speelt een centrale rol bij het bevorderen van verschillende doelstellingen op het gebied van duurzame ontwikkeling door de terugwinning van energie en hulpbronnen uit organische resten en afvalwater naadloos te integreren, terwijl de vervuiling effectief wordt beheerd. Het vermogen van AD om hernieuwbare gasvormige energie te produceren, essentiële voedingsstoffen te recyclen en overtollige slibproductie te minimaliseren, gecombineerd met een beter begrip van de microbiologie en ecofysiologie, heeft AD-technologieën op de voorgrond gebracht. Deze technologieën dienen nu als milieuvriendelijke behandelingsopties voor een breed scala aan afval en afvalwater, zoals blijkt uit de wijdverbreide toepassing ervan op mondiaal niveau (van Lier et al., 2020).

De duurzame en efficiënte omzetting van deze afvallipiden in methaan in anaerobe reactoren stuit op belemmeringen zoals adsorptie, flotatie van slib, uitspoeling en remming. Deze complicaties kunnen echter worden omzeild door voedingsprotocollen, geoptimaliseerde menging en bedreven scheidingsmethoden voor vaste stoffen, ondersteund door geavanceerde reactorontwerpen en operationele methodologieën. Meer recentelijk zijn ontwikkelingen zoals de anaerobe membraanbioreactor (AnMBR) en op

flotatie gebaseerde bioreactoren naar voren gekomen als oplossingen op maat voor lipidenintensieve afvalwaterbehandeling (Cavaleiro et. al., 2008).

AnMBR, een combinatie van anaerobe vergisting en membraanfiltratie, is bijzonder geschikt gebleken voor de behandeling van zuivelafvalwater. Het verlicht de uitdagingen die gepaard gaan met scheiding op basis van zwaartekracht, waardoor afvalwater ontstaat zonder zwevende vaste stoffen en van superieure kwaliteit (Judd, 201).

De centrale focus van dit onderzoek lag op de beoordeling van de retentietijd van vaste stoffen (SRT) en de cruciale rol ervan in de operationele parameters van AnMBR. Dit werd bereikt door de slibfiltreerbaarheid en membraanfiltratieprestaties te bestuderen. Daarnaast onderzochten we hoe de acclimatisatie van biomassa de transformatie van langeketenvetzuren (LCFA) in lipiderijk afvalwater beïnvloedde.

Initiële evaluaties benadrukten de rol van SRT op de AnMBR-efficiëntie tijdens de behandeling van synthetisch zuivelafvalwater beladen met lipiden. Door gebruik te maken van twee verschillende AnMBR-configuraties met SRT's van 20 en 40 dagen, vertoonden beide systemen een efficiëntie van ongeveer 99% bij het verwijderen van afval bij een organische laadsnelheid van 4,7 g CZV L-1 d-1. Het is veelbetekenend dat lipidensedimentatie afwezig was, wat hun voortdurende anaërobe afbraak mogelijk maakte. De accumulatie van LCFA was in beide systemen minimaal, waarbij de 40-daagse SRT-configuratie een licht verbeterde biologische conversie en stabiliteit vertoonde.

Vervolgens werd in de studie dieper ingegaan op de effecten van SRT op de filtratie-effectiviteit van AnMBR met behulp van lipiderijk synthetisch zuivelafvalwater. Toen het systeem werd geconfronteerd met een SRT van 40 dagen, ondervond het systeem verhoogde druk en weerstand, vermoedelijk als gevolg van geëscaleerde niveaus van verontreinigende stoffen, waaronder vetten, oliën en LCFA's. Hoewel beide systemen een analoge filtreerbaarheid vertoonden, vertoonde de 20-daagse configuratie superieure membraanprestaties, wat mogelijke operationele verfijningen van het membraan voor de 40-daagse SRT suggereert.

Tenslotte werd de invloed van LCFA op anaerobe slibprocessen onderzocht. Bij het testen van drie verschillende slibmonsters – twee lipide-geacclimatiseerd en één niet-geacclimatiseerd – werden ze blootgesteld aan variërende olie- en palmitinezuurconcentraties, variërend van 50 tot 600 mg CZV/l. Oliezuur vertoonde in alle monsters superieure afbraakmogelijkheden vergeleken met palmitinezuur, met verhoogde methaanproductie. Aan lipiden geacclimatiseerd slib vertoonde a vergroot het afbraakpotentieel van LCFA. Bij het bereiken van LCFA-concentraties boven 400 mg/l werd de afbraak van beide zuren tot tussenproducten echter geremd, zij het zonder de methaanproductie te beïnvloeden. Intrigerend genoeg werden specifieke bacteriële taxonomieën geassocieerd met de afbraak van LCFA geïdentificeerd in aan lipiden geacclimatiseerde slibmonsters, wat het potentieel onderstreept van

slibadaptatiestrategieën bij het verbeteren van de anaerobe behandeling van lipidenrijke effluenten.

In dit doctoraatsonderzoek hebben we de vooruitzichten en uitdagingen verduidelijkt die gepaard gaan met het gebruik van AnMBR voor de behandeling van lipiderijk zuivelafvalwater. We benadrukten het cruciale belang van Solid Retention Time (SRT), een belangrijke operationele parameter die een diepgaande invloed uitoefent op zowel de biologische als membraanaspecten van het systeem. Bovendien onderstreepte onze studie de cruciale rol die de twee meest voorkomende langeketenvetzuren (LCFA's), namelijk oliezuur en palmitinezuur, spelen binnen het domein van de anaerobe vertering.

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1

INTRODUCTION

1.1 GENERAL INTRODUCTION

The global population has been increasing steadily, and along with it, there has been an increase in resource consumption. Unsustainable patterns of resource consumption pose significant challenges for the future. These challenges include the potential for food and water scarcity, social inequity, and adverse impacts on public and environmental health (Corcoran, 2010). Water consumption, for example, has been on the rise since the 1980s, with an average annual growth rate of 1%. This trend is projected to continue, leading to an estimated total increase of 20-30% in water consumption, exacerbating water scarcity issues worldwide (UN, 2019). While the population growth rate has decreased over time, resource consumption remains a concern due to the cumulative impact of a growing global population. Sustainable practices and equitable resource management are crucial to address the challenges posed by population growth and the corresponding strain on resources. It is essential to promote responsible consumption and adopt sustainable strategies to ensure a more balanced and secure future.

The increased industrialization has led to a growing demand for fresh water. The industrial wastewater is one of the significant sources of water pollution globally. Industries such as textiles, pulp and paper, food processing, and chemical manufacturing discharge significant amounts of contaminated industrial wastewater and the untreated or inadequately treated industrial wastewater discharged into water bodies can have severe environmental and public health consequences (Corcoran et al., 2010).

The wastewater generated from these industries contains a variety of pollutants, including heavy metals, organic compounds, and nutrients. Heavy metals, such as lead, cadmium, and mercury, can be toxic to aquatic life and accumulate in the food chain, ultimately affecting human health (Kumar et. al., 2019). Organic compounds, such as pesticides and solvents, are often persistent in the environment and can cause long-term harm to wildlife and human populations. Nutrients, such as nitrogen and phosphorus, can lead to eutrophication in water bodies, resulting in algal blooms and oxygen depletion, leading to fish kills and other adverse ecological impacts (Shah, 2017).

Moreover, industries' high volumes of wastewater production can also cause physical and biological changes to aquatic ecosystems. For example, the discharge of warm wastewater into rivers or lakes can increase the water temperature, affecting aquatic organisms' metabolic rates and potentially causing thermal shock. The high organic load in industrial wastewater can also result in oxygen depletion, causing hypoxia, which can lead to the death of aquatic organisms (UNIDO, 2018).

The effects of industrial wastewater contamination are not limited to aquatic ecosystems alone; they can also significantly impact human health. For example, consuming contaminated fish or shellfish can lead to mercury poisoning or other toxicities. Additionally, the discharge of untreated or inadequately treated industrial wastewater into

groundwater can contaminate drinking water sources, posing a significant public health risk (WHO, 2018).

Contamination by industrial wastewater is complex, requiring the involvement of various stakeholders to address it. Governments can play a crucial role in regulating industrial wastewater discharge by imposing strict discharge standards and monitoring. Industries themselves can also take steps to reduce their wastewater generation and treat their wastewater before discharge. Additionally, technological advancements in wastewater treatment can enhance treatment techniques, making them more effective and efficient (UNIDO, 2018).

The United Nations has identified 17 Sustainable Development Goals (SDGs) as part of its 2030 Agenda for Sustainable Development (UN, 2015). These goals aim to end poverty, protect the planet, and ensure prosperity for all. The following are the SDGs related to industrial wastewater contamination:

> Goal 6: Clean Water and Sanitation - This goal aims to ensure the availability and sustainable water and sanitation management for all. This includes ensuring access to safe and affordable drinking water and improving water quality by reducing pollution and increasing water treatment.

In conclusion, industrial wastewater contamination is a significant environmental and public health issue. Therefore, it is imperative that all stakeholders, including governments, industries, and individuals, take steps to mitigate the negative impacts of industrial wastewater discharge on the environment and human health.

1.2 THE DAIRY INDUSTRY

The dairy industry plays a critical role in the global food system and economy. Milk and dairy products are essential parts of a healthy diet. Their consumption is encouraged by the World Health Organization (WHO) guidelines since. These products are basic components of diets around the world, providing valuable nutrients such as protein, calcium, and vitamins. (WHO, 2005). The rapid industrial expansion brings not only increased productivity but also a greater release of potentially toxic substances into the environment, both on land and in water bodies. This discharge can lead to environmental degradation and pose significant health risks to humans (Porwal et al., 2015).

The dairy industry produces yogurt, cheese, butter, ice cream, milk powder, and various types of desserts through several manufacturing processes from raw milk. These processes require water usage in all stages; hence the amount of wastewater produced is significant, (Sarkar et al., 2006; Karadag et al., 2015), around 6-10 L of waste effluent per liter of processed milk.

The components of dairy effluent are diverse. They include milk and milk products that are lost during the technological cycle, such as skimmed milk, spoiled milk, spilled milk,

and pieces of curd. They also contain by-products of processing operations like whey, whey permeates, and milk, as well as the starter cultures employed in the creation of fermented products. Additionally, they consist of reagents used in Cleaning-In-Place (CIP) procedures, contaminants from the washing of trucks, cans, equipment tanks, and floors, and various additives used in the manufacturing process (Slavov, 2017).

The waste from dairy industries, due to its high organic content, presents a significant threat to the environment. Consequently, there are strict regulations in place to control the discharge of dairy industrial effluents. These regulations aim to mitigate the potential environmental consequences associated with dairy wastewater and ensure responsible and a sustainable management.

1.3 CHARACTERISTICS OF DAIRY INDUSTRY

The composition of untreated dairy wastewater depends largely on the types of products and processes involved (Carvalho et al., 2013). Dairy industry effluents are characterized by high concentrations of organic matter, fats and oils, fatty acids, and significant quantities of nitrogenous phosphorus, and potassium compounds coming from dissolved organic materials, such as lactose, minerals, fats, and whey protein. Furthermore, cleaning agents and sanitizing products used in cleaning contribute to the wastewater content. (Porwal et al., 2015; Singh et al., 2013).

The fats, oils, and grease, commonly characterized as “lipids”, are significant components of wastewater from dairy industries (Cavaleiro et al., 2008). Fats, oils, and grease (FOG) are a sub-group of organic contaminants that include lipids consisting of triglycerides, composed of glycerol esterified with long chain fatty acids (LCFA) (Alves et al., 2009). According to (Hwu et al., 1998), the more common LCFAs found in lipid-rich wastewaters are palmitic acid (C16:0) and oleic acid (C18:1).

1.4 TREATMENT OF DAIRY WASTEWATER

The treatment of dairy wastewaters can be conducted using either physicochemical or biological methods. However, due to the high costs associated with reagents and the insufficient removal of COD in the physicochemical approach, the biological method is often favored (Demirel et al., 2005). The biological treatment has emerged as the most popular strategy for handling dairy wastewater, including trickling filters, aerated lagoons, activated sludge, up-flow anaerobic sludge blanket (UASB), anaerobic filters, and sequential batch reactors (SBR) (Yonar, 2018). Known for its efficacy in dealing with organic materials from dairy waste, the biological method offers a considerable promise (Carvalho et al., 2013).

1.4.1 Biological treatment

Biological treatment can be divided into two main types: Aerobic and Anaerobic. Most dairy wastewater treatment plants utilize the aerobic treatment, though its efficiency is often limited due to quick acidification and filamentous growth, which arise from the low buffer capacity and elevated lactose levels, respectively. The aerobic biological treatment is reliant on microorganisms, which thrive in oxygen-rich environments and convert organic compounds into carbon dioxide, water, and cellular material (Britz et al., 2006). This method is highly effective in degrading nitrogen from ammonia (NH_3); however, its effectiveness dwindles when it comes to phosphorus removal, which heavily depends on environmental conditions (Rosenwinkel et al., 1999). In a continuous mode (CSTR), the aerobic biological system demonstrates satisfactory results in treating synthetic dairy wastewater, achieving over 96% degradation with a COD, TKN and pH of 4 g/L, 1 g/L, and 11.5, respectively (Carta-Escobar et al., 2004).

1.4.2 Anaerobic digestion

Anaerobic digestion is considered one of the oldest wastewater treatment processes (Pavlostathis & Giraldo-Gomez, 1991). Anaerobic wastewater treatment is a cost-effective technology, when compared to aerobic treatment, and is applicable to a large diversity of wastewaters, ranging from low, medium to high strength, including sewage and industrial wastewater (Wijekoon et al., 2011; Karadag et al., 2015). It is now considered a consolidated technology with more than 4000 high-rate reactors implemented worldwide (Van Lier, 2020). The advantages of anaerobic treatment include i) a low biomass yield, ii) high loading potential, iii) less maintenance demand, iv) smaller reactors, and v) low operational and maintenance costs, in addition to vi) biogas recovery and vii) greenhouse gases emission reduction; makes anaerobic treatment a very attractive solution for the removal of organic matter from industrial wastewater (Hawkes et al., 1995; Pretti et al., 2011; Dereli et al., 2012; van Lier et al., 2020). These anaerobic treatment techniques include: anaerobic digestion (AD), upward-flow anaerobic sludge blanket (UASB), upward-flow anaerobic filters, completely stirred tank reactors (CSTR), anaerobic contact processes, expanded and/or fluidized-bed digesters, membrane anaerobic reactor systems (AnMBRs), and fixed-bed digesters (Goli et al., 2019).

The anaerobic degradation of complex organic matter to methane (CH_4) and carbon dioxide (CO_2) involves the collaboration of different microbial species and has been described as a multistep process of series and parallel reactions. According to Angelidaki et al. (2011), the syntrophic relationships between the hydrogen producers (acetogens) and hydrogen scavengers (homoacetogens, hydrogenotrophic methanogens) are of extreme importance to this process. The first step of anaerobic digestion is the hydrolysis of complex polymeric materials. Next, polysaccharides, proteins, and lipids (fat, oils, and grease) are hydrolyzed by extracellular enzymes to soluble products of small size to allow their transport across the cell membrane. These relatively simple, soluble compounds are

fermented or anaerobically oxidized to short-chain fatty acids, alcohols, carbon dioxide, hydrogen, and ammonia. Finally, the short-chain fatty acids (other than acetate) are converted to acetate, hydrogen gas, and carbon dioxide. Lastly, methanogenesis can take place from carbon dioxide reduction by hydrogen and acetate (Pavlostathis & Giraldo-Gomez, 1991). Acetate is the most important precursor of methane in an anaerobic digestor, representing 70% of the total carbon flow to methane. In comparison, the remaining 30% is formed from hydrogen and carbon dioxide or formate.

As can be observed in Figure 1.1 is described the anaerobic digestion pathway.

In addition to hydrolysis of biopolymers and fermentation of intermediates, the activity and performance of methanogenic archaea is of major importance for methane production (Demirel & Scherer 2008). To obtain stable and efficient methanogenesis, all these conversion steps and microorganisms needs to be synchronized and it is very important to fulfil the environmental and thermodynamic requirements of all the microorganisms involved.

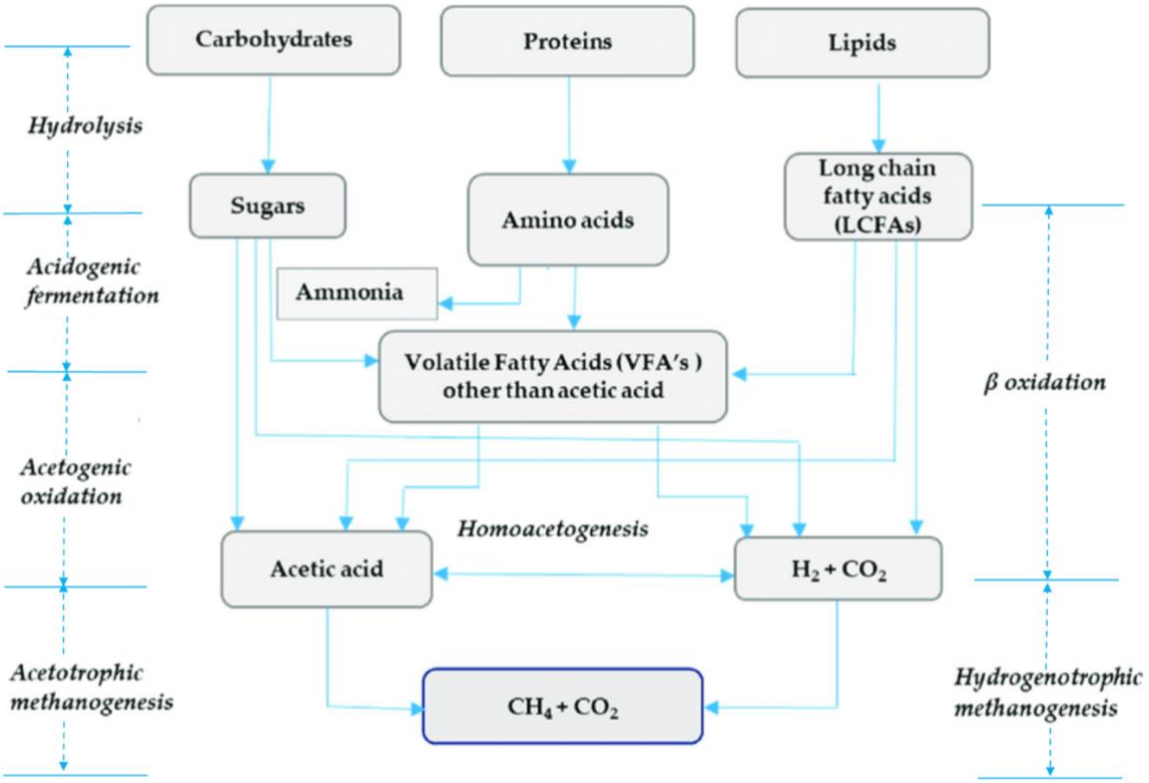

Figure 1.1 – Anaerobic digestion scheme.

1.4.3 FOG and Anaerobic Digestion

FOG are potentially inhibitory compounds, which are commonly present in dairy wastewater and may restrict anaerobic treatment. In anaerobic digestion, the lipids are hydrolyzed by extracellular lipases to yield glycerol and LCFAs (Figure 1.1). Glycerol, which is further degraded via acidogenesis, has been reported as a non-inhibitory compound (Perle et al. 1995). The LCFA are degraded to acetate, H_2, and CO_2 through the β-oxidation process (syntrophic acetogenesis) (Cavaleiro et al. 2008). This pathway has a relatively slow reaction rate and is hindered by the low solubility of LCFAs (Kim et al. 2004). The presence of LCFA may induce toxic and inhibitory effects on acetoclastic methanogens (Hanaki & Nagase, 1981; Lalman & Bagley, 2000). Considering the average low oxidation state of carbon, the treatment of lipids/LCFA-rich wastewaters in anaerobic bioreactors yields methane-rich biogas (Alves et al., 2009). However, the two major problems in the anaerobic treatment of fats and lipids containing wastewaters are, in the first place, sludge flotation and biomass wash-out due to adsorption of lipids/LCFA onto the sludge (Hwu et al., 1998; Hanaki & Nagase, 1981; Angelidaki & Ahring, 1995) and in second place the inhibition of the microbial activity by LCFAs (Hanaki & Nagase, 1981; Rinzema et al., 1994). According to Pereira et al. (2005), the decrease in methanogenic activity during anaerobic digestion after cell contact with LCFA is related to the mass transfer limitation of substrates and products due to LCFA accumulation onto the sludge. In the cited study of Pereira et al. (2005), the metabolic inhibition of LCFA has been encountered as a temporary effect, being eliminated after depletion of LCFA linked to the biomass. Since conventional anaerobic reactor systems like sludge bed reactors do not achieve successful performance in terms of COD converted to methane and are frequently confronted with reactor failure, the treatment of dairy wastewater is considered a challenge (Cavaleiro et al., 2008). Therefore, specific reactor designs and process operational conditions are required to optimize methane production from LCFA-rich wastewaters by mixed methanogenic communities (Sousa et al., 2009).

A better insight in microbial community composition and dynamics is of particular interest since, thus far, anaerobic bioprocesses have been operated as “black boxes” without properly accounting for the relationship between microbiology and process function (Seib et al. 2016). With recent advances in genomics and sequencing technologies, the microbial community analyses using culture-independent molecular techniques have initiated a new era of microbial ecology (Rastogi & Sani 2011). Increased knowledge of key microbial players is crucial to understand the potentials and limitations of the various sequential steps constituting anaerobic digestion, i.e., hydrolysis, fermentation, acetogenesis, and methanogenesis (Mckeown et al. 2015). Such insights, linking microbial community composition and biochemical functionality, could be used to match inoculum biomass to specific operating conditions, including temperature or waste type (McKeown et al., 2012).

Kougias et al. (2016) and Ziels et al. (2016) reported the importance of microbial community adaptation for the proper degradation of lipids. The limitations observed in the degradation of LCFAs is often due to the low presence of syntrophic bacteria in the anaerobic biomass community (Cavaleiro et al., 2016). Pereira et al. (2002) reported a better oleic acid degradation on biomass pre-exposed to lipids compared to biomass non-exposed to lipids. Silva et al., (2014) observed that a long-term acclimation of sludge to LCFAs is essential to obtain a bacterial community, able to effectively convert LCFA to methane. Cavaleiro et al. (2009), were able to degrade a LCFA-rich wastewater, at a very high OLR of approximately 21 kg COD/m^3 d, in a continuous reactor acclimating the microbial community by using a pulse feeding strategy. Moreover, Cavaleiro et al. (2010) bioaugmented non-acclimated sludge with a LCFA degrading bacterium (*Syntrophomonas zehnderi*) when treating oleic acid; by applying that strategy the authors doubled the methane production. Silva et al. (2014) and Cavaleiro et al. (2010) concluded that both the presence of a long-term acclimated biomass, as well as avoiding LCFA accumulation in the reactors are the two most influential factors for maximizing LCFA degradation to methane.

1.4.4 Anaerobic treatment technologies

The continuous stirred tank reactor (CSTR) and anaerobic contact process (ACP) were the first anaerobic reactors developed for the treatment of industrial wastewater (Tauseef et al., 2013). However, completely mixed reactor systems require an enormous footprint, are characterized by high hydraulic retention times (HRT) and have electromechanical equipment installed for mixing purposes. As a result, investment, and operational costs of CSTR and ACP reactors are very high for the more dilute types (< 10 g COD/L) of industrial wastewater; gradually these types of reactors were substituted by the high-rate anaerobic reactors (HRAT).

Anaerobic filters have been successfully applied for the wastewater treatment of dairy streams with low concentrations of suspended solids (Viraraghavan & Kikkeri, 1990). Several studies have been reported where the organic loading rate varied from 5.5 up to 21 kg COD/(m^3 day) and removal efficiencies between 75-90% (Viraraghavan & Kikkeri, 1991; Ince, 1998).

The up-flow anaerobic sludge blanket (UASB) reactor, developed in the seventies (Lettinga et al., 1980), has been successfully applied to treat many kinds of industrial wastewater, mainly in the agro-food sector. Nowadays, there are different types of granular sludge-based reactors, such as, the UASB reactor, the expanded granular sludge bed (EGSB) reactor, and the internal circulation (IC) reactor, which dominated the market in the past decades (van Lier, 2008; van Lier et al. 2020) and are worldwide applied. UASB reactors have been effectively applied to dairy wastewater treatment for over two decades (Demirel et al., 2005). The reported studies applied organic loading rates from 8.5 to 31 kg COD/(m^3 day), and the removal efficiencies were 87 to 90%. The treated

wastewater had a content of suspended solids varying from 0.4-5 g TSS/L (Gutirrez, 1991; Hwang et al., 1992; Yan et al., 1989; Gavala & Kopsinis, 1999).

According to Demirel et al. (2005), FOG and the high concentrations of suspended solids and in dairy wastewaters can affect the performance of this treatment technology. It is commonly observed that anaerobic sludge granules are encapsulated by FOG and form a floating layer, prone to wash-out; moreover, studies reported granule disintegration and biomass loss with the effluent (Alves et.al. 2009). A proper granulation process is conditional for the successful operation of high-rate anaerobic sludge bed reactors.

Complex industrial wastewaters, characterized by a high content of FOG, salt, particulate matter such as the dairy industry, may hamper granule formation or may lead to de-granulation and loss of biomass, and flotation; so, an alternative technology is required (Dereli et al., 2012; van Lier et al., 2015; Skouteris et al., 2012; Jeison et al., 2008; Lin et al., 2013; Visvanathan & Abeynayaka, 2012).

1.5 ANAEROBIC MEMBRANE BIOREACTORS (ANMBR)

The anaerobic membrane bioreactor (AnMBR) combines anaerobic digestion and membrane filtration. AnMBR technology for dairy industry wastewater treatment eliminates the operational and biological issues associated with gravity separation and produces a suspended solid-free effluent with excellent quality (Judd, 2011).

The AnMBR was first introduced in the late 1980s and offers a method of solid–liquid separation and biomass retention, allowing the successful decoupling of HRT and SRT (Visvanathan & Abeynayaka, 2012). It is an advantageous technology over traditional AD systems, offering a smaller physical footprint, superior effluent quality, and enhanced biogas production efficiency (Dereli et. al., 2012). According to Ng (2006), the AnMBRs can operate at high biomass concentrations, high SRTs and OLR, and relatively short HRTs, independent of the sludge flocculation state. Moreover, it produces biogas, which can be recovered as a renewable energy source (Liao et al., 2006). The energy recovered must be balanced against the increased system energy consumption related to the membrane filtration process (Smith et al., 2014).

AnMBRs are used for the treatment of effluents from the dairy industry (Arros-Aileche et al, 2008; Al-Malack, 2015), the beverage industry (winery, brewery, and distillery), (Dereli, 2013; Torres, 2011) and slaughterhouses (Chen, 2012; Saddoud, 2017). High COD removal efficiencies (up to 94%) were achieved in these studies and the organic loading rate varied in the range 2-15 kgCOD/(m^3 d). Dereli et al. (2013) conducted an experiment using a laboratory-scale AnMBR to treat lipid-rich thin stillage, a byproduct of corn-to-ethanol production, attained removal efficiencies as high as 99% with an OLR up to 8 kg/(m^3 day). In addition, Ramos et al. (2011) evaluated the effectiveness of a pilot AnMBR in treating lipid-rich wastewater generated from a snack manufacturing facility. They obtained satisfactory results with the OLR below 2 kgCOD/(m^3 d). Most of these

studies used completely mixed bioreactors with external cross-flow membranes, while the others used a submerged membrane.

There are two principal approaches to membrane design and operation (Liao et al., 2006; Stuckey,2012; Robles et al., 2018), sidestream and immersed. The sidestream configuration is typically utilized for treating high-strength wastewaters, such as those produced by industrial activities. On the other hand, the immersed configuration is commonly used for the treatment of low-strength wastewaters, like sewage.

The sidestream configuration is where the membrane can be operated under pressure or vacuum, as shown in Figure 1.2a. The membrane is separate from the bioreactor, and a feed pump pressurizes the feed flow towards the membrane unit. While passing the membrane, a fraction of the feed flow permeates through the membrane, while the remainder is returned to the bioreactor. The cross-flow velocity of the liquid across the membrane allows scouring of the membrane surface to reduce membrane fouling (Liao et al., 2006; Stuckey, 2012). In practice, cross-flow velocities between 1-4 m/s are applied, brought about by an external feed pump that circulates the biomass through the membrane skid. The resulting energy costs are high, which is compensated by the recovered biochemical energy in the form of CH_4. Moreover, the external feed pump may induce floc and cell shear, decreasing the average sludge particle size (PSD) and increasing the soluble organics (Kim et. al., 2011).

The second configuration (Figure 1.2b and 1.2c) is when the permeate side of the membrane is operated under vacuum, and is called submerged or immersed, since the membrane is placed into the liquid. A pump or plain gravity is used to extract the permeate through the membrane. The build-up of a fouling layer on the membrane surface, also referred to as cake formation, can be mitigated by gas bubbling across the membrane surface (Liao et al., 2006, Robles et al., 2018). The submerged approach can be used in two configurations: immersed directly into the bio-reactor (Figure 1.2b) or immersed in a separate membrane tank (Figure 1.2c), which facilitates the cleaning and maintenance of the submerged membranes. The advantage of submerging the membrane in the reactor is that the energy for pumping is minimalized. However, for fouling mitigation, the produced biogas must be recycled using a gas pump for sparging the membrane surface. In addition, the biomass is exposed to less severe shear compared to a side stream configuration. The moderate shear leads to increased membrane resistance and thus, lower fluxes and higher membrane area demand (Stuckey, 2012; Robles et. al., 2018).

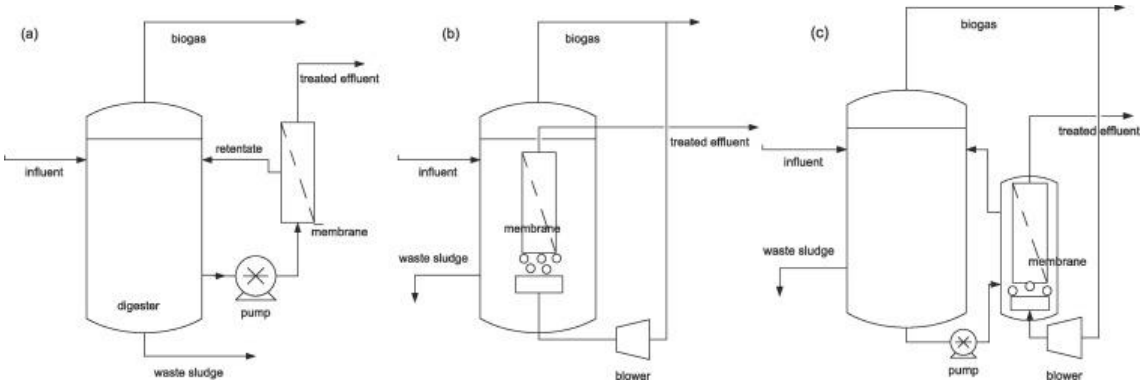

Figure 1.2 – Schematic representation of AnMBR configurations (a) Sidestream, (b)Inmersed, (c) combination of sidestream and inmersed. Source: Robles et. al., (2018).

1.6 MEMBRANE FOULING

AnMBR reactors are characterized by very high treatment efficiencies. However, there is a need for a deeper understanding of fouling mechanisms involved and a more detailed knowledge of the fouling process that could greatly improve the optimization of control strategies (Ramos et. al., 2018). Fouling hinders the flux, causing an increase in the required membrane area per reactor volume, which subsequently leads to higher capital costs (Stuckey, 2012). Over the past decades, numerous research efforts have been dedicated to understanding and mitigating fouling phenomena, primarily related to aerobic MBRs, as reviewed by Le-Clech et al. (2006) and Meng et al. (2009).

Membrane fouling in Anaerobic Membrane Bioreactors (AnMBRs) is a combined process that comprises biofouling, organic, and inorganic fouling. All these mechanisms generally occur concurrently, although the impact of each one varies based on factors such as membrane and sludge characteristics, environmental conditions, reactor design, and operational strategies (Liao et al., 2006).

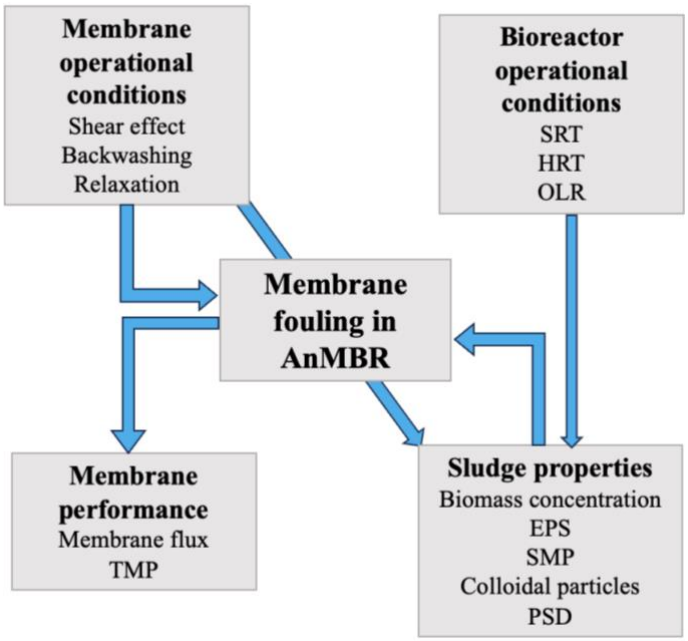

Figure 1.3 – Factors affecting membrane fouling in AnMBRs

Traditionally, membrane fouling is categorized into reversible and irreversible fouling, based on the cleaning techniques used, although the definitions vary across different sources. In this context, is used the classification proposed by Meng et al. (2009), which divides reversible fouling into removable and irremovable fouling. Removable fouling refers to the type that can be eliminated using physical methods such as backflushing or relaxation under cross flow conditions. In contrast, irremovable fouling needs chemical cleaning for removal. Irreversible fouling represents a permanent type of fouling that cannot be eliminated through any cleaning methods. Generally, removable fouling happens due to the loose external deposition of material, while irremovable fouling is triggered by pore blocking and the adherence of strongly bound foulants during the membrane filtration process (Meng et al., 2017). The formation of a robust fouling layer matrix with the solute during continuous filtration can transform removable fouling into an irremovable fouling layer. Given the nature and causes of irremovable fouling, numerous studies have been conducted to examine the cake layer (Jeison and van Lier, 2009).

From the perspective of the constituents causing fouling, membrane fouling can be categorized into biological, organic, and inorganic types (Liao et al, 2006; Meng et al., 2009). Biological fouling is specifically associated with the interaction between the membrane and biomass. The onset of membrane fouling is typically linked to pore blockage, triggered by cell debris and colloidal particles. Even before the deposition of biomass begins, passive adsorption of colloids and organic substances has been observed, even under zero-flux operations (Di Bella et al., 2007).

The mechanisms of biofouling can be grouped into three main categories: pore clogging, sludge cake formation, and the adsorption of extracellular polymeric substances (EPS) (Liao et. al., 2006). Pore clogging occurs when cell debris and colloidal particles, similar to the membrane pore size, accumulate within the pores, thus reducing the filtration surface area. Studies by Choo and Lee (1996) suggest that colloids, rather than dissolved and cellular fractions, are the principal contributors to fouling in Microfiltration (MF) and Ultrafiltration (UF) membranes. Pressure-driven, external crossflow filtration has been linked with increased fouling, as pump-induced shear stresses can reduce particle size and free up colloids, leading to pore clogging (Chang et al., 1994). The restoration of membrane flux after backwashing (Chang et. al., 1994; Beaubien et. al., 1996; Zeigler, 2007; Ye et. al., 2006; Ho et. al., 2009) is evidence of the role of pore-clogging in membrane fouling and presents a strategy to counter flux losses due to this mechanism.

Sludge cake formation happens when the shear stress at the membrane surface is insufficient to remove solids. Research by Choo and Lee (1996) showed that a thick cake layer comprising biomass and struvite forms on polymeric membrane surfaces, causing significant hydraulic resistance. The increase in membrane flux, brought about by enhanced shear forces through gas circulation and gas-liquid two-phase flow in cross-flow membranes (Kim et al., 2007; Zhang et al., 2005; Padmasiri et al., 2007; Ye et al., 2006), underpins the involvement of sludge cake formation in membrane fouling (Jeison et al., 2009). The degree of biofouling due to cake deposition will depend on the concentration of suspended material presented to the membrane (Lin et al., 2013).

Lastly, the accumulation and adsorption of extracellular polymeric substances (EPS) and soluble microbial products (SMP) on membrane and pore surfaces is another biofouling mechanism. Cho and Fane (2002) noticed that a lower membrane flux was associated with a larger quantity of EPS per unit membrane surface area, with a clear correlation between EPS deposition load and fouling resistance. Autopsies of membranes also unveiled significant fouling by EPS and an uneven distribution of EPS (Lin et al., 2013).

Organic fouling arises from the buildup and attachment of organic constituents on the surfaces of membranes. While fouling resulting from extracellular polymeric substances (EPS) can also be viewed as a form of organic fouling, it was previously discussed under biofouling to underline the biological origin of EPS and soluble microbial products (SMP) (Liao et al., 2006).

Operating at higher Solid Retention Times (SRT) may help diminish organic fouling by reducing the effluent COD concentration. Though impractical at a full scale, additives like powdered activated carbon and zeolites have been used in AnMBRs to absorb soluble organic compounds, reducing organic fouling, and enhancing membrane flux (Liao et al., 2006).

Inorganic fouling, on the other hand, results from inorganic colloids and crystals on the membrane and pore surfaces. Struvite ($MgNH_4PO4 \cdot 6H_2O$) precipitation is the most prevalent inorganic foulant, occurring on organic and inorganic membranes. Other

inorganic foulants may include substances like $K_2NH_4PO_4$ and $CaCO_3$. AnMBRs may be more prone to inorganic fouling than aerobic MBRs, partly due to the greater likelihood of pH shifts caused by changes in carbon dioxide partial pressure and the production of high concentrations of ammonia and phosphate, especially during sludge digestion (Lin et al., 2013; Dereli et al., 2015).

1.7 OPERATIONAL PARAMETERS OF ANMBR

Considered as modifiable operational parameters, HRT and SRT hold paramount importance in the functioning of an AnMBR. They influence treatment performance and biomass characteristics, which in turn significantly impact the development of membrane fouling (Liao et al., 2006; Huang et al., 2011; Lin et al., 2013).

1.7.1 Sludge retention time (SRT)

The Solid Retention Time (SRT) is a crucial operational parameter significantly influencing treatment performance and membrane fouling. Unlike UASB reactors, an AnMBR ensures complete biomass retention, thus providing more straightforward SRT control. Studies by Trzcinski and Stuckey (2010) evaluated the performance of two submerged AnMBRs treating municipal solid waste leachate at psychrophilic temperatures with SRTs of 300 and 30 days, respectively. They found that a longer SRT was linked with better soluble COD removal. Generally, operating an AnMBR with relatively longer HRTs and SRTs is beneficial for enhancing methane recovery, improving treatment performance, and reducing sludge production (Ho et al., 2009).

Sludge age is one of the most critical design and operational parameter in anaerobic treatment systems and is expected to affect SMP formation significantly (Ince et al., 2000). As a rule of thumb, SRT in high-rate anaerobic bioreactors is equal or exceeds three times the doubling time of the rate-limiting biomass, which commonly is the slow-growing heterotrophic methanogenic biomass with doubling times of 4-10 days. Therefore, an SRT of more than 20 days is generally applied in mesophilic (30-35^{o}C) anaerobic high-rate reactors (Dereli et al., 2012). However, in most anaerobic high-rate reactors, the applied SRTs generally vary from 30 to 300 days, which results in less sludge production (Dereli et al., 2013; Zhen et al. 2019).

The SRT directly affects the biological performance and also the biological characteristics of the suspension, such as biomass concentration, EPS content, and SMP content (Ng et al., 2006). For example, high SRTs may results in increased cell lysis and increasing release of inert decay products; the increased sludge concentrations and SMP content leads to a rapid cake build-up (Dereli et al., 2012; Jeison and van Lier, 2006). Therefore, SRT appears as a determining criterion in AnMBRs, not only for the bacterial capacity to transform organic matter in volatile fatty acids and biogas, but also for its influence on the filterability of the suspension. Regarding the latter, two main criteria are indentified,

i.e., i) solids in suspension and ii) SMP/ EPS concentrations, and their role on fouling dynamics (Ng et al., 2006). Evidently, at long SRT, the sludge will be highly mineralized; however, the biomass associated products (BPAs), which here refer to the SMPs in the digester, tend to increase with increasing SRTs, thus resulting in increased effluent COD (Barker & Stuckey, 1999).

1.7.2 Hydraulic retention time (HRT)

In AnMBR applications, the Hydraulic Retention Time (HRT) has ranged from 2.6 hours to 14 days. When treating high-strength wastewater and dilute wastewater, the typical HRT falls between 1-10 days and 0.25-2 days, respectively. Lengthening the HRT usually enhances pollutant removal, but the improvements reach a plateau at a certain point. For instance, Hu and Stuckey (2006) noted only a slight decrease in COD removal (around 5% overall) when the HRT was reduced from 48 hours to 24, 12, 6, and 3 hours during the treatment of synthetic dilute wastewater.

As the Hydraulic Retention Time (HRT) increases within the reactor, there is a simultaneous accumulation of Soluble Microbial Products (SMP) and Extracellular Polymeric Substances (EPS). This build-up can lead to membrane fouling (Berkessa et al., 2018). The fouling triggered by this SMP accumulation can manifest through various mechanisms, such as standard, intermediate, or complete pore blockage, as well as the formation of a cake layer, as observed by Herrera-Robledo et al. (2011).

Optimal AnMBR operations typically employ a Hydraulic Retention Time (HRT) ranging from 4 hours, as noted by Salazar-Peláez et al. (2011), to 2 days, as found by Baek et al. (2010). Simultaneously, the sludge concentration should be between 8 and 12 g/L. However, if the organic matter is not readily biodegradable, a longer HRT can help alleviate the problem, although this approach necessitates a larger reactor footprint. The work of Qiao et al. (2013) underscores the importance of an extended HRT for specific waste categories; their study involved an HRT between 30 and 70 days to examine the thermophilic anaerobic digestion of coffee grounds, with and without waste-activated sludge as a co-substrate, using a submerged AnMBR.

1.8 RESEARCH HYPOTHESIS AND OBJECTIVES

In this study, the AnMBR is researched as a viable technological alternative to conventional anaerobic wastewater treatment for the treatment of dairy industrial wastewater with high concentrations of lipids. Based on the research gaps outlined in the previous sections, the research was particularly focused on the evaluation of SRT as most crucial operational parameter of AnMBRs, determining SRT, sludge filterability, and membrane filtration performance. Additionally, the effect of biomass acclimation on LCFA conversion in the lipid rich wastewater was researched.

The overall objective of this study was to assess the treatment performance of anaerobic membrane bioreactors treating lipid rich wastewater from dairy industry and to research the operational factors affecting the biological and membrane performance.

This research addresses the following hypothesis:

i. Applying anaerobic digestion to lipids presents promising prospects for sustainable and efficient lipid valorization, enabling the long-term operation of stable systems.
ii. A high SRT will result into a high sludge concentration, a better substrate removal efficiency, and less production of waste sludge. An increased substrate conversion rate is expected at high SRT due to a low F/M ratio, corresponding to a better bioconversion of organic matter and lipids to methane.
iii. Sludge filterability is negatively affected by an increase in SRT, resulting in an increase in transmembrane pressure (TMP). At high SRT, the SMP and EPS are expected to increase due to a higher biomass concentration and cell lysis in the AnMBR. High SMP concentrations might result into cake compaction and pore blocking of the membrane, which would lead to an increase in the TMP when working at a constant flux.
iv. The acclimation of sludge to dairy lipid-rich wastewater is critical for achieving exceptional methane production, mainly when the feed contains oleic and palmitic acids.

The specific objectives of this research are as follows:

i. Provide a critical review containing a synthesis of the recent advancements in anaerobic digestion of lipids pertaining to anaerobic wastewater treatment[1].
ii. Determine the most critical aspects relate to the microbiology and technology of efficient lipids conversion in anaerobic reactors[1].
iii. Assess and evaluate the biological performance of an AnMBR treating synthetic lipid-rich dairy wastewater at different SRTs[2].
iv. Assess the impact of the presence and accumulation of LCFAs in AnMBR operated at different SRTs[2].
v. Evaluate the membrane filtration performance when treating lipid-rich wastewater from a dairy industry in an AnMBR at different SRTs[3].
vi. Study the effect of dairy influent wastewater characteristics and operational SRTs on the sludge characteristics, i.e., TSS, dynamic viscosity, PSD, CST,

[1] This specific objective is addressed in the hypothesis nr. i

[2] This specific objective is addressed in the hypothesis nr. ii

[3] This specific objective is addressed in the hypothesis nr. iii

SRF, SF, EPS, and SMP, and the impact of the sludge features on the membrane filtration performance, i.e., total resistance to filtration, flux decline, and TMP[3].

vii. Study the effect of biomass acclimation on LCFA biomethanation using acclimated and non-acclimated sludge and assess the methanogenic activity utilizing different concentrations of oleic and palmitic acids[4].

viii. Study the microbial population in AnMBR sludge using metagenomic analysis and discuss the possible role of the predominant microorganisms in the LCFA degradation pathway[5].

1.9 RESEARCH APPROACH

The study aimed to assess the feasibility of AnMBR technology for the treatment of dairy lipid-rich wastewater in a laboratory scale reactor. A 10 L anaerobic reactor with a PVDF cross-flow tubular ultrafiltration membrane was used to achieve the objectives.

In the first experiments, the system was treating synthetic dairy industrial wastewater (diluted whole milk). The research was primarily focused on the AnMBR biological and filtration performance, by assessing the sludge's physicochemical characteristics, linked to the AnMBR's operational parameters. In addition, batch assays were performed using 120 mL serum bottles for assessing the biochemical characteristics of the different sludges in the presence of different types of LCFA. Moreover, the microbial community composition was assessed to further research the role of the different microorganisms in the anaerobic degradation of LCFAs under the different conditions.

1.10 OUTLINE OF THE THESIS

This thesis is divided into six chapters. The graphical representation of the thesis structure and connection of the chapters with the hypothesis of the doctoral thesis is shown in Figure 1.4. Chapter 1 provides a general introduction to the thesis, knowledge gaps, and an introduction to the research topic. The second chapter critically assesses the most important principles underpinning the anaerobic degradation process and discusses recent discoveries, coupling fundamental and applied aspects. The third chapter studies the effect of the SRT on biochemical performance, and the fourth chapter evaluates the membrane filtration performance under the different conditions. The fifth chapter studies the effect and importance of inoculum source on LCFA anaerobic degradation. The final chapter concludes and summarizes the findings of this research and provides further recommendations and perspectives on this topic.

[4] This specific objective is addressed on hypothesis nr. v

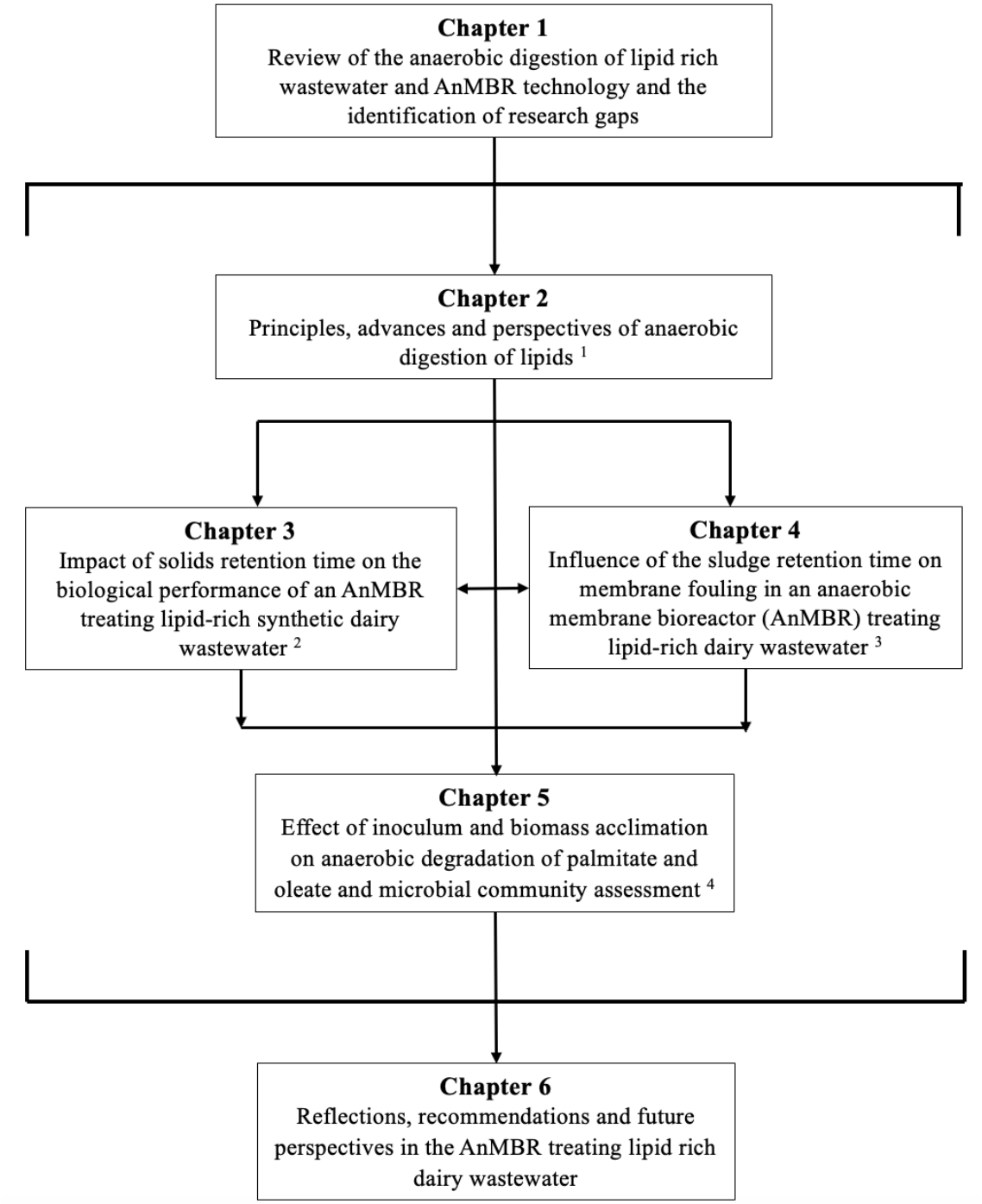

Figure 1.4 – Structure of the thesis and links between chapters and hypothesis

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2

PRINCIPLES, ADVANCES AND PERSPECTIVES OF ANAEROBIC DIGESTION OF LIPIDS

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ABSTRACT

Several problems associated to the presence of lipids in wastewater treatment plants are usually overcome by removing them ahead of the biological treatment. However, because of their high energy content, waste lipids are interesting, yet challenging pollutants in anaerobic wastewater treatment and co-digestion processes. The maximal amount of waste lipids that can be sustainably accommodated, and effectively converted to methane in anaerobic reactors, is limited by several problems including adsorption, sludge flotation, washout, and inhibition. These difficulties can be circumvented by appropriate feeding, mixing and solids separation strategies, provided by suitable reactor technology and operation. In recent years, membrane bioreactors and flotation-based bioreactors have been developed to treat lipid-rich wastewater. In parallel, the increasing knowledge on the diversity of complex microbial communities in anaerobic sludge, and on interspecies microbial interactions, contributed to extend the knowledge, and to understand more precisely the limits and constraints influencing the anaerobic biodegradation of lipids in anaerobic reactors. This paper reviews the most important principles underpinning the degradation process, the recent key discoveries, and outlines the current knowledge, coupling fundamental and applied aspects. A critical assessment of knowledge gaps in the field is also presented, by integrating sectorial perspectives of academic researchers and of prominent developers of anaerobic technology.

2.1 INTRODUCTION

Anaerobic digestion (AD) contributes to several sustainable development goals by combining energy and resource recovery from organic wastes and wastewater with pollution control. The generation of a gaseous renewable energy source, the recycling of nutrients and the low surplus sludge production, aligned with the increasing knowledge on microbiology and ecophysiology, has promoted the development of AD technologies as a sustainable treatment solution for a diverse range of wastes and wastewaters, with a significant number of worldwide full-scale implementations (van Lier et al., 2015; Van Lier et al., 2020). Considering the main components of organic matter in wastes/wastewaters, lipids present a high COD/TOC (chemical oxygen demand/total organic carbon) ratio and are, theoretically, ideal substrates for methane production via AD, since their degradation produces more biogas per weight of substrate, than others, i.e., 1.4 L of biogas per gram of lipids, compared to 0.9 and 0.8 L/g for proteins and carbohydrates, respectively (Alves et al., 2009).

High-rate anaerobic treatment (HRAT) technologies, mostly based on well settling granular sludge, have been established for the treatment of biodegradable industrial wastewaters, such as those from food and drink processing, pulp, and paper amongst others, primarily applied directly on industry' sites (van Lier et al., 2015)

However, when dealing with lipid-rich wastewaters this HRAT technology is inappropriate because the lipids and long chain fatty acids (LCFA) strongly adsorb to the sludge, leading to sludge flotation and washout, and potential microbial inhibition (Chen et al., 2008; Lalman & Bagley, 2001; Palatsi et al., 2009; Rodríguez-Méndez et al., 2017). Therefore, the economically feasible utilisation of lipids in HRAT, and the resulting resource recovery (i.e., biogas), has been challenging (Cavaleiro et al., 2015; Dereli et al., 2015).

The low-rate anaerobic treatment (LRAT) of solid wastes such as agricultural residues and municipal sludge is also an established practice, applying variations on the well-known continuously stirred tank reactor (CSTR). The potential to boost biogas production in these systems through the addition of lipids (fat, oil, and grease (FOG) wastes) has been demonstrated (Alves et al., 2009). However, similarly to the HRAT, the addition of lipids can cause problems in these co-digestion processes, requiring proper feeding and mixing strategies, coupled with effective monitoring of the system performance, mandatory to avoid microbial inhibition and to enhance biogas production (Long et al., 2012; Wallace et al., 2017).

This review provides a synthesis of recent advancements in the AD of lipids, both in anaerobic wastewater treatment and co-digestion processes, including examples of full-scale applications. Critical aspects on the microbiology and technology, linked to efficient lipids conversion, have been identified, and support is given to the more widespread utilisation of lipids from wastes and wastewaters as a sustainable resource for biogas production.

2.1.1 Occurrence and composition of waste lipids

Lipids are ubiquitous in nature and are found in most waste/wastewaters. The classification of 'lipids' includes an extremely diverse range of compounds, which can be divided into four main groups of those most commonly found in wastewaters: triacylglycerols including LCFAs, glycolipids, phospholipids, and cholesterol (Abraham, 2010). From these, the most abundant are LCFAs and triacylglycerols, commonly referred to as fats and oils (Gunstone & Harwood, 2007). LCFAs have been characterised with a myriad of different chain lengths, configurations, and degrees of (un)saturation, however, only 20 LCFAs appear widely in nature, and, of these, palmitic, oleic, and linoleic acids make up to 80 % of common oils and fats (Gunstone & Harwood, 2007; Lalman & Bagley, 2001; Novak & Carlson, 1970). Unsaturated LCFAs are components of vegetable oils, while fats are normally composed of saturated fatty acids. Generally, the lipids that are present in the wastewater from industries, that use fats or oils as raw materials, are simple esters of straight chains, even-numbered long chain fatty acids and linear polyols (triglycerides, phospholipids), as well as their hydrolysis resulting products. Their typical fatty acids composition was reviewed by Alves et al. (2009), being palmitic (C16:0) and oleic (C18:1) acids the most abundant saturated and unsaturated fatty acids respectively.

Several food and other processing industries have wastewater streams characterised by high FOG contents namely dairy, slaughterhouses, edible oils production, fish canning factories, bioethanol and diesel production, and wool scouring (Table 2.1). The FOG content of these wastewaters is highly variable and dependent on the production process. For example, dairy processing industry wastewaters have high concentrations of fats, along with carbohydrates and proteins, which come from the milk. Since the dairy industry produces many different kinds of products, the characteristics of the wastewater vary significantly according to the specific industry and the processing methods (Demirel et al., 2005; Karadag et al., 2015; Traversi et al., 2013) as can be observed in the Table 2.1, varying from 0.3 to approximately 40 g FOG/L (Arbeli et al., 2006; Ince, 1998; Omil et al., 2003; Saddoud & Sayadi, 2007; Szabo-Corbacho et al., 2019). For slaughterhouse wastewater, the composition of the suspended fraction is characterized by a complex mixture of fats, proteins, and fibres, and varies considerably on the type of animals slaughtered and on the production process (Núñez & Martínez, 1999; Ruiz et al., 1997). Regarding the fish industry, the FOG concentration is around 1 to 1.5 g FOG/L (Achour et al., 2000; Maya-Altamira et al., 2008). The extraction and purification of palm oil generates different kinds of wastewaters, commonly known as palm oil mill effluent (POME), where the separator sludge and sterilizer effluent are the two most important fractions of POME (Igwe & Onyegbado, 2007), which contribute to the highly polluting characteristics of this wastewater. The literature reports values from 1.4 to 27 g FOG/L in this type of wastewater (Igwe & Onyegbado, 2007; Ismail et al., 2010; Poh et al., 2010). The olive oil mill wastewaters produced by the traditional mill and press processes have a high organic fraction made up of sugars, polyphenols, polyalcohols, proteins and lipids (Boari et al., 1984), with characteristic values of 0.3 – 100 gFOG/L (Angelidaki & Ahring, 1997; Azbar et al., 2004).

Bioethanol production from corn generates a lipid-rich stream called thin stillage, a complex wastewater containing high concentrations of carbohydrates, proteins, lipids, glycerol and lactic acid (Dereli et al., 2014; Kim et al., 2008) with FOG values accounting from 11 to 13 g/L. According to Becker et al. (1999) the major constituents of wool scouring effluent are fats and oils, and effluent characteristics vary largely between processes and materials, with values ranging from 0.6 to 55 gFOG/L (Bisschops & Spanjers, 2003; Lapsirikul et al., 1994).

Table 2.1- Typical FOG concentrations in wastewater of different industrial wastewaters

Industrial wastewater	FOG concentration (g /L)	Reference
Dairy		
Milk and cream bottling plant	0.3 – 0.5	(Ince, 1998)
Dairy industry overall	0.3 – 40	(Arbeli et al., 2006)
	1.7	(Szabo-Corbacho et al., 2019)
Cheese production	0.8	(Omil et al., 2003)
Cheese whey production	9.4	(Saddoud et al., 2007)
Ice cream	0.88 – 5.12	(Frijters et al., 2014)
Slaughterhouse		
Cattle	35.8	(Jeganathan et al., 2006)
	0.2 – 0.3	(Saddoud & Sayadi, 2007)
	1.3	(Maya-Altamira et al., 2008)
Sheep and goat	0.1 – 0.4	(Manjunath et al., 2000)
Poultry	0.2 – 0.7	(Del Nery et al., 2007)
	38.8	(Nakhla et al., 2003)
Food industry		
Tank cleaning company	0.1 – 2.2	(Frijters et al., 2014)
Fish	1	(Maya-Altamira et al., 2008)

	1.5	(Achour et al., 2000)
POME		
POME	1.4 - 15	(Igwe & Onyegbado, 2007)
	8.8 – 11.4 (in COD)	(Ismail et al., 2010)
	2.2 – 27.2	(Poh et al., 2010)
Olive oil mill		
Olive oil mill	0.3 – 100	(Azbar et al., 2004)
	17.2	(Angelidaki & Ahring, 1997)
Bioethanol		
Corn-to-ethanol thin stillage	10.8 – 11.8	(Dereliet al., 2014)
	13	(Kim et al., 2008)
Wool scouring		
Wool scouring	0.6 – 55	(Bisschops & Spanjers, 2003)
	10.8	(Lapsirikul et al., 1994)

Often, the lipids present in these industrial wastewaters are removed by pre-treatment systems, such as screening, centrifugation, sedimentation, dissolved air flotation, flocculation, or precipitation (Sayed et al., 1987), producing a more concentrated waste-stream commonly referred to as FOG-waste. With this procedure, a more diluted wastewater, with significantly less FOG, is obtained that can be more easily treated in traditional anaerobic and aerobic processes. The FOG-waste is still frequently disposed in landfills along with waste activated sludge. However, it is also co-digested via LRAT with sewage sludge, manure, or the organic fraction of municipal solid waste, in order to increase the biogas production. The fraction of FOG removed in the pre-treatment can account for up to 85 % of the total organic fraction with potential for biogas production, highlighting their importance for industry as a potential renewable energy source. This value has been reported by NVP Energy Ltd, at the Arrabawn Dairies Group's WWTP (Ireland), with dissolved air flotation pre-treatment employed, based on the wastewater generated from a milk processing industry.

Municipal wastewater streams in industrialized countries are generally characterized by a relatively low FOG content, estimated between 50 and 150 mg/L (Pastore et al.,

2015). This relatively low number can be attributed to the common practice in these countries to collect FOG at source in commercial cooking premises, to prevent blockages in drains due to solidification of fats. Interception of FOG can be achieved via grease traps, plumbing devices at source points before it enters the municipal wastewater systems. The produced waste stream is referred to as grease trap waste (GTW) and its composition is highly diverse, mainly dependent on the source (Kobayashi et al., 2014). These biosolids have started to be utilised in co-digestion systems, to boost the biogas yield in LRAT systems (Grosser, 2017; Kashi et al., 2017).

2.1.2 Ad of lipids: a historical perspective

During AD, triacylglycerols are hydrolysed to glycerol and LCFA (Figure 2.1), in a step catalysed by extracellular lipases produced by acidogenic bacteria (Hanaki et al., 1981; Heukelekian & Mueller, 1958; Masse et al., 2002). The released LCFA are further degraded to acetate (and propionate, in the case of odd-numbered LCFA) and hydrogen via β-oxidation, which is the rate-limiting step in the degradation of lipids (Hanaki et al., 1981; Hwu et al., 1996; Novak & Carlson, 1970; Rinzema et al., 1994; Weng & Jeris, 1976). These compounds are then finally converted to methane and carbon dioxide by methanogens (Bryant, 1979).

Lipids hydrolysis is a surface related process, and its rate may vary depending on the fatty acid chain length, substrate physical state (solid or liquid) and specific surface area (Alves et. al., 2009). When fat concentration is very high, hydrolysis can become the rate-limiting step in the whole anaerobic degradation process (Masse et al., 2003). For example, in wastewater treatment systems, large insoluble droplets can be formed with concomitant low surface area for hydrolysis. However, when the lipid-water interface area is large, because of the small particle size (e.g., lipids emulsions or micelles), hydrolysis is not necessarily the rate-limiting step. In this case, lipids conversion to glycerol and LCFA is regarded as a fast process, and the overall degradation of lipids is limited by LCFA degradation.

Efficient methane production from FOG-containing wastewater is not easily achieved with existing conventional HRAT, mainly due to the formation of a thick layer of sludge enclosed by a whitish greasy matter on the top of the water surface (Hwu et al., 1997; Pereira et al., 2004, 2005). Consequently, an important fraction of the sludge is lost by washout, and methane production decreases over time. Biogas bubbles are frequently retained in the floating hydrophobic layer, leading to foam formation, which may cause problems in the biogas line. Moreover, lipids and LCFA have been reported as toxic for the anaerobic microbial communities (Hanaki et al., 1981).

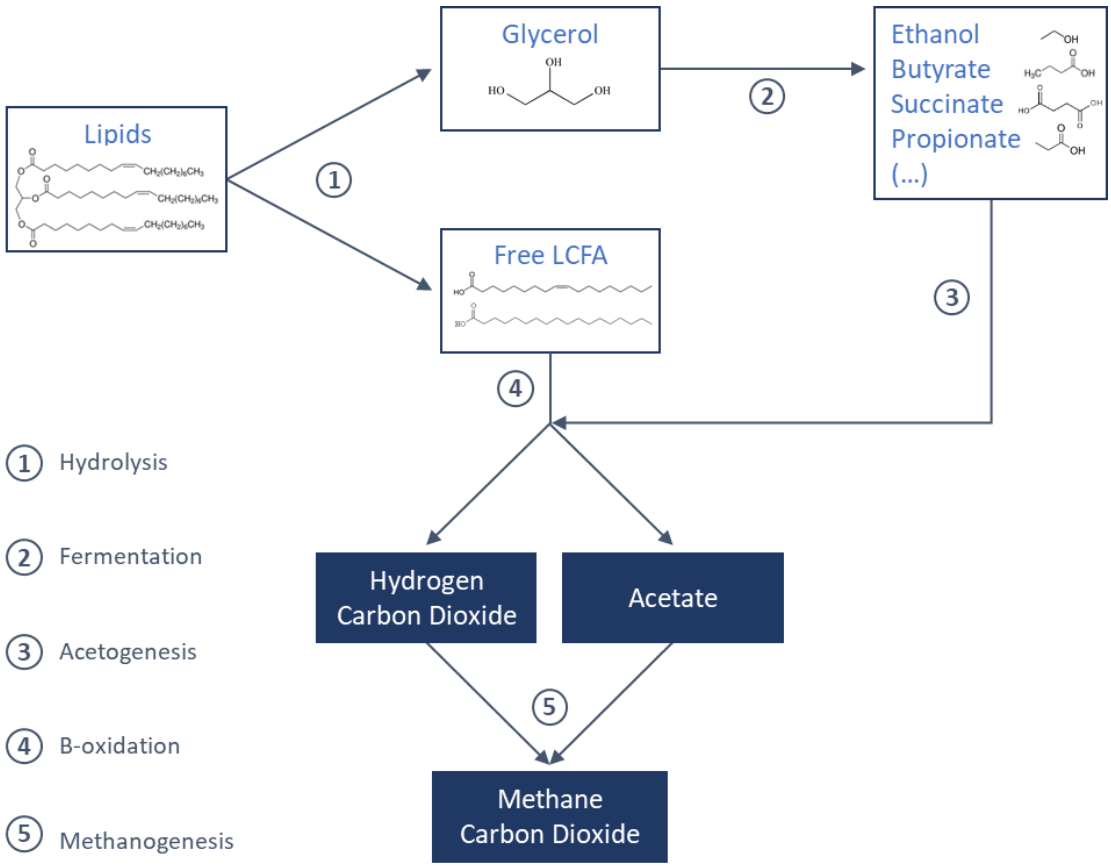

Figure 2.1- Pathway of anaerobic triacylglycerol biodegradation.

The identification of these problems promoted the practice that, for a long time, lipids and LCFA have been separated from the wastewater before AD, with the consequent loss of their energy potential. A significant amount of research has been performed to understand the complex phenomena of lipids biodegradation by anaerobic communities, aiming to overcome process limitations and enhance methane production from these compounds. The key milestones in the microbiology and process research on AD of lipids are summarized in Figure 2.2.

In 1981, pioneering work by Hanaki et al., (1981) showed that lag phases preceding methane production were a consequence of LCFA accumulation and inhibition, rather than an effect of neutral lipids. In the early 90's, LCFAs were considered to be bactericidal, exerting a permanent and irreversible toxic effect, particularly towards methanogens (Angelidaki & Ahring, 1992; Rinzema et al., 1994). Both acetoclastic and hydrogenotrophic methanogens were reported to be inhibited in the presence of LCFA (Hanaki et al., 1981), but acetoclasts were reported as more sensitive to LCFA than hydrogenotrophs (Hanaki et al., 1981; Hwu & Lettinga, 1997; Koster & Cramer, 1987; Lalman & Bagley, 2001; Rinzema et al., 1994). In these studies, acetoclastic methanogens were unable to adapt to LCFA, after repeated exposure to toxic concentrations as well as after extended exposure to sub-toxic concentrations (Rinzema et al., 1994). More recently, however, Silva et al. (2016) showed that pure cultures of

Methanothrix (Methanosaeta) concilii and *Methanosarcina mazei* tolerated LCFA concentrations similar to those previously reported for hydrogenotrophic methanogens (Sousa et al., 2013), showing that these acetoclastic methanogens are more robust than considered previously, which may explain the observed prevalence of microorganisms from *Methanosarcina and Methanothrix* genera in anaerobic bioreactors treating LCFA-rich wastewater.

It has also been shown, however, that the nature of the lipids also influences the extent of toxicity. Unsaturated fatty acids, containing one or more double bonds (e.g., oleate, C18:1), are more toxic to microbial cells than saturated fatty acids, such as stearate (C18:0) or palmitate (C16:0) (Demeyer & Henderickx, 1967; Lalman & Bagley, 2001, 2002), and the toxicity also increases with the carbon chain length (Galbraith & Miller, 1973).

In general, most studies have been performed within the mesophilic range (30 ºC – 37 ºC), but the anaerobic digestion of LCFA under thermophilic conditions (40 ºC – 60 ºC) has also been studied (Angelidaki et al., 1990; Angelidaki & Ahring, 1992; Hwu & Lettinga, 1997; Hwu et al., 1997). Hwu et al. (1997, 1998) reported higher oleate conversion rates in high temperature reactors (55 °C), but oleate toxicity towards acetoclastic methanogens was also higher than at 30 ºC. Since the composition of cell membranes of thermophilic and mesophilic microorganisms is different, responses to LCFA toxicity may vary (Hwu & Lettinga, 1997). Moreover, lipids/LCFA solubilisation increases with the temperature, thus enhancing their bioavailability (Becker et al., 1999) and possibly their toxicity. AD of lipids at low temperatures (12 ºC – 20 °C) remains seldom studied. Recently, Singh et al. (2019) showed the potential of mesophilic sludge to produce methane from a synthetic dairy wastewater containing LCFA (33 % in COD) at low temperatures (10 ºC and 20 °C) over a 150-day bioreactor trial. Petropoulos et al. (2018) assessed the lipase activity in the treatment of municipal wastewater at 4, 8 and 15 °C, and concluded that, although lipases were produced at these temperatures, their activities were low, and even became undetectable at 4 °C. Interestingly, these authors found that the raw wastewater presented high levels of lipase activity, that was unaffected by the temperature and, as was shown by Keating et al. (2018), no hydrolytic-based limitation was expected. Nonetheless, lipid-rich wastewater digestion at low-temperatures should be investigated. Furthermore, comparing rates of lipid and LCFA conversion to methane across temperatures ranges coupled with the identity of active microbial community members would prove valuable.

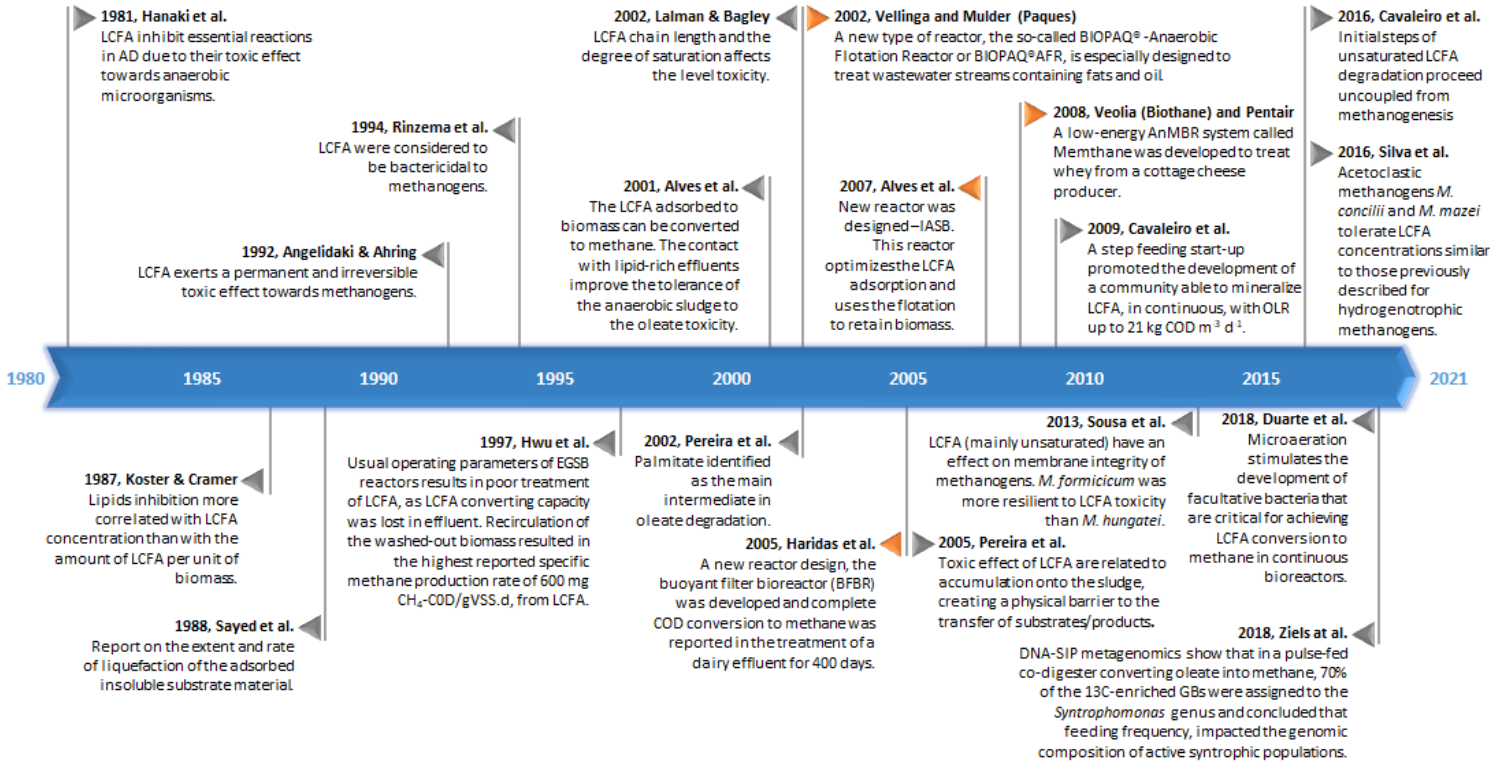

Figure 2.2 - Timeline of key milestones in the microbiology and process research on AD of lipids. The orange markers represent the main reactors developed for AD of lipid-rich wastewaters (Alves et al., 2001, 2007; Angelidaki & Ahring, 1992; Bouman & Heffernan, 2016; Cavaleiro et al., 2009, 2016; Duarte et al., 2018; Hanaki et al., 1981; Haridas et al., 2005; Hwu, et al., 1997; Koster & Cramer, 1987; J. Lalman & Bagley, 2002; Pereira et al., 2005; Pereira, et al., 2002; Rinzema et al., 1994; Sayed et al., 1988; Silva et al., 2016; Sousa et al., 2013; Vellinga & Mulder, 2002; Ziels et al., 2018).

It has also been shown, however, that the nature of the lipids also influences the extent of toxicity. Unsaturated fatty acids, containing one or more double bonds (e.g., oleate, C18:1), are more toxic to microbial cells than saturated fatty acids, such as stearate (C18:0) or palmitate (C16:0) (Demeyer & Henderickx, 1967; Lalman & Bagley, 2001, 2002), and the toxicity also increases with the carbon chain length (Galbraith & Miller, 1973).

In general, most studies have been performed within the mesophilic range (30 ºC – 37 ºC), but the anaerobic digestion of LCFA under thermophilic conditions (40 ºC – 60 ºC) has also been studied (Angelidaki et al., 1990; Angelidaki & Ahring, 1992; Hwu & Lettinga, 1997; Hwu et al., 1997). Hwu et al. (1997, 1998) reported higher oleate conversion rates in high temperature reactors (55 °C), but oleate toxicity towards acetoclastic methanogens was also higher than at 30 ºC. Since the composition of cell membranes of thermophilic and mesophilic microorganisms is different, responses to LCFA toxicity may vary (Hwu & Lettinga, 1997). Moreover, lipids/LCFA solubilisation increases with the temperature, thus enhancing their bioavailability (Becker et al., 1999) and possibly their toxicity. AD of lipids at low temperatures (12 ºC – 20 °C) remains seldom studied. Recently, Singh et al. (2019) showed the potential of mesophilic sludge to produce methane from a synthetic dairy wastewater containing LCFA (33 % in COD) at low temperatures (10 ºC and 20 °C) over a 150-day bioreactor trial. Petropoulos et al. (2018) assessed the lipase activity in the treatment of municipal wastewater at 4, 8 and 15 °C, and concluded that, although lipases were produced at these temperatures, their activities were low, and even became undetectable at 4 °C. Interestingly, these authors found that the raw wastewater presented high levels of lipase activity, that was unaffected by the temperature and, as was shown by Keating et al. (2018), no hydrolytic-based limitation was expected. Nonetheless, lipid-rich wastewater digestion at low-temperatures should be investigated. Furthermore, comparing rates of lipid and LCFA conversion to methane across temperatures ranges coupled with the identity of active microbial community members would prove valuable.

The perceived toxicity of LCFA is also influenced by the structure of the reactor' inoculum, i.e., granular sludge is generally more resistant to LCFA than suspended or flocculent sludge (Hwu et al., 1996). The higher perceived toxicity observed for flocculent sludge was ascribed to its higher surface area, and therefore to a higher adsorption capacity. In the granules, their three-dimensional structure offers higher protection to the methanogens, generally located in the inner layers. However, lipids also have a negative effect on granulation and maintenance of granular sludge integrity, which is critical for most HRAT reactor types (Hawkes et al., 1995; C. Hwu & Lettinga, 1997; Sam-Soon et al., 1991).

LCFA adsorption was initially reported as the main cause of cell damage and toxicity, by limiting cell membrane transport and decreasing its protective function (Demeyer & Henderickx, 1967; Galbraith & Miller, 1973; Heukelekian & Mueller, 1958). Even so, Koster and Cramer (1987) suggested that microbial inhibition was more correlated with

the fatty acid concentration than with the amount of LCFA per unit of biomass and proposed that adsorption is an essential step preceding LCFA degradation. Further developments showed that microbial inhibition caused by LCFA is not permanent (Alves et al., 2001; Pereira et al., 2004) and that biomass adaptation to LCFA can occur (Alves et al., 2001), which was striking and opened new perspectives for the AD of lipids (Figure 2.2).

Considering the various observations made by the different authors we may postulate on two different mechanisms for LCFA interference on the microbes, previously perceived as 'toxicity', in which we can differentiate between bactericidal toxicity and temporary inhibition. Bactericidal toxicity would then refer to the impact of the long hydrophobic alkyl-chain on the archaeal membrane, leading to membrane leakages, lysis, and decay of cells. This irreversible loss of the methanogenic activity of the biomass can then only be restored by growth of new cells as suggested by Rinzema et al. (1994). This concept of permanent inhibition or bactericidal toxicity was questioned, by Pereira et al., (2005), who observed that the accumulation of LCFAs on the methanogenic biomass, prevented mass-transfer from the liquid broth to the microbial cells, but cells integrity and viability was maintained. These authors showed that biomass-associated LCFA up to 5 kg/kg (expressed in COD per mass unit of volatile solids - VS) could be degraded to methane in batch, eliminating the mass transfer limitations and restoring the methanogenic activity. Therefore, the physical inhibition related to mass transfer limitations, imposed by the LCFA layer adsorbed onto the sludge, were proposed as the main causes for the transient inhibitory effects observed during AD of lipids (Pereira et al., 2005). By concluding that a temporary inhibition could be overcome by incubating the LCFA-loaded biomass in batch mode, thus promoting the degradation of the accumulated substrate to methane (Alves et al., 2001; Pereira et al., 2005), a strategy based on reactors operation in cycles of adsorption followed by degradation was proposed as a first suggestion for the treatment of wastewater with high LCFA content (Pereira et al., 2001). Later, Cavaleiro et al. (2009) demonstrated that by sequencing continuous feeding phases and batch reaction phases in the start-up of an anaerobic reactor, a microbial community able to efficiently mineralize LCFA was established. In a subsequent continuous operation with high organic loading rates (OLR), up to 21 kg/(m^3 day) (expressed in COD, 50 % of the COD being LCFA), stable COD to methane conversion of 80 % was observed. More recently, it has been shown that long-term sludge acclimatisation to lipids or LCFA-rich wastewater and limitation of excessive LCFA accumulation are beneficial for the efficient degradation of LCFA to methane (Nakasaki et al., 2019, 2020; Silva et al., 2014). Ideally, specific biomass-associated substrate should be kept below 1 kg/kg (expressed in COD per mass unit of VS of inoculum) (Pereira et al., 2004), although well-adapted sludge could still have a good performance with approximately three times this value (Silva et al., 2014).

In co-digestion processes, microbial adaptation has been recently shown to be important for the degradation of FOG. Ziels et al. (2016) highlighted the enrichment of syntrophic LCFA-degrading bacteria during the co-digestion of FOG with municipal wastewater

sludge. Similar observations were reported in other studies of FOG co-digestion (Amha et al., 2017; Kurade et al., 2019, 2020). Moreover, Ziels et al. (2017) showed that, during the anaerobic digestion of cattle manure, oleate pulse feeding (every 48 hours) resulted in higher conversion rate and functional stability than when oleate pulses were performed every 6 hours. Syntrophic LCFA-degrading bacteria were significantly enriched in both co-digesters relative to the control (without oleate), being more abundant in the co-digester that was 48 h pulse-fed. In the same line of research, Kougias et al. (2016) showed that during the digestion of cattle manure, a thermophilic inoculum previously exposed to LCFA was capable of degrading oleate pulses more efficiently than a non-acclimatized inoculum, due to the specialization of the microbial consortium.

Over the years, several other strategies have been studied to overcome the LCFA/lipids toxicity, namely bioaugmentation with LCFA-degrading bacteria (Cavaleiro et al., 2010; Silva et al., 2014), emulsification of LCFAs (Eftaxias et al., 2020), addition of adsorbents like bentonite (Angelidaki et al., 1990) and LCFA precipitation with calcium salts (Hanaki et al., 1981). Different bioreactor designs have also been tested to overcome the problems of sludge flotation and washout.

2.2 METABOLIC PATHWAYS AND MICROBIOLOGY

To achieve an efficient anaerobic digestion of lipids, comparable with easier degradable substrates, the kinetics, metabolic pathways, and the microorganisms involved need to be fully understood. Targeted "omics" approaches and improved analytical methodologies have recently offered new insights into complex microbial communities in natural and engineered environments, and contributed to the understanding of microbial diversity, function, and interactions in lipid degrading communities. However, despite these recent advances, many aspects remain poorly understood, including the initial steps of unsaturated LCFA degradation, and the interactions among the microorganisms involved, as for example the role of anaerobic facultative microorganisms.

2.2.1 Metabolic pathways – β-oxidation of LCFA

Long chain fatty acids are degraded via β-oxidation. Fatty acids are actively transported inside bacterial cells (Mackie et al., 1991) and activated to acyl-CoA thioesters by acyl-CoA synthetase. After this step, the fatty acyl-CoA undergoes β-oxidation. This oxidation pathway acts in a cyclic way, with each cycle resulting in the shortening of the input acyl-CoA by two carbon atoms, thus producing acetyl-CoA and hydrogen (DiRusso et al., 1999). More detail on the biochemical features of LCFA biodegradation can be found in Sousa et al. (2009).

Due to thermodynamic constraints, acetogenic reactions are only energetically feasible when the hydrogen concentration is kept low (Table 2.2) (Schink, 1997; Stams et al., 2006), which is generally accomplished through syntrophic cooperation of acetogenic

bacteria and hydrogenotrophic methanogens (Schink, 1997) (reactions 4, 5 and 6, Table 2). This obligate relationship is essential to achieve complete LCFA conversion to methane. Alternatively, to hydrogen interspecies electron transfer, direct interspecies electron transfer (DIET) may also occur. However, many claims for DIET and electrotrophy have only been suggested without adequate experimental validation (Lovley, 2022). Although the reactions efficiencies may be different for both situations, the overall Gibbs free energy change is the same (Wang et al., 2021).

In fact, Cavaleiro et al., (2016) showed that in bioreactors in which methanogenesis was inhibited, the degradation of different unsaturated LCFA (namely C18:2, C18:1 and C16:1) lead to the accumulation of two carbons shorter saturated LCFA. From these observations it could be hypothesized that hydrogenation of unsaturated LCFA (e.g., oleate) is followed by one β-oxidation cycle (reactions 2 and 3, Table 2.2), after which two carbons shorter saturated LCFA (e. g. palmitate in oleate-fed bioreactors) would be expelled from the bacterial cells. However, this hypothesis is unlikely, as no immediate energy gain is derived from this uptake and excretion process. One alternative hypothesis is that the chain saturation step and first β-oxidation cycle might occur membrane bound, possibly outside the cell, in which the reducing equivalents generated from β-oxidation are used to reduce the double carbon bond of the unsaturated chain, producing palmitate. This, however, has never been shown and remains speculative, since the LCFA molecule needs to be activated prior to β-oxidation. Therefore, the reason why palmitate accumulates in oleate-based wastewater treatment still represents a knowledge gap. Moreover, it is still not clear whether conversion of oleate to palmitate (involving the two possible steps of hydrogenation and β-oxidation) is performed by only one or by more than one microorganism (Sousa et al., 2009). The accumulated palmitate can be further degraded by different or by the same microorganisms that performed the oleate bioconversion, since bacteria that degrade unsaturated fatty acids are also able to degrade saturated fatty acids, whereas the opposite generally does not occur (Sousa et al., 2007). However, in anaerobic reactors with mixed communities, oleate consumption is generally fast, while palmitate degradation is slow, which underpins the hypothesis that two different metabolic routes may be involved in the complete oleate degradation to methane. Therefore, the build-up of palmitate during oleate biodegradation must be deeply studied since it is directly linked with potential solutions to increase the conversion rate of full-scale lipids' AD systems. Palmitate was also the main LCFA identified in floating fat-balls that were formed during the treatment of high lipid concentrations. These aggregates were mainly composed by calcium LCFA salts and were essentially unavailable to microbes (Dereli et al., 2014; Pitk et al., 2014).

Table 2.2 - Gibbs free energy changes for some of the acetogenic and methanogenic reactions presumably involved in the conversion of fatty acids (adapted from Sousa et al. and Cavaleiro et al. (Cavaleiro et al., 2016; Sousa et al., 2009)).

Reaction	$\Delta G^{0'}$ (kJ reaction^{-1})	$\Delta G'$ (kJ reaction^{-1})
Hydrogenation		
(1) $oleate^- + H_2 \rightarrow stearate^-$	-79	-50
One β-Oxidation cycle		
(2) $stearate^- + 2\ H_2O \rightarrow palmitate^- + acetate^- + 2\ H_2 + H^+$	+51	-23
Hydrogenation + one β-Oxidation cycle		
(3) $oleate^- + 2\ H_2O \rightarrow palmitate^- + acetate^- + H_2 + H^+$	-28	-73
Complete β-Oxidation		
(4) $oleate^- + 16\ H_2O \rightarrow 9\ acetate^- + 15\ H_2 + 8\ H^+$	+326	-190
Methanogenic reactions		
(5) $acetate^- + H_2O \rightarrow HCO_3^- + CH_4$	-31	-
(6) $4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$	-136	-

$\Delta G^{0'}$ – Gibbs free energy change at standard conditions (solute concentrations of 1 mol L^{-1}, gas partial pressure of 10^5 Pa, 25 °C) and pH 7. $\Delta G'$ – Gibbs free energy change at non-standard conditions (1 mmol L^{-1} for reagent LCFA, products stoichiometric accumulation, H_2 depletion to 1 Pa partial pressure, 25 °C and pH 7).

2.2.2 Microbiology of lipids and LCFA anaerobic degradation

In the absence of external electron acceptors (other than CO_2), LCFA biodegradation is associated with a syntrophic cooperation between LCFA-consuming bacteria and methanogens, with the latter consuming the acetate and hydrogen formed by the bacteria and producing methane (reactions 4, 5 and 6, Table 2.2). None of these groups can degrade LCFA alone, so this obligate relationship between syntrophic bacteria and methanogens (especially hydrogenotrophic methanogens) is essential to achieve LCFA degradation to methane. In the absence of a hydrogen scavenger, hydrogen partial pressure increases and LCFA conversion becomes thermodynamically unfeasible (reaction 4, Table 2.2) (McInerney et al., 2008; Schink, 1997).

Syntrophomonas sapovorans was the first LCFA-degrading syntrophic bacterium isolated in co-culture with *Methanospirillum hungatei* (Roy et al., 1986). To date, twelve syntrophic strains able to convert C4 and longer fatty acid have been described (Sousa et al., 2009; Zhang et al., 2012). Five of these microorganisms can grow with unsaturated LCFA (Sousa et al., 2009; Zhang et al., 2012), and only one,

Thermosyntropha lipolytica, is also able to hydrolyse lipids (Svetlitshnyi et al., 1996). In general, the syntrophic LCFA degraders are also able to degrade short chain fatty acids (SCFA), while the opposite is not true. Information on syntrophic species degrading propionate and butyrate (the most important SCFA) are compiled by (Schink & Stams, 2012; Stams et al., 2012; Stams, 1994). Besides methanogens, other microorganisms can act as syntrophic partners for LCFA degrading bacteria, e.g., hydrogen- and acetate-consuming sulphate reducing bacteria (SRB) (Sousa et al., 2009). For example, Salvador et al. (2018) reported a novel syntrophic relationship between an oleate-degrading bacterium, closely related to *Syntrophomonas zehnderi*, and a hydrogenotrophic sulfonate-reducing *Desulfovibrio*. Recently, a new study suggested that the anaerobic degradation of LCFAs could be enhanced by the presence of other electron acceptors such as iron. Cavaleiro et al. (2020) investigated the effect of different sub-stoichiometric amounts of Fe (III) on the anaerobic degradation of oleate in suspended and granular sludge. In that study, faster LCFA biodegradation was observed by suspended sludge in the presence of iron, but no noticeable effect of iron was observed with granular sludge. Regarding the microbial community composition, the results obtained suggest the occurrence of a novel microbial interaction in LCFA oxidation, involving microorganisms of the *Syntrophomonas, Geobacter* and *Methanobacterium* genera (Cavaleiro et al., 2020).

The microbiome of the anaerobic LCFA-degrading communities was initially studied using traditional molecular techniques (e.g., cloning and sequencing), targeting the 16S rRNA gene, focusing on the phylogenetic and taxonomic characterization (Baserba et al., 2012; Pereira et al., 2002; Sousa et al., 2007). In general, the relative abundance of fatty acid-degrading syntrophic bacteria in high-rate methanogenic bioreactors is low (between 0.2 − 3 %), despite their importance in lipids/LCFA degradation (Sousa et al., 2009; Stams et al., 2012). Besides the genus *Syntrophomonas* from the phylum *Firmicutes* (known as a syntrophic fatty acid degrading bacteria), also *Clostridium* species were detected in several studies (Cavaleiro et al., 2016; Francisci et al., 2015; Hatamoto et al., 2007; Palatsi et al., 2011; Solli et al., 2014). Members of phyla *Bacteroidetes*, *Synergistetes*, *Spirochaetes* and *Proteobacteria* were also detected, even though their direct involvement in LCFA degradation was never demonstrated (Table 2.3) (Hatamoto et al., 2007; Pereira et al., 2002; Salvador, 2013; Shigematsu et al., 2006). These microbial groups were also detected by Nakasaki et al. (2020), which examined microbial community changes during the degradation of oil, LCFA and glycerol. Their results showed that *Leptospirales, Thermobaculaceae, Synergistaceae* and *Syntrophaceae* were the most abundant bacteria in both oil and LCFA experiments (Nakasaki et al., 2019).

In the last decades, the development of new methodologies for the detection and identification of uncultivated microorganisms has contributed to increase the knowledge about microbial diversity, their functions, and interactions in complex communities. In Table 2.3, microorganisms found in lipid/LCFA-rich environments are shown. Nevertheless, the role of most of these microorganisms in LCFA conversion is unknown.

While it is clear that acclimatisation of the microbial community to LCFAs or lipids, both in wastewater and co-digestion processes, benefits degradation and decreases inhibition through the development of specialized microbial communities, as previously described (Cavaleiro et al., 2009; Ziels et al., 2016, 2017), it is now important for future studies to further link microbial identification to function. Metagenomics, metatranscriptomics, metaproteomics and metametabolomics are different approaches to address that challenge (Salvador, 2013). Treu et al. (2016) studied the metatranscriptome of an anaerobic microbial community during LCFA exposure. Besides confirming the importance of *Syntrophomonas* species in fatty acids degradation, authors also noted the upregulation of genes involved in "peptidoglycan biosynthesis" and in "lipopolysaccharides biosynthesis" by bacteria belonging to order *Clostridiales*, to *Rykenellaceae* families and to *Halothermothrix* and *Anaerobaculum* genera. This may indicate that, by modifying their cell wall and the composition of the lipopolysaccharides, the bacteria promote a protective mechanism to counteract the toxic/inhibitory effect of LCFA (Treu et al., 2016). Kougias et al. (2016) studied the microbial community dynamics during an inhibitory shock load induced by single pulses of oleate, using high throughput shotgun sequencing (metagenomics). They showed that only the microorganisms associated with LCFA degradation could encode proteins related to "chemotaxis" and "flagellar assembly", which allow these microbes to move towards LCFA. Recently, Ziels et al., (2018) used DNA-SIP metagenomics and showed that in a pulse-fed co-digester converting oleate into methane, 70 % of the 13C-enriched genome bins were assigned to the *Syntrophomonas* genus and concluded that feeding frequency impacted the genomic composition of active syntrophic populations.

Table 2.3. Phylogenetic composition of LCFA/lipids/FOG degrading microbial communities in enrichment cultures or in bioreactors.

Substrate and culture conditions	**Techniques applied**	**Bacterial community**	**Archaeal community**	**Reference**
Thermophilic oleate degrading enrichment cultures	ARDRA; sequencing	*Firmicutes* (*Clostridia*) *Synergistetes* (*Sinergistia*)	*Methanobacterium thermoautotrophicum* (added to the enrichment culture)	(Menes et al., 2001)
Bioreactors with granular and suspended sludge fed with oleate	PCR-DGGE, sequencing, FISH	*Firmicutes* (*Syntrophomonas* and others) *Proteobacteria (Pseudomonas* and others*)* *Spirochaetales*	*Methanobacterium* sp. *Methanobacterium formicium* *Methanosaeta concilii*	(Pereira, et al., 2002b)
Bioreactors with granular sludge fed with increasing loads of oleic acid	PCR-DGGE, cloning, sequencing	*Pseudomonas* *Desulfovibrio*	*Methanobacterium* sp. *Methanobacterium formicium* *Methanosaeta concilii*	(Pereira et al., 2003)
Stearate degrading enrichment cultures	Culture dependent; RFLP; FISH; sequencing	*Deltaproteobacteria* (*Syntrophus gentianae*) *Bacteroidetes* (*Cytophaga* sp. BHI60-95B)	*Methanocalculus taiwanensis* *Methanosaeta concilii*	(Grabowski et al., 2005)
Synthetic LCFA wastewaster containing oleate and palmitate (chemostat cultivation)	Real-time PCR; FISH; DGGE; cloning; sequencing	*Firmicutes* (*Syntrophomonadaceae* and others) *Proteobacteria* *Bacteroidetes* *Spirochaetes*	*Methanosarcina* *Methanosaeta* *Methanospirillum*	(Shigematsu et al., 2006a)

Batch degradation of oleate or palmitate accumulated during continuous feeding in bioreactors	DGGE; real-time PCR; cloning; sequencing; FISH	*Firmicutes* (*Clostridiaceae*; *Syntrophomonadaceae*; uncultured) *Proteobacteria* *Bacteroidetes*	*Methanobacterium aarhusense* *Methanobacterium formicicum* *Methanosaeta concilii* *Methanosarcina mazei*	(Sousa, et al., 2007)
Oleate or palmitate enrichment cultures	Culture dependent; PCR-DGGE; cloning; sequencing	*Syntrophomonas* – in both enrichments *Bacteroidetes/Chlorobi* group (*Chlorobium*) – in oleate enrichment *Proteobacteria* (*Desulfovibrio*)- in oleate enrichment *Proteobacteria* (*Syntrophobacter*; *Halothiobacillus*) – in palmitate enrichment	Archaeal community was not studied	(Sousa, et al., 2007)
Thermophilic or mesophilic palmitate, stearate, oleate or linoleate enrichment cultures	RNA-SIP; cloning; FISH; RFLP; Culture dependent;	*Firmicutes* (*Syntrophomonas*; *Syntrophothermus*; others) *Proteobacteria* (*Deltaproteobacteria*)	Archaeal community was not studied but *Methanosaeta* was detected by microscopic observation	

Substrate and culture conditions	**Techniques applied**	***Bacterial community***	**Archaeal community**	**Reference**
Incubations with palmitate under mesophilic or thermophilic conditions	RNA-SIP; RFLP sequencing	*Bacteroidetes* *Firmicutes (Clostridium; Syntrophomonas; Syntrophothermus; Tepidanaerobacter; Desulfotomaculum; Coprothermobacter)* *Deltaproteobacteria (Syntrophaceae; Geobacteraceae)*	Archaeal community was not studied	(Hatamoto, et al., 2007)

		Synergistetes; Deferribacteres; Bacteroidetes/Chlorobi; Thermotogae; *Acidobacteria; Spirochaetes;* Others		
Thermophilic bioreactor fed with manure with successive pulses of a LCFA mixture (oleate, stearate, palmitate)	PCR-DGGE; sequencing	*Firmicutes (Clostridium; Syntrophomonadaceae)* *Synergistetes*	*Methanosarcina*	(Palatsi et al., 2010)
Thermophilic co-digestion of organic fraction of municipal solid wastes with FOG wastes	PCR-DGGE; cloning; sequencing	*Firmicutes (Clostridiales; Thermoanaerobacterales)* *Bacteroidetes* *Thermotogales* *Synergistetes* *Thermotogae*	*Methanobacterium, Methanoculleus* *Methanosarcina* *Methanothermobacter wolfeii*	(Martín-González et al., 2011)
Co-digestion of dairy and poultry wastes	Cloning; sequencing	Bacterial community was not studied	*Methanocorpusculum sp.* *Methanosardna barkeri* *Methanosaeta concilii* *Methanoculleus palmolei* *Methanomethylovorans sp.*	(Zhang et al., 2011)
Biodegradability batch tests of fresh pig/cattle slaughterhouse waste mixtures	PCR-DGGE; sequencing	*Firmicutes (Thermodesulfobiaceae; Syntrophomonadaceae)* *Synergistetes (Anaerobaculum sp)* *Bacteroidetes (Porphyromonadaceae)* *Chloroflexi (Anaerolineaceae)*	*Methanosaeta concilii* *Methanosarcina siciliae*	(Palatsi et al., 2011)

Thermophilic bioreactor fed with manure with continuous addition of oleate	PCR-DGGE; sequencing	*Firmicutes* (*Clostidiaceae*; *Bacillaceae*; *Syntrophomonas*) *Bacteroidetes* *Proteobacteria (Pseudomonas)* *Thermotogae*	*Methanococcus* *Methanosarcina* *Methanobacterium* *Methanosaeta*	(Baserba et al., 2012)

Substrate and culture conditions	**Techniques applied**	**Bacterial community**	**Archaeal community**	**Reference**
Oleate-rich wastewater treated in a bioreactor based on a sequence of step feeding and reaction cycles	PCR-DGGE; sequencing	Bacterial community was not studied	*Methanobacterium* *Methanosaeta*	(Salvador et al., 2013)
Low-Temperature (10 °C) Anaerobic Digestion of Dilute Dairy Wastewater in an EGSB Bioreactor	Real-time PCR; PCR-DGGE	*Firmicutes* *Proteobacteria* *Spirochaetes* *Bacteroidetes* *Trichococcus* *Proteobacteria*	*Methanocorpusculum* *Methanospirillum hungatei* *Methanosaeta concilii*	(Bialek et al., 2013)
CSTR co-digesting fish waste and cow manure	Pyrosequencing	*Firmicutes (Clostridium; Syntrophomonas;* others*)* *Proteobacteria* *Actinobacteria* *Synergistetes*	*Methanobrevibacter*, *Methanoculleus* *Methanosarcina* *Methanosaeta*	(Solli et al., 2014)

		Tenericutes *Cloacimonetes (Candidatus Cloacimonas acidaminovorans)*		
Batch-fed methanogenic bioreactors degrading oleic acid	Quantitative PCR; PCR; sequencing	*Clostridiales (Syntrophomonas)* *Anaerolineales (Levilinea)* *Synergistales (Synesgistes)* *Enterobacteriales (Escherichia;Shigella)*	*Methanomicrobiales* *Methanosaetaceae*	(Ziels et al., 2015)
Biogas reactors disturbed with pulses of lipids	Sequencing;	*Megamonas* *Flectobacillus* *Clostridium* *Syntrophomonas sapovorans*	*Methanoculleus* *Methanocorpusculum* *Methanocella*	(Francisci et al., 2015)
Sequencing batch reactors treating dairy wastewater and cattle manure	PCR-DGGE; sequencing	*Bacteroidales* *Syntrophomonas* *Thermovirga*	*Methanospirillum* *Methanosarcinales* *Methanobacteriales*	(Jihen et al., 2015)

Substrate and culture conditions	**Techniques applied**	**Bacterial community**	**Archaeal community**	**Reference**

Bioreactors continuously operated with Palmitoleate (C16:1) and oleate (C18:1)	16S rRNA Gene Pyrosequencing	*Levilinea* *Clostridium* *Syntrophomonas* *Spirochaeta* *Longilinea* *Bellilinea* *Thermanaerovibrio* *Thermanaerothrix* *Anaerolinea* *Syntrophobacter* *Pseudomonas* *Delftia* *Curtobacterium* *Rheinheimera* *Petrotoga* *Candidatus* Odyssella	*Methanosaeta* *Methanospirillum* *Methanobacterium* *Methanolinea*	(A. J. Cavaleiro et al., 2016b)
Bioreactors subjected to inhibitory shock load induced by single pulses of unsaturated LCFA	Illumina HiSeq sequencing	*Clostridia (Syntrophomonas; Desulfotomaculum; Syntrophothermus)* *Gammaproteobacteria*	*Methanosarcina* *Methanoculleus*	(Kougias et al., 2016)
Reactors co-digesting three agro-industrial wastes underwent abrupt and gradual changes of LCFAs concentrations	PCR-DGGE; FISH; Illumina MiSeq sequencing	*Clostridiales* *Syntrophomonadaceae (Syntrophomonas)* *Synergistetes*	*Methanosarcina*-related methanogens	(Regueiro et al., 2016)

		Anaerobaculaceae *Tissierellaceae* *Peptococcaceae*		
Comparison of bioreactors fed with cattle manure after oleate addition to the feeding	RNA Illumina MiSeq sequencing; shotgun reads	*Alcaligenaceae sp* *Eubacteriaceae sp* *Rikenellaceae sp.* *Clostridiales sp.* *Porphyromonadaceae sp.* *Halothermothrix* *Anaerobaculum*	*Methanoculleus sp.* *Methanosarcina sp.* *Methanothermobacter sp.*	(Treu et al., 2016)

Substrate and culture conditions	**Techniques applied**	**Bacterial community**	**Archaeal community**	**Reference**
Anaerobic co-digestion of fats, oils, and grease with municipal sludge	Quantitative PCR; rRNA Illumina MiSeq sequencing;	*Syntrophomonas* *Petrimonas* *Mahella* *Levilinea* *Sedimentibacter* *Ornithobacterium* Others	*Methanosaeta* *Methanospirillum*	(Ziels et al., 2016a)

Digesters were exposed to a subsequent OLR increase with FOG and glycerol	Pyrosequencing Phospholipids and ether-linked isoprenoids analysis	*Firmicutes (Cloacibacillus)* Sulphur reducing bacteria (SRB) *Bacteroidetes*	Archaeal community was not studied	(Ferguson et al., 2016)
Bioreactors subjected to inhibitory shock load induced by single pulses of unsaturated LCFA	Illumina HiSeq sequencing	*Clostridia (Syntrophomonas; Desulfotomaculum; Syntrophothermus)* *Gammaproteobacteria*	*Methanosarcina* *Methanoculleus*	(Kougias et al., 2016)
Reactors co-digesting three agro-industrial wastes underwent abrupt and gradual changes of LCFAs concentrations	PCR-DGGE; FISH; Illumina MiSeq sequencing	*Clostridiales* *Syntrophomonadaceae (Syntrophomonas)* *Synergistetes* *Anaerobaculaceae* *Tissierellaceae* *Peptococcaceae*	*Methanosarcina*-related methanogens	(Regueiro et al., 2016)
Comparison of bioreactors fed with cattle manure after oleate addition to the feeding	RNA Illumina MiSeq sequencing; shotgun re	*Alcaligenaceae sp* *Eubacteriaceae sp* *Rikenellaceae sp.* *Clostridiales sp.* *Porphyromonadaceae sp.* *Halothermothrix* *Anaerobaculum*	*Methanoculleus sp.* *Methanosarcina sp.* *Methanothermobacter sp.*	(Treu et al., 2016)
Anaerobic co-digestion of fats, oils, and grease with municipal sludge	Quantitative PCR; rRNA Illumina MiSeq sequencing	*Syntrophomonas* *Petrimonas* *Mahella*	*Methanosaeta* *Methanospirillum*	(Ziels et al., 2016)

		Levilinea *Sedimentibacter* *Ornithobacterium,* Others		

Substrate and culture conditions	**Techniques applied**	**Bacterial community**	**Archaeal community**	**Reference**
Sequential bench-scale respirometry experiments (thermophilic) with FOG (30 % - 60 %) and food waste.	rRNA sequencing	unclassified *Clostridiales* unclassified *Thermotogales* *Anaerobaculum* *Syntrophomonas* *Coprothermobacter* *Lactobacillus* *Tepidimicrobium* *Syntrophothermus* Tepidanaerobacter	*Methanoculleus* *Methanosarcina*	(Amha et al., 2017)
Anaerobic codigesters treating manure and oleate (continuous-fed and pulse-fed, at 35 °C)	16S rRNA gene amplicon sequencing of DNA-SIP samples	*Syntrophomonas* *Thermovirga* *Aminivibrio* Candidatus *Cloacamonas,* *Anaerofustis*	*Methanosaeta* *Methabacterium*	(Ziels et al., 2018)

		Syntrophothermus *Ruminococcaceae* *Firmicutes* Candidatus *Parcubacteria* unclassified *Planctomycetes, Spirochaetae, Synergistes, Actinobacteria* and *Bacteriodetes*		
Reactors treating oleate-based effluent under different redox conditions	16S rRNA Gene Illumina MiSeq sequencing	*Stenotrophomonas* *Delftia* *Leptothrix* *Comamonas* *Pseudomonas* *Acinetobacteria* *Azoarcus* *Aeromonas* *Microvirgula* *Ochrobactrum* *Aquamicrobium*	*Methanosaeta* *Methabacterium*	(Duarte et al., 2018)

Substrate and culture conditions	**Techniques applied**	**Bacterial community**	**Archaeal community**	**Reference**
EGSB for the treatment of mixed LCFA-containing synthetic dairy wastewater at 20 °C.	16S rRNA amplicon sequencing	*Bacteroidia* *Clostridia*	*Methanomicrobia (Methanobacterium)* *Methanobacteria (Methanosaeta)*	(Singh et al., 2019)

		Synergistia		
Lab-scale mesophilic (37 °C) and thermophilic (54 °C) continuous stirred tank reactors fed with cheese whey	High-throughput 16S rRNA gene amplicon sequencing	*Clostridiales (Clostridiales sp. M2 and M3) Aminobacterium colombiense M1* *Bacteroidetes sp. M9* *Blautia producta sp. M20* *Erysipelatoclostridium ramosum M6* *Pelotomaculum schinkii M44* *Bacteroidaceae sp. M42* *Syntrophomonas sapovorans M45* *Verrucomicrobia* *Clostridia sp.T3, Clostridium sp. T7* *Defluviitoga tunisiensis T1* *Anaerobaculum sp. T2* *Clostridium thermopalmarium T13* *Sporanaerobacter acetigenes T24* *Alkalispirillum sp. T21* *Sinibacillus sp. T30* *Syntrophomonas bryantii T17* *Tepidimicrobium xylanilyticum T12* *Bacteroidetes sp. T36* *Clostridium ultunense T6*	*Methanoculleus palmolei M8* *Methanomassiliicoccaceae sp. M46 and M48* *Methanimicrococcus sp. M41* *Methanobacterium formicicum T20* *Methanothermobacter sp. T22*	(Treu et al., 2019)
Batch reactors treating cooking oil, LCFA and glycerol	16S rRNA gene Ion Torrent PGM	*Enterobacteriaceae (Enterobacter; Raoultella; Citrobater; Klebsiella)*	*Methanocorpusculum* *Methanobrevibacter*	(Nzila et al., 2019)

	sequencing platform	*Shewanellaceae* *Clostridiaceae* *Ruminococcaceae* *Porphyromonadaceae* *Bacteroidaceae* *Spirochaetes* *Spirochaetaceae*		

Substrate and culture conditions	**Techniques applied**	**Bacterial community**	**Archaeal community**	**Reference**
Anaerobic sequencing batch reactor treating synthetic lipid-rich wastewater, which comprised of glucose, acetic acid, lactic acid, and soybean oil	16S rRNA gene Illumina MiSeq sequencing	*Synergistales* *Anaerolineales* *Actinomycetales* *Nitrospirales*	*Methanobacteriales* *Methanosarcinales*	(Nakasaki et al., 2019)
Fed-batch anaerobic digestion of synthetic wastewater containing oil, glycerol or LCFAs	16S rRNA gene Illumina MiSeq sequencing	*Rikenellaceae* *Thermobaculaceae* *Anaerolineaceae* *Anaerolineaceae* *Clostridium* *Desulfovibrio* *Desulfovibrio aminophilus*	*Methanobacterium* *Methanosaeta*	(Nakasaki et al., 2020)

		Syntrophophaceae *Syntrophobacter* *Leptospirales* *Treponema* *Dethiosulfovibrionaceae* *Synergistaceae* *Kosmotoga*		
Batch codigestors treating anaerobic digestion sludge and FOG	16S rRNA gene Illumina MiSeq sequencing	*Firmicutes (Syntrophomonas)* *Bacteroidetes (Fermentimonas)* *Proteobacteria* *Synergistetes*	*Methanobacteriales* *Methanomicrobiales* *Methanosarcinales (Methanosaeta; Methanosarcina)* *Methanomassiliicoccales*	(Usman et al., 2020)

When studying the hypothesis that different microorganisms may be involved in the accumulation and further degradation of palmitate in oleate-fed bioreactors, Cavaleiro et al., (2016) concluded that the initial steps of unsaturated LCFA degradation can happen independently from methanogenic activity. Because facultative anaerobic bacteria became abundant, these authors suggested that these bacteria might have a role in these biochemical reactions, thus opening new possibilities besides the classical syntrophic degradation pathway (Cavaleiro et al., 2016) (Figure 2.2). To further investigate the role of facultative anaerobic bacteria, Duarte et al. (2018) studied oleate conversion in continuous bioreactors, one operated with microaeration (-250 mV), and other under strict anaerobic conditions (-350 mV). That difference in the oxidation-reduction potential (ORP) was correlated to a higher abundance of facultative anaerobic bacteria, particularly *Pseudomonas* spp. Interestingly, microaeration also promoted the transformation of oleate to palmitate, avoiding the long-term methanogenic inhibition observed in the strict anaerobic control experiment, possibly because palmitate is less toxic to methanogens than oleate (Figure 2.2). In fact, the theoretical ORP value of oleate to palmitate reaction is -270 mV (calculated at standard temperature and pressure conditions, using $\Delta G^{0'}$ from Table 2.2, and according to Thauer et al. (1977) this value is close to the ORP measured in the microaerophilic reactor (-250 mV), where the oleate to palmitate reaction was favoured. However, ORP in bioreactors (under non-standard conditions) will vary with the soluble concentration of compounds, which for LCFA is generally difficult to determine with accuracy. Moreover, the presence of other soluble species, such as sulphur compounds or oxygen, will also influence the ORP conditions. In anaerobic bioreactors treating oleate-based wastewater, the presence of facultative anaerobic bacteria was also shown to be important because they accelerate oleate conversion to methane, by protecting strict anaerobes from oxygen toxicity and also by acting as alternative hydrogen/formate and acetate scavengers for LCFA- degrading anaerobes (Duarte et al., 2020). From an applied point of view this is very important, since at industrial scale, the feeding tanks/pipelines are not kept under strict anaerobic conditions and small amounts of oxygen can be introduced to the system. The potential role of facultative bacteria in the conversion of unsaturated to saturated LCFA is still to be disclosed, and further studies are needed to better understand the interactions between facultative anaerobic bacteria and other microorganisms within methanogenic communities in continuous bioreactors. The addition of vestigial levels of oxygen and the fine regulation of redox potential are new perspectives to investigate in this field (Duarte et al., 2018).

The microbial communities developed during the co-digestion of lipids also have been the focus of recent studies. Hao et al. (2020) reported that in the co-digestion of waste activated sludge and FOG, an important increment of methane production was observed, probably due to the abundance of *Geobacter* species, indicating the role of direct interspecies electron transfer in FOG and activated sludge co-digestion. In another study, Salama et al. (2020) assessed the effect of calcium on FOG degradation. The addition of calcium promoted an increase in methane production and a shift in the microbial community, increasing the growth of bacteria from the *Clostridium,*

Syntrophomonas and *Sedimentibacter* genera. The genus *Methanosaeta* increased after the addition of 0.5 % calcium, which is one of the factors responsible for high methane production, avoiding the inhibitory growth and toxic effects of high concentrations of FOG. In the study of Usman et al. (2020), *Syntrophomonas* and *Fermentimonas* were abundant. *Methanosaeta* were dominant in the beginning, owing to the increased presence of LCFA, but afterwards were replaced b*y Methanosarcina* genus, likely because of the increase in acetate concentration due to the LCFA conversion. Kurade et al. (2019) compared acclimatised (fed-batch over 160 days – 10 batch cycles) to non-acclimatised sludge and showed an increased LCFA degradation efficiency in the former of up to 64 %, albeit LCFA degradation was still not complete within 30 days and 56 % oleate remained unconverted in the acclimatised reactor. Amha et al. (2017) thoroughly evaluated the microbial community under thermophilic conditions treating a waste with up to 60 % FOG. These authors highlighted that syntrophic bacteria were enriched and promoted the successful co-digestion process with FOG. Moreover, their approach of jointly utilising sequencing technology with qPCR analysis (and quantification) on specific groups (e.g., methanogens, syntrophic bacteria) was shown to be robust and beneficial for future studies in the field.

2.3 BIOREACTOR CONFIGURATIONS IN HIGH-RATE WASTEWATER TREATMENT

Since the 1970s the field of anaerobic digestion is commercially active treating waste/wastewaters from various industries with different bioreactor configurations. To ensure the uptake of AD by industry, the costs need to be competitive, both capital and operational per m^3 of waste treated. This can be achieved if the rate of degradation is increased, along with the biogas yield per m^3, especially in respect to wastewater treatment.

For several biodegradable industrial wastewaters, HRAT have enabled high rates of degradation and biogas yields. The superior performance of these systems is based on the retention of slow-growing microorganisms inside the bioreactor, requiring a successful decoupling of solids retention time (SRT) and hydraulic retention time (HRT). The three most common mechanisms to achieve this are physical separation (e.g., by settling and/or filtration), attachment to fixed or non-fixed inert supports and auto-immobilisation or granulation (Lettinga et al., 1997; van Lier et al., 2015). Among these mechanisms, microbial granulation dominated the implementation of anaerobic technology in the last decades, following the development of the upflow anaerobic sludge blanket (UASB), the expanded granular sludge bed (EGSB) and the internal circulation (IC) reactors (van Lier et al., 2015). However, the improved performances of these HRAT designs did not translate across to the treatment of lipid-rich wastewaters. In these systems, the COD removal efficiencies are generally high, but the substrate conversion to methane tends to be incomplete (Alves et al., 2009), mainly due to lipids/LCFA adsorption onto the sludge.

2.3.1 High-rate anaerobic technologies (HRAT) for lipid-rich wastewater treatment

Operational parameters of diverse first-generation reactors treating lipid-rich wastewater are summarized in Table 2.4. The anaerobic contact process (ACP) is one of the original developments of HRAT, and is constituted by a continuous stirred anaerobic digester and an external clarifier, where the anaerobic sludge is settled and returned back to the reactor (Shin et al., 1990). In this type of system, the successful operation relies on the operation of the clarifier, and problems with sludge settleability can be partially addressed through the degasification of the reactor effluent, where the biogas is released from the sludge often allowing it to settle again. Sludge separation through flotation and not through settling is an alternative way.

The anaerobic filter (AF), upflow or downflow, is another type of HRAT, in which the reactor has support media (e.g. PVC or ceramic rings, for example) for biomass attachment. AF have a relatively simple construction, since there are no moving parts, however a large reactor volume is required. Moreover, AF generally suffers from severe clogging issues due to suspended solids entrapment and biomass growth in the filter, resulting in the occurrence of channelling and short circuiting. Moreover, high concentration of lipids in the wastewater will aggravate the clogging process (Borja & Banks, 1994; Rajeshwari et al., 2000) and lipids may act as a soap, decreasing the biomass adhesion to the support (Alves et al., 2001).

Table 2.4 - Operational parameters of first-generation reactors treating lipid-rich wastewaters.

Type of reactor	Type of wastewater	Scale	Volume	OLR (in COD) ($g\ L^{-1}\ d^{-1}$)	HRT (days)	T (ºC)	Influent (in COD) ($g\ L^{-1}$)	FOG ($g\ L^{-1}$)	Methane yield or Methane production	COD removal (%)	Trial duration (months)	Ref.
ACP	Bakery industry	Full-scale	1 860 m^3	3	7.8	35	23.7	5.8	ND	97	4	(Shin et al., 1990)
	Ice- cream	Pilot	5.4 m^3	1.09	5.51	35	4.9	0.8	0.39 ($L\ CH_4\ g^{-1}$ CODr)*	81.8	9	(Hawkes et al., 1995)
AF	Slaughterhouse	Lab	2 L	0.88 – 11.21	7.1 – 0.5	37	5.2 – 11 .4	0.2 – 0.7	0.05 – 1.10 ($L\ CH_4\ L^{-1}\ d^{-1}$)	28 – 82	13.6	(Ruiz et al., 1997)
	Dairy Industry	Full-scale	12 m^3	2 – 4.7	7.00 – 1.85	35 – 37	10.5	1.8	30.94 ($m^3\ CH_4\ d^{-1}$)	67 – 93	20.84	(Omil et al., 2003)
	Ice-cream	Pilot	5 m^3	6.38	0.93	35	4.9	0.8	0.36 ($L\ CH_4\ g^{-1}$ CODr)*	81.8	15 (period of stability)	(Hawkes et al., 1995)
UASB	Slaughterhouse	Lab	10 L	2.2 – 5.9	5	35	10.7 – 29.4	3.9 – 16.4	0.42 – 0.15 ($L\ CH_4\ g^{-1}$ CODr)*	90	5.5	(Jeganathan et al., 2006)

			15 L	1.2 – 8.9	2.5 – 1.25	35	3 – 11.1	1.1 – 4.9	0.55 – 0.18 ($L\ CH_4\ g^{-1}$ CODr)*	80 – 88	8.4	
		Lab	33.5 L	2.5 – 19.5	0.38 – 0.07	30	1.5 – 2.2	0.05 – 0.10	0.24 – 1.87 ($L\ CH_4\ L^{-1}\ d^{-1}$)	53 – 67	2.3	(Sayed et al., 1987)
			33.5 L	3 – 12	0.42 – 0.21	20	1.5 – 2.2	0.05 – 0.10	0.22 – 1.12 ($L\ CH_4\ L^{-1}\ d^{-1}$)	40-62	4.7	
		Lab	2 L	1 – 6.5	6.5 – 1.2	37	5.2 – 11.4	0.2 – 0.7	0.22 – 1.34 ($L\ CH_4\ L^{-1}\ d^{-1}$)	59 – 91	13.7	(Ruiz et al., 1997)
	Ice-cream	Pilot	5 m^3	2.19	1.62	35	4.9	0.8	0.19 ($L\ CH_4\ g^{-1}COD_{in}$)**	49	8.5 (period of stability)	(Hawkes et al., 1995)
UASB+ AF	Dairy (UHT and cheese production)	Lab	10 L	0.98 – 15.7	1	30	10.9 – 20.5	0.2	ND	76 – 95	7.2	(Gomes et al., 2011)
	Slaughterhouse	Lab	2 + 10 L	1.2 – 4.5	2.5 – 1.25	35	2.8 – 5.6	0.5 – 1.6	0.52 – 0.09 ($L\ CH_4\ g^{-1}$ CODr)*	80	5.5	(Jeganathan et al., 2006)
EGSB	Slaughterhouse	Lab	2.7 L	2.1 – 15.8	0.79 – 0.22	35	1.4 – 4.2	0.05 – 0.28	ND	47 – 91	10	(Núñez & Martínez, 1999)
	POME	Lab	20.5 L	10	3	35	32.5	11	ND	93	4.3	(Y. Zhang et al., 2008)

	Oleic acid	Lab	1 L	2.7 – 1.18	1	37	3.9	ND	0.16 – 0.28 ($L\ CH_4\ L^{-1}\ d^{-1}$)	65 – 93	2.5	(Pereira et al., 2005)
	Palmitic acid	Lab	1 L	2.7 – 1.14	1	37	3.7	ND	0.13 – 0.25 ($L\ CH_4\ L^{-1}\ d^{-1}$)	62 – 93	2.5	

*OLR - Organic loading rate (expressed in COD), ND - not determined, * Expressed relatively to the COD removed, ** Expressed relatively to the COD added.*

In the UASB reactor, developed by Lettinga et al., (1980), formation of highly settleable sludge aggregates (granules) takes place, combined with gas separation and sludge settling (van Lier et al., 2015). However, several reports describe difficulties when applying granular sludge reactors to lipid containing wastewaters. The granules are structurally unstable when lipids or LCFA adsorb to their surface, suffering breakage, loss of density and thus process inhibition. Sayed et al., (1987) studied the UASB reactor performance in the treatment of a slaughterhouse wastewater containing 50 % of insoluble suspended COD and 5 % of grease in the total solids. The process could not handle OLRs exceeding 3.5 g/(Ld) (in COD) at an HRT of 8 h (Table 2.4). At the same time, there was a deterioration of the COD removal of the system under high loading conditions. Further to this, other studies found that the operation of UASB or other granular systems is limited by components, such as milk fat and proteins, presenting low rate of anaerobic degradation and microbial inhibition problems (Hawkes et al., 1995; Perle et al., 1995; Vidal et al., 2000). Hawkes et al., (1995) reported the performance of a pilot scale UASB reactor treating ice-cream wastewater at an OLR of 2.19 g/(Ld) (in COD), giving a poor performance with less than 50 % COD removal efficiency (Table 2.4). Jeganathan et al. (2006) studied the treatment of a complex oily wastewater from a slaughterhouse in two different UASB reactors and verified that, at an OLR of 3 g/(Ld) (in COD), FOG and COD removal efficiencies were higher than 80 % (Table 2.4). However, the reactors performance deteriorated sharply at higher loading rates, and the presence of FOG caused a severe sludge flotation resulting in process failure. Fat, protein, and cellulose components of the POME wastewater were also reported to have an adverse impact on UASB reactors performance and caused deterioration of microbial activity and biomass washout (Zinatizadeh et al., 2007).

In the EGSB reactor design, problems have also been noted when treating lipid-rich wastewaters. In the study of Núñez & Martínez, (1999), an EGSB was used for the treatment of slaughterhouse wastewater obtaining a COD removal efficiency of 65 -80 %, applying an OLR (in COD) up to 15 g/(Ld) with a fat influent concentration of 0.15 g /L. In this study 85 % of the fats present in the wastewater were removed and no accumulation of fats on the sludge was observed. Zhang et al., (2008) treated POME wastewater in a laboratory scale EGSB reactor at OLR (in COD) from 1.45 to 17.5 g/(Ld) and an HRT of 2 - 3 days, obtaining 90 % – 95 % of COD removal efficiency. In this study, scum formation and sludge flotation were reported due to the presence of FOG in the raw POME and its adsorption to the granules. Pereira et al., (2005) studied LCFA inhibition in a lab scale EGSB treating oleate at an OLR (in COD) of 8 g/(Ld), with a COD removal efficiency around 80 % and a biogas containing 55 % of methane.

From these studies, it becomes clear that these HRAT reactors do not successfully deal with the commonly reported problems related to lipid-rich wastewater, namely the loss of granular structure or unsuccessful granulation, sludge flotation and washout. Therefore, different solutions were evaluated to overcome these problems. For example, the two-phase reactor concept (Kim et al., 2004; Saddoud & Sayadi, 2007) was applied to improve

process stability and efficiency due to physical separation of the rate limiting methanogenic phase. However, considering that saturated LCFA biodegradation requires syntrophic cooperation with methanogens, phase separation may not be advantageous. Inverse fluidized reactors were also used for the treatment of a dairy wastewater by Arnaiz et al., (2003) with good COD removal efficiencies, but methane yields were not reported. Haridas et al., (2005) developed a new reactor design, the buoyant filter bioreactor (BFBR), for the treatment of fat-rich wastewater. In this system, buoyant polystyrene beads form a granular filter bed that allows the decoupling of the SRT from the HRT. An almost complete COD conversion to methane was reported during the treatment of a dairy effluent for 400 days. When the OLR was increased, scum accumulation was observed, followed by further solubilisation and degradation to methane.

2.3.2 Second generation reactors for AD of lipids

In the last decades, novel reactor designs based on alternative sludge retention strategies have been developed up to technology readiness levels (TRL) of 8-9, which are able to deal with the main problems associated to the AD of lipids. The core developments include sludge flotation as a strategy to prevent the washout of biomass.

Nowadays there are several commercially available bioreactors suitable to treat lipid-rich wastewaters: *Evoqua*'s ADI-BVF®, *Paques B.V.*'s anaerobic flotation reactor (AFR), trading as BIOPAQ®AFR, and both *Biothane-Veolia*'s Memthane® (anaerobic membrane bioreactor - AnMBR) and recently Sparthane® (anaerobic sequencing batch reactor - AnSBR). All these bioreactors use flocculent sludge. In Table 2.5, a summary of reported operational conditions of second-generation reactors treating lipid-rich wastewater is presented.

The **ADI-BVF®** system provides low-rate treatment for complex wastewaters, operating at lower volumetric loading rates and higher HRT than HRAT. The large volume of the reactor, the low-rate operation mode and the sludge recycle system avoids biomass washout and guarantees very long SRT. The tank has a simple design and operation, however due to its size, it represents a large capital investment.

The **BIOPAQ®AFR** reactor by *Paques B.V.* (Vellinga & Mulder, 2002) is especially designed to treat wastewater streams containing fats and oil, for example from the dairy, poultry and food industries. It utilises the flotation properties of the FOG-sludge mixtures, assisting it with white-water microbubbles, derived from a small part of the system's produced biogas, that is compressed and solubilised in the feed water to be released in the lower part of the internally mounted anaerobic floatation unit. The effluent is withdrawn from the suspended solids free zone below the flotation layer. The flotation unit, integrated with the reactor system, retains the sludge up to concentrations of 15 – 30 kg per m^3 of reactor volume (Frijters et al., 2014). Therefore, it saves the biomass and the substrate solids from washout, and also increases the biological activity through increasing contact with the substrate and allowing the sludge to degrade absorbed lipids.

Ultimately, through an engineered robust retention system for the sludge, the BIOPAQ®AFR reactor overcomes one of the common bottlenecks related to sludge washout during the anaerobic treatment of lipid-rich wastewater. A recent improvement is based on pressurising the effluent flow of the bioreactor (including the biomass), instead of the effluent of the flotation unit only, which enhances the efficiency of the flotation process. Furthermore, less pressure is required for biomass floatation and increased solids loading rates on the flotation unit can be applied, resulting in an even more compact system. In pilot and full-scale treatment of the complex wastewater it was observed that during the first half-day after a non-feeding period (e.g., a weekend) filamentous sludge developed. However, within a day after restarting feeding, the sludge becomes more compact again (Paques personal communication). While this issue is easily addressed, the rapid change in biomass morphology is unclear. Since the phenomenon of developing filamentous biomass after a restart seems a generic observation in other type of plants as well (Paques personal communication), the microbiological knowledge regarding on floc-formation and composition, its thickening and exopolysaccharides formation should be further explored. In the AFR system itself, this filamentous biomass is retained, as sludge floatation is very efficient by directly pressurising the biomass as explained above.

Full scale studies performed with this reactor design showed that extremely high concentrations of fats could disturb the system, but the inhibition was reversible (Frijters et al., 2014). Therefore, managing the waste streams (for example the high concentrated FOG streams, like ice cream, in a small buffer tank and the low to medium concentrated stream in a large buffer tank) is necessary. Both streams can be pumped in the reactor in a controlled way, avoiding extreme peaks of fat (Frijters et al., 2014). Despite the possible requirement for separate buffers, the reactor has strong buffering capacity against spike-loading of lipids. It is hypothesised that this buffering capacity is due to the adsorption of the lipids to the sludge and the degradation of the excess lipids at a later time. The reactor has a high COD removal efficiency of 90 – 95 %, applying an HRT of 1 – 8 days, dependent on substrate and volumes (Table 2.5). It has the ability to treat wastewater with COD concentrations of 5 up to 70 g/L, with a maximum of 50 % of the COD being lipids (Frijters et al., 2014). Microbiologically, the flocculent biomass has proven ideal for this reactor system, with high methanogenic activities recorded, despite the complex substrates treated. The AFR system is applied for full scale treatment of various fat or oil containing wastewaters as dairy wasters, meat processing wastewater, tank cleaning wastewater and fish processing wastewater. The system is very robust, and the sludge is well retained, even if there is an upset in load. The sludge has, in case of a higher fat concentration in the reactor, a tendency to float which is an advantage in this system as it is designed to retain by means of flotation. Therefore, the system shows a high flexibility for changes in loading rates and types of waste (Frijters et al., 2014).

Other commercially available technologies to treat lipid-rich wastewaters include the anaerobic membrane system offered by *Biothane-Veolia B.V.*, the **Memthane**®. Saddoud

& Sayadi, (2007) who studied the application of an AnMBR for the treatment of slaughterhouse wastewater, with an operational OLR (in COD) from 4.34 – 15.8 g/(Ld), achieved a COD removal efficiency up to 94 %. Dereli, et al., (2014) studied the performance of a lab scale AnMBR treating lipid-rich corn to ethanol thin stillage at different SRTs, achieving removal efficiencies up to 99 % with an OLR (in COD) up to 8 g/(Ld). These results were obtained applying SRTs of 20 and 30 days, where LCFA precipitation with cations or adsorption onto biomass of LCFA were the dominant mechanism for LCFA removal. Results showed that high amounts of COD originating from lipids accumulated as very large LCFA precipitates (denominated fat balls) at short SRTs, meaning that COD bioconversion was, in fact, less. Ramos et al. (2014) studied the performance of a pilot AnMBR treating lipid rich wastewater from a snacks factory, where satisfactory results were obtained with an OLR below 2 g/(Ld) (in COD) with acclimated sludge, without inhibitory effects. Szabo-Corbacho et al., (2019) studied the performance of an AnMBR treating synthetic dairy wastewater, at 2 different SRTs (20 and 40 days), with a working OLR of 4.7 g/(Ld) (in COD), obtaining efficiencies of more than 99 % organic matter removal and a very low LCFA accumulation inside the system. *Biothane* commissioned 9 full-scale AnMBRs (Memthane® systems), using tubular inside-out polymeric membranes in cross-flow skids (van Lier et al., 2015). Other companies, e.g., Kubota (Kanai et al., 2010), are implementing submerged AnMBRs in which the membranes are mounted inside the bioreactor or in a separate membrane tank. While membrane-based bioreactors offer a solution for lipid-rich wastewaters, their economic viability due to high operation costs related to membrane filtration proves difficult for standard treatment of wastewater, unless downstream water reuse, where high effluent quality is demanded, and other membrane systems (i.e., reverse osmosis) are in operation. Therefore, further novel systems have been developed and tested at pilot scale. The **Sparthane**®, a sequencing batch reactor (AnSBR) also by *Biothane* takes another approach to address the problem of lipid-degradation through a patented batch sequence of a stirred reactor, batch degassing tank and semi-continuous settling tank (Veolia, 2020). Similar in set-up to the ACP, it can, however, accept high-loading rates of 8 to 10 g/(Ld) (of total COD), under mesophilic conditions. Pre-acidification is core to the process, ensuring a balanced liquid matrix of compounds that are degradable by the flocculent microbial community, avoiding denaturation of proteins and temporary inhibition of lipid degradation. Stringent monitoring positively influences the separation and clarification steps, limiting the growth of filamentous bacteria, to ensure sludge settleability and thus easy clarification, solving previously documented issues with the contact reactor process (van Lier et al., 2015). The batch sequencing is further supported by the microbial findings of Cavaleiro et al., (2009) and Ziels et al., (2017), suggesting that this approach increased the ability to rapidly degrade lipids. Ziels et al., (2017) further supported this work with quantification of the syntrophic communities and their resulting increase from batch feedings, albeit in a co-digestion system. Overall, as the system is expanded with full-scale reference sites presently, it offers a full degradation of complex lipid-rich wastewaters in strategic yet operationally candid manner.

Another technology especially designed for the treatment of wastewater with high lipids content, not yet commercially available, is the **Inverted Anaerobic Sludge Bed (IASB)** reactor (Alves et al., 2007, 2009; Cavaleiro et al., 2015). Similar to the BIOPAQ®AFR, the IASB reactor uses the sludge flotation properties, resulting from lipids/LCFA adsorption, to retain the sludge and the LCFA in the system. Adsorption is promoted by mixing the feed with the recycled sludge, and this mixture is fed from the top. The recycle line and a gas lift effect assist in the internal mixture of the reactor content. Sludge separation is performed at the bottom. A pilot scale IASB reactor (1.2 m^3) was operated for the treatment of a slaughterhouse wastewater, at an OLR (in COD) from 0.5 to 16 g/(Ld), with 63 % as fat (Picavet & Alves, 2010). COD removal efficiencies higher than 80 % were achieved and excessive LCFA accumulation was prevented, showing its capacity for the treatment of complex wastewater with high quality fluctuations.

The commercial need for the treatment of complex lipid-rich wastewaters has driven the field towards market ready systems and technologies, as listed and detailed above. The possibility of directly treating lipid-rich wastewater anaerobically has been accomplished, and the high return in biogas coupled with the savings in pre-treatments for FOG separation, contributes to counterbalance any extra operating and capital expenses. With the implementation of these second-generation AD reactors, the main issues of the first-generation are solved, i.e., LCFA inhibition, sludge washout, low removal efficiency, allowing to treat high OLR (up to 16 g/(Ld) in COD) with high removal efficiencies and keeping a more stable reactor performance.

Table 2.5- Operational parameters of second-generation reactors treating lipid-rich wastewaters.

Type of reactor	Type of wastewater	Scale	Volume	OLR (in COD) ($g\ L^{-1}\ d^{-1}$)	HRT (d)	SRT (d)	T (ºC)	Influent (in COD) ($g\ L^{-1}$)	Influent FOG ($g\ L^{-1}$)	Methane yield or Methane production	COD removal (%)	Trial duration (months)	Ref.
AFR	Ice-cream	Full-scale	511 m^3	2 – 6	3	90	38	4.5 – 25.6	2.2 – 12.8	0.33 (L CH_4 g CODr)*	90	8.1	(Frijters et al., 2014)
	Food cleaning stream	Full-scale	430 m^3	0.1 – 4.6	5	90	38	2.3 – 29.8	0.1 – 2.2	ND	98	10	(Frijters et al., 2014)
	Slaughterhouse	Full-scale	9000 m^3	3.3	3	20-50	28-35	11	0.6	0.33 (L CH_4 g CODr)*	94	12	Personal communication (Paques)
AnMBR	Corn to ethanol thin stillage	Lab	10 L	8.3	10	20	35	63.6 – 80.8	10.8 – 11.8	0.26 (L CH_4 g CODr)*	99 99 98	3	(Dereli et al., 2014)
			10 L	7.8	10	30	35	63.6 – 80.8	10.8 – 11.8	0.28 (L CH_4 g CODr)*	99	3	
			10 L	6.1	10	50	35	63.6 – 80.8	10.8 – 11.8	0.29 (L CH_4 g CODr)*	98	3	
	Snacks factory	Pilot	760 L	1 – 2	4	25	30-36	8.6 – 14.8	0.1 - 0.4	0.5	91 – 75	0.8	(Ramos et al., 2014)

										(g COD-CH_4 L^{-1} d^{-1})			
			760 L	2 – 16	2.8	95	30-36	11.6 – 98.0	2.7 – 36	2.75 (g COD-CH_4 L^{-1} d^{-1})	81 – 99	3.2	
	Slaughterhouse	Pilot	50 L	4.34 – 15.8	3.33 – 1.25	ND	37	7.1 – 20.4	0.2 – 0.3	0.31 -0.13 (L CH_4 g^{-1} CODr)	97 – 60	2.37	(Saddoud & Sayadi, 2007)
	Dairy	Lab	10 L	2.3 – 4.7	2.2	20	35	2.6 – 17.6	1.7	0.31 (L CH_4 g^{-1} CODr)*	99	6.5	(Szabo-Corbacho,et al., 2019)
			10 L	2.3 – 4.7	2.2	30	35	2.6 – 17.6	1.7	0.32 (L CH_4 g^{-1} CODr)*	99	6.6	
IASB	Slaughterhouse	Pilot	1.2 m^3	0.5 – 16	1.5 – 1.4	ND	30 – 35	0.001 – 44	6.7	ND	80 – 85	12	(Picavet & Alves, 2010)

*OLR - organic loading rate (expressed in COD), ND - not determined, * Expressed relatively to the COD removed*

2.4 ALTERNATIVE STRATEGIES FOR IMPROVING AD OF LIPIDS

Besides the development of novel reactor configurations, other strategies have been studied to improve the AD of LCFA/lipids. For example, addition of calcium ions (Roy et al., 1985) or inert materials, e.g., activated carbon, bentonite, or other clays (Angelidaki et al., 1990), was tested, considering that these materials can reduce LCFA/lipids bioavailability through mechanisms of precipitation or adsorptions, thus decreasing their potential toxicity. These strategies intended to reduce LCFA bioavailability and thus decrease their toxicity. The mitigation of LCFA inhibition by the addition of cations and natural adsorbents has been recently reviewed by Elsamadony et al. (2021). For example, recently, Salama et al., (2020) tested the application of calcium (0.1 to 1 %) in order to overcome the inhibition caused by 2 % of FOG in bioreactors. The addition of 0.5 % calcium was best, promoting a 6-fold increase in the biomethane production and a reduction in the outlet COD from 131 to 14 – 64 g/L. Mixing the calcium with FOG before feeding the reactor was advantageous, since it reduced the growth-inhibitory effects of FOG at the process start-up.

Also, the use of conductive materials (e.g., ferric oxyhydroxide, magnetite and granular activated carbon) recently have improved the methane production rate from dairy wastewaters (Baek et al., 2017; Martins et al., 2018). Also, biomethane potential assays using oleate and granular activated carbon (GAC) (0 - 33 g/L) were performed by Tan et al. (2021). The authors suggested that GAC addition promotes the faster consumption of both volatile fatty acid and LCFA, particularly palmitate. During oleate degradation, the presence of GAC decreased the lag-phase for methane production. These authors postulate that since the electron transfer via DIET is higher than via hydrogen, the potential shift from indirect hydrogen transfer to the DIET pathway, induced by the presence of GAC, may result in a more efficient conversion of LCFA to methane (Tan et al., 2021). Despite of the use of conductive materials to promote direct interspecies electron transfer (DIET) in processes of AD of lipids is recently being studied, their application in both HRAT or LRAT systems needs to be further explored.

Additionally, the implementation of microaeration also has been shown as a promising strategy to enhance the digestion of lipids/LCFA-rich wastewaters, since it promotes oleate conversion to palmitate (which is less toxic to the microorganisms than oleate) avoiding a severe inhibition of methanogens (Duarte et al., 2018).

Biogas upgrading from anaerobic digestion of waste frying oils (WFO) was obtained in a biogas-lift bioreactor in which gas and liquid recirculation was applied. In this reactor 1.4 times more biogas, with higher methane content (79 %), was obtained when compared with the control reactor without gas recirculation (67 %). This improvement resulted from the enrichment of hydrogenotrophic methanogens. Biogas recirculation thus appear as a promising strategy to enhance biomethane production from lipids (Duarte et al., 2021).

Despite of all the achievements, the basic issue of LCFA inhibition and palmitate accumulation are still not clearly understood, and their comprehension might boost process performance. This could allow true high-rate (< 24 h) digestion, larger energy gains (even at low, psychrophilic, temperatures) and ultimately lead to further implementation of resource recovery from lipids in wastewater.

2.5 CONCLUSIONS AND FUTURE PERSPECTIVES

AD of lipids is a complex process that proceeds close to the thermodynamic minimum of life, being highly dependent on specific and complex microbial interactions. Significant progress has been made in the past two decades regarding fundamental knowledge in microbiology, biochemical pathways, and new reactor configurations, which have been translated into the market. The main challenges of the field have been tackled, allowing to overcome the classical problems of microbial inhibition and sludge flotation and washout at higher loads. Therefore, AD of lipids is now a mature technology, which offers excellent opportunities for successful lipids valorization over long-term operation of stable full-scale systems.

Nevertheless, some issues are still challenging and constrain a wider implementation of the AD of lipids:

i. The equilibrium between LCFA accumulation and biodegradation to methane is still not mastered. Extremely high concentrations of fat lead to LCFA accumulation, that hinder the bioconversion. Therefore, managing the waste streams is currently necessary. This is a main critical point observed in pilot/full-scale operation of AD systems, that may be tackled through the development of novel strategies that accelerate LCFA biodegradation and further conversion to methane (e.g., microaeration or addition of conductive materials).

ii. The effect of lipids/LCFA on the structure and integrity of the sludge is only poorly perceived yet, which most likely have a direct impact on process performance. In-depth studies on flocs formation, spatial organisation within microbial aggregates and exopolysaccharides formation are essential.

These issues call for further research, development, and innovation, targeting high-rate methane production from lipids, and promoting AD of lipids as a hub in the bioenergy market. The production of medium chain fatty acids and/or other valuable compounds also represents an interesting alternative to biogas, which is highly relevant in the quest for a carbon-neutral world.

New strategies such as microaeration or addition of conductive materials are promising to boost methane production from lipids. Regarding microaeration in AD of lipids, fine tuning the redox potential conditions can promote the partial detoxification of LCFA, likely triggering a more active methanogenic community thriving on lipids. Yet, the

mechanisms involved, and the interactions between facultative anaerobes and methanogens, are new research topics in the field that still require additional studies, e.g., by using pure cultures or synthetic microbial consortia. Concerning the application of conductive materials, those may act upon interspecies electron transfer or/and methanogenic activity which, otherwise, will rate-limit the process. A deeper comprehension of the pathways and functional regulations in the mixed microbial communities performing AD of lipids in the presence of conductive materials is essential for an effective management of this approach.

Coupling the current methods used in the field with multi-omics and advanced visualization, isotope probing, and detailed reactor data will increase the knowledge of AD of lipids. However, it is worth noting that reference genomic databases for the field need to be expanded, as only limited data is currently available. Thus, further holistic metagenome and meta-transcriptome studies need to be performed.

Above mentioned research directions, together with novel strategies to improve the efficiency and interaction of the microorganism involved in the degradation of lipids, as well as the close collaboration between industry and academia, will most likely bring the AD of lipids to a higher maturity level.

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3

ANMBR TREATING LIPID RICH WASTEWATER IMPACT OF SOLID RETENTION TIME ON BIOLOGICAL PERFORMANCE

This chapter is based on: Szabo-Corbacho, M. A., Pacheco-Ruiz, S., Míguez, D., Hooijmans, C. M., García, H. A., Brdjanovic, D., & van Lier, J. B. (2021). Impact of solids retention time on the biological performance of an AnMBR treating lipid-rich synthetic dairy wastewater. Environmental Technology, 42(4), 597-608.

ABSTRACT

In this study, the impact of applied solids retention time (SRT) on the biological performance of an anaerobic membrane bioreactor (AnMBR) treating synthetic dairy wastewater with high lipid content was assessed. Two side-stream AnMBR systems were operated at an SRT of 20 and 40 days (R20 and R40, respectively), equipped with an inside-out tubular membrane operated in cross-flow mode under full-scale operational conditions, i.e. crossflow velocity, transmembrane pressure, membrane flux. Successful operation was achieved, and removal efficiencies of both reactors were up to 99% applying an organic loading rate (OLR) of 4.7 g COD/(Ld). No precipitation of lipids was observed throughout the operational period, keeping the lipids available for the anaerobic degradation. Long chain fatty acid (LCFA) accumulation was very modest and amounted 148 and 115 mg LCFA-COD per gram of volatile suspended solids (VSS) for R20 and R40, respectively. At an SRT of 40 days, a slightly better biological conversion was obtained. Periodically performed specific methanogenic activity (SMA) tests showed stabilization of the SMA for R40 sludge, whereas for R20 sludge the SMA continued to decrease. This study revealed a more stable reactor performance operating the AnMBR at an SRT of 40 days compared to 20 days.

3.1 INTRODUCTION

The dairy sector produces large quantities of wastewater, approximately 0.2 to 10 liters of wastewater per liters of processed milk (Karadag et. al., 2015). The main constituents of dairy industrial wastewater include easily biodegradable carbohydrates (mainly lactose), as well as proteins and lipids (Angelidaki et. al, 1995; Fang et. al., 2000). The exact composition of dairy wastewater considerably differs per location (Table 3.1), depending both on the type of dairy product being produced, such as milk, butter, yoghurt, ice-cream, desserts, and/or cheese, and on the production methods, operations, and technologies available at each particular industry. Most dairy wastewaters are characterized by considerable amounts of fats, oil, and grease (FOG) (Table 3.1) (Demirel et. al., 2005). Karadag et al. (2015), reported FOG concentrations varying from 0.5 to 9.5 g/ L and reported a detailed analysis of the long-chain fatty acids (LCFA) being present in dairy wastewater, mainly consisting of palmitic acid (23.5%), oleic acid (21%), and myristic acid (10.5%).

Table 3.1 – Dairy industrial wastewaters

Dairy industry	**pH**	**COD** (g /L^{-})	**BOD_5** (mg /L)	**Solids** (g /L)	**Volatile solids** (g /L)	**Nitrogen** (mg /L)	**Phosphorus** (mg /L)	**FOG** (g /L)	**Reference**
Cheese whey	4.9	68.6	7.71	1.95 (TS)	NA	1120 (TKN)	500	9.44	Traversi et. al., 2013
Ice-cream	5.2	5.2	2.45	3.9 (TS)	2.6	60 (TKN)	14	NA	Borja et. al., 1995
Ice-cream	6.96	4.94	NA	1.1 (TSS)	0.99	NA	NA	NA	Hawkes et. al., 1995
Milk processing	4.0-7.0	5-10	3-5	3-7 (TS)	NA	20-150 (TKN)	50-70	NA	Pretti et. al., 2011
Dairy	8-11	2-6	1.2-4	0.35-1 (TSS)	0.33-0.94	50-60	20-50	0.3-0.5	Ince et. al., 1998

Mixed dairy processing	6-11	1.2-9.2	NA	0.3-1.7 (TSS)	0.3-0.8	14-272 (TKN)	8-68	NA	Demirel et. al., 2005
Cheese	5.5-9.5	1-7.5	0.6-5	0.5-2.5 (TSS)	NA	NA	NA	NA	Monroy et. al., 1995
Milk processing	NA	1.5-6	NA	0.3-2 (TSS)	NA	200-300 (TKN)	< 100	<0.5	(*)
Milk powder	NA	0.5-2	NA	<0.3 (TSS)	NA	<100 (TKN)	<100	<0.5	(*)
Fresh cream	NA	8-19	NA	7-8 (TSS)	NA	300-600 (TKN)	<100	0.1-0.3	(*)
Yoghurt	NA	5-20	NA	2-4 (TSS)	NA	200-400 (TKN)	0.2	0.3-1	(*)
Cheese	NA	2-13	NA	0.5-2 (TSS)	NA	200 (TKN)	0.1	0.3-1	(*)
Ice cream	NA	5-36	NA	5-10 (TSS)	NA	150-200 (TKN)	0.3	0.3-4	(*)

NA: Not available; FOG: Fats and Oil and Grease; COD: Chemical oxygen demand; (*) Internal data of Biothane-Veolia

The anaerobic treatment of industrial wastewater provides several advantages, such as high organic matter removal efficiencies, energy recovery through biogas production, and low sludge production and wastage (van Lier, 2008; Rajeshwari et. al., 2000). Dairy wastewaters have a high concentration of organics and lipids, being an ideal substrate for anaerobic treatment (Alves et. al., 2009). However, there are also negative aspects associated to the anaerobic conversion of lipids, which adds to the major complexity of treating lipid-rich wastewater such as dairy wastewater. During anaerobic digestion, triacylglycerol lipids are firstly hydrolyzed to glycerol and LCFAs. In general, hydrolysis of lipids occurs relatively fast, and the degradation of LCFA is considered the rate limiting step, potentially leading to the accumulation of LCFA in the system (Pavlosthatis et. al., 1991). Even at low concentrations, the LCFA are toxic to methanogens and acetogens,

whereby the unsaturated LCFA are more inhibitory than the saturated LCFA (Lalman et. al., 2000). Moreover, LCFA adsorb onto the biomass causing mass transfer limitations affecting the biomass uptake of substrates and nutrients (Pereira et. al., 2005). In addition, the adsorption of LCFA onto the biomass surface causes biomass flotation and washout, which particularly limits the application of sludge bed reactor systems such as the upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactor (Hwu et. al., 1988; Hanaki et al., 1981).

Completely mixed reactor systems with a high biomass surface to liquid ratio are increasingly considered for the full-scale anaerobic treatment of FOG-rich wastewaters. However, the effectiveness of these systems fully depends on the effectiveness of the sludge separation device preventing sludge wash-out. Some systems combine an internal gas floatation unit for improved sludge retention such as the Biopaq® AFR reactor (Frijters et. al., 2014). Other reactors rely on the complete retention of biomass using a membrane separation device (van Lier et. al., 2015). At present, anaerobic membrane bioreactors (AnMBR) are indeed increasingly applied for the treatment of FOG-rich wastewaters such as dairy wastewater (Pacheco-Ruiz et. al., 2017). However, the required physical separation device is an additional and sometimes considerable cost factor to the anaerobic bioreactor. Therefore, process optimization is required that allows for minimizing the required filtration area in the membrane units. Previous research has shown that sludge filterability is determined by the prevailing sludge characteristics, which are impacted by the operational solids retention time (SRT) (Dereli et. al., 2014; Pacheco-Ruiz et. al., 2017). In the treatment of lipid-rich wastewater, the SRT is considered a crucial operational parameter, because it will not only determine the degree of scavenged LCFA and thus extent of lipid conversion, but it will also determine the resulting specific methanogenic activities (SMA) of the sludge. The accumulation of LCFA in the system is directly related to the SRT or cell residence time of the biomass with contradictory effects: (i) slow growing microorganisms, such as those involved in the biodegradation of LCFAs would benefit from a high residence time in the system increasing the opportunities for degrading such compounds and reducing their accumulation in the system (Alves et al., 2009); and (ii) the higher the SRTs, the higher the chances of accumulating LCFAs due to the reduced wastage of these compounds with the sludge waste. As a result, the SRT may significantly contribute to set the appropriate conditions for the accumulation or not of LCFAs in the system. Dereli et al., (2014) reported a severe LCFA inhibition on the biological performance and methanogenic activity when working at 50 days SRT when treating corn-to-ethanol thin stillage; this is the only research reported in the literature relating the effects of the SRT to the anaerobic lipid degradation and LCFA accumulation in an AnMBR system. However, this research was performed with a very specific industrial wastewater with a different LCFA profile (corn-to-ethanol thin stillage), compared to dairy wastewater (Alves et. al., 2009). Moreover, the main conclusions of that study, such as the formation of round shape fat precipitates (called fat balls by the authors) and the biological inhibition when operating

at high SRTs may be strictly related to that specific wastewater, making it very difficult to extrapolate such behavior to other types of wastewaters. Therefore, there is a need for a better understanding of the SRT effects on the biological performance of an AnMBR, fed with lipid-rich wastewater such as dairy wastewater. Our research directly addresses those needs.

The objective of this research was to evaluate the biological performance of an AnMBR treating synthetic (lipid-rich) dairy wastewater at different SRTs. In addition to assessing the overall performance of the anaerobic system, the impact of the presence and accumulation of LCFAs at different SRTs is evaluated.

3.2 MATERIALS AND METHODS

3.2.1 Synthetic wastewater

The synthetic dairy wastewater was prepared by diluting whole milk up to a COD and FOG concentration of approximately 10 g COD/L and 1.7 g FOG/L, respectively. Moreover, additional nutrients and micronutrients were added to the system (Zoutberg & de Been, 1997). The synthetic wastewater was prepared periodically (three times per week); the average wastewater composition for the entire evaluation is presented in Table 3.2.

Table 3.2 – Wastewater characterization

Parameter	Unit	Value
COD	g /L	10.1 ± 7.5
SCOD	g/ L	3.3 ± 0.7
FOG	g /L	1.7
TS	g /L	6.0 ± 0.4
VS	g/ L	5.4 ± 0.3
TSS	g /L	2.6 ± 0.5
VSS	g /L	2.7 ± 0.6
TKN	mg /L	273.5 ± 15.2
NH_3–N	mg/ L	94.3 ± 0.3
TP	mg /L	27.6 ± 0.4

3.2.2 Reactor setup

Two AnMBRs were operated, each with an effective volume of 10 L equipped with a full-scale length (3 m) cross-flow tubular PVDF ultrafiltration membrane (Pentair X-Flow, The Netherlands) with a surface area of 0.049 m^2 and a mean pore size of 0.03 µm. The reactor was gently mixed at 35 rpm by a top entry mechanical mixer and via sludge recirculation with a recirculation pump. The reactor was fed by a peristaltic pump (Watson-Marlow, 120U/DV) from the influent tank. The filtration membrane was operated at a cross-flow velocity of 1 m s^{-1} applying a feed cycle of 890 seconds filtration and 10 seconds backwash. The backwash was done by reversing the flow of the peristaltic

pump (Watson Marlow, 530S). Both reactors were double jacketed and a water bath was used to control the temperature at 35 ºC. The pH was kept constant at pH (7.0 ± 0.5) using a pH controller. The biogas production was measured by a biogas flow meter (Drum-type gas meter Ritter, Germany). The entire reactor systems were controlled by a programmable logic controller (PLC) and the transmembrane pressure (TMP) was monitored throughout the operational time. The membrane filtration unit was operated at a flux of 10 L/(h m^2). The cross-flow velocity was set to 0.5 m/s. The operational TMP averaged at 300 mbar and 400 mbar for reactors R20 and R40, respectively. Figure 3.1 shows the reactor set-up.

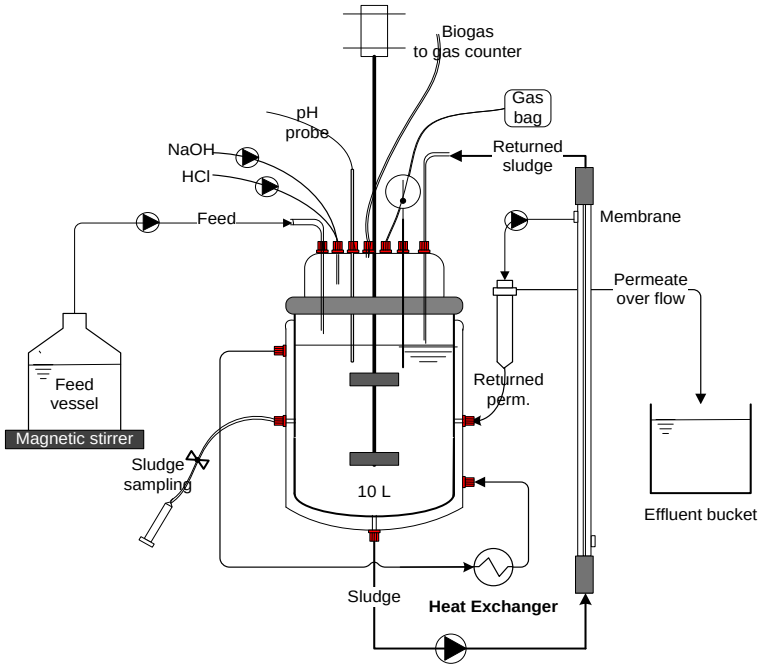

Figure 3.1 – Experimental set-up

3.2.3 Experimental procedures

The reactors were inoculated with crushed and sieved (600 µm mesh size) granular sludge from a full scale EGSB system (DSM; Delft, The Netherlands). Mesophilic conditions (35 ± 1) ºC were maintained. Both reactors were operated initially at an SRT of 30 days; the OLR was increased stepwise at 0.5 g COD /(Ld) every 5 days until reaching the targeted OLR of 4.7 g COD /(Ld). After 82 days of operation, the reactors were decoupled; hereafter, they were operated in parallel at different SRTs, i.e., 20 (R20) and 40 days (R40) for a period of 3 SRTs each. The reactors were operated at an HRT of 2.2 days. Once week analyses were performed on the feed and the sludge, whereas and on the effluent, three times a week the following parameters were assessed: total solids (TS), suspended solids (SS), total Kjeldahl nitrogen (TKN) and ammonium nitrogen, which were measured according to Standard Methods of APHA of 1998. In addition, chemical oxygen demand (COD) and soluble COD were measured with Hach-Lange test kits. The volatile

fatty acids (VFA) were analyzed by gas chromatography (GC, Varian 3900) equipped with a silica column (25 m and 0.53 mm internal diameter) and a flame ionization detector. Injector, column, and detector temperatures were 250, 140 and 275 ° C respectively. Lipid content of the feed and sludge were determined by the norm ISO 1443. The individual LCFA composition of sludge were measured according to Neves et al. (2005).

The specific methanogenic activity (SMA), using acetate, propionate and butyrate as the substrate was measured in sealed serum bottles (120 mL) by following the pressure increase with a pressure transducer (Centre Point Electronics PSI-30). The initial food mass ratio (F/M) of the tests was 1 gCOD/gVSS. The liquid volume of the bottles was 50 mL, and the biomass concentration was 2 gVSS/L. The anaerobic medium was prepared by dissolving sodium bicarbonate 3.5 g/L with tap water. The head space was flushed with a mixture of N_2:CO_2 (70:30%). The SMAs were carried out in batch tests using as substrates different volatile fatty acids (acetic, propionic, and butyric acid). Linear regression of the slope of the methane production curve was performed and expressed as mg CH4-COD/(g VSS d). The SMA experiments were performed every two weeks.

3.3 RESULTS

3.3.1 Operational performance

Both reactors were kept at an SRT of 30 days for the first 82 days of operation, denominated as the “coupled period”. In this phase the OLR was increased stepwise until reaching 3.5 g COD /(Ld) Afterwards both systems were decoupled and the OLR was increased up to (4.7 ± 0.7) g COD/(Ld) in R20 and (4.7 ± 0.8) gCOD/(Ld) in R40. As can be seen in Figure 3.2a, throughout the entire evaluation (coupled and decoupled period), the COD removal efficiency of both reactors was higher than 99%, (99.3 ± 0.3) % for R20 and (99.6 ± 0.2) % for R40 and remained constant until the end of the experiment. That is, the biological performance of the systems was similar for both reactors. The effluent COD concentration was on average (67 ± 17) mg COD/L in R20 and (54 ± 10) mg COD/L in R40 (Figure 3.2).

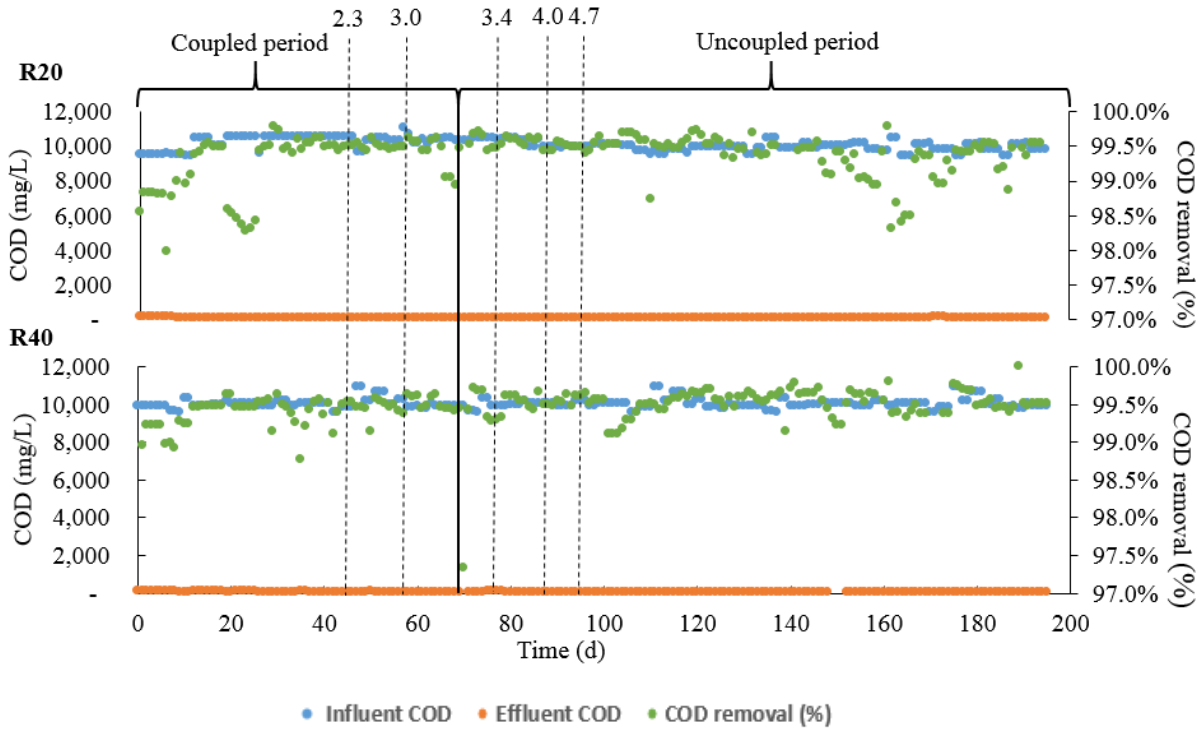

Figure 3.2 -Influent, effluent, and COD % removal throughout the operational time of R20 and R40 (dotted lines correspond to the OLR at the different stages).

The VFA concentration in the reactor/effluent is a good indicator of the anaerobic treatment performance; moreover, it can be used to monitor the activity of the acetogenic and methanogenic bacteria (Hanaki et. al., 1981; Wijekoon et. al., 2011). Figure 3.3 shows the effluent VFA concentration as a function of the operation time of the reactors. At the beginning, in the coupled phase, the VFA concentrations average values of 3.3 and 0.3 mg VFA-COD/L for the R20 and R40, respectively. Then, after the decoupled period and up to an OLR of 4.7 g COD/(Ld), the VFA concentration increased in both systems at average values of 14 mg VFA-COD/L and 3.7 mg VFA-COD/L for the R20 and R40 reactors, respectively. When both systems reached steady operational performance, at an OLR of 4.7 g COD/(Ld), the VFA concentrations in the effluent were 26 mg VFA-COD/ L and 3.1 mg COD/L for the R20 and R40 reactors, respectively. GC analysis showed that the VFA composition was acetate, propionate, and butyrate, with acetic and butyric acids being the major VFA constituents throughout the entire evaluation. As shown in Figure 3.3, an increase in the organic loading rate resulted only in a slight increase in the VFA concentration.

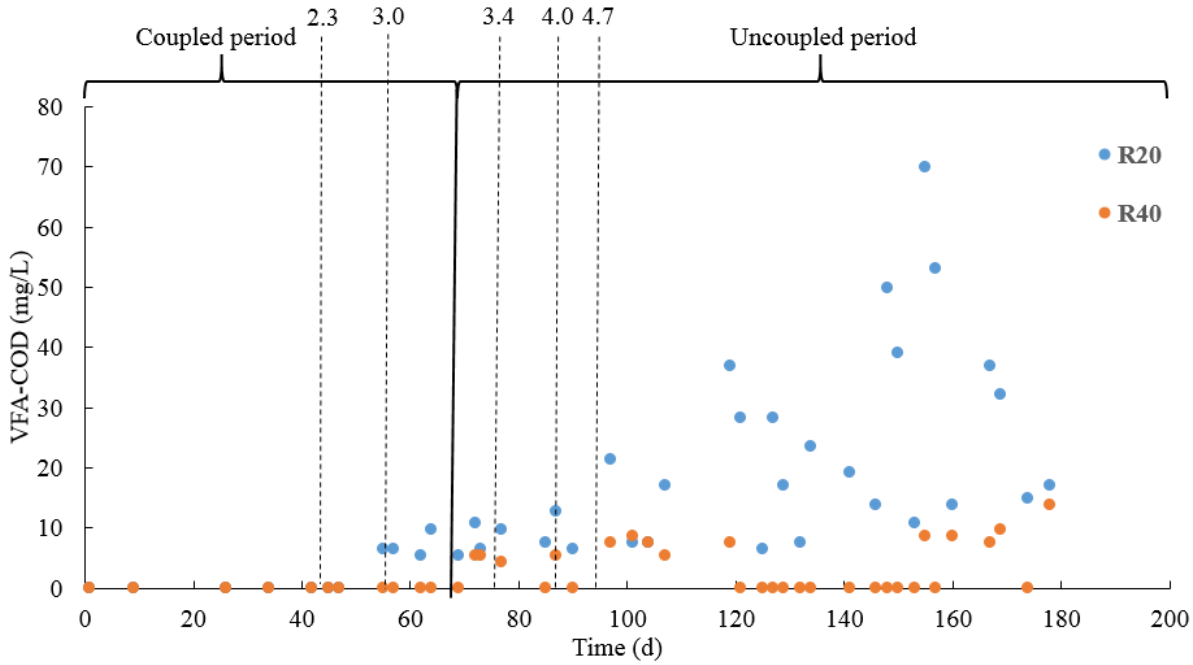

Figure 3.3 – VFA-COD effluent concentration over operational time

The specific methane production for the two reactors was on average 0.31 ± 0.02 NL CH_4 /(g COD removed) and 0.32 ± 0.02 NL CH_4 /(g COD removed) for R20 and R40 reactors, respectively. These values are lower than the maximum stoichiometric amount that could be obtained, i.e. 0.35 NL CH_4/(gCOD removed). The small difference might be attributed to biomass growth (anabolic COD uptake) and some non-biodegraded COD that accumulates in the sludge.

3.3.2 COD mass balance analysis

The COD mass balance in both reactors showed negligible differences of 0.4% and 1.1% for the R20 and R40 reactors, respectively as shown in Figure 3.4 and Table 3.3. Dereli et al. (2014) reported differences on the COD mass balance which were larger at shorter SRTs. They described the formation of aggregates in the sludge, described as LCFAs clumps (denominated 'fat balls' by the authors), that accumulated in the reactor at an SRT of 20 days and to lesser extent 30 days. At 50 days SRT these clumps were absent. Those particular sort of fat balls or LCFA clumps were not observed in our research.

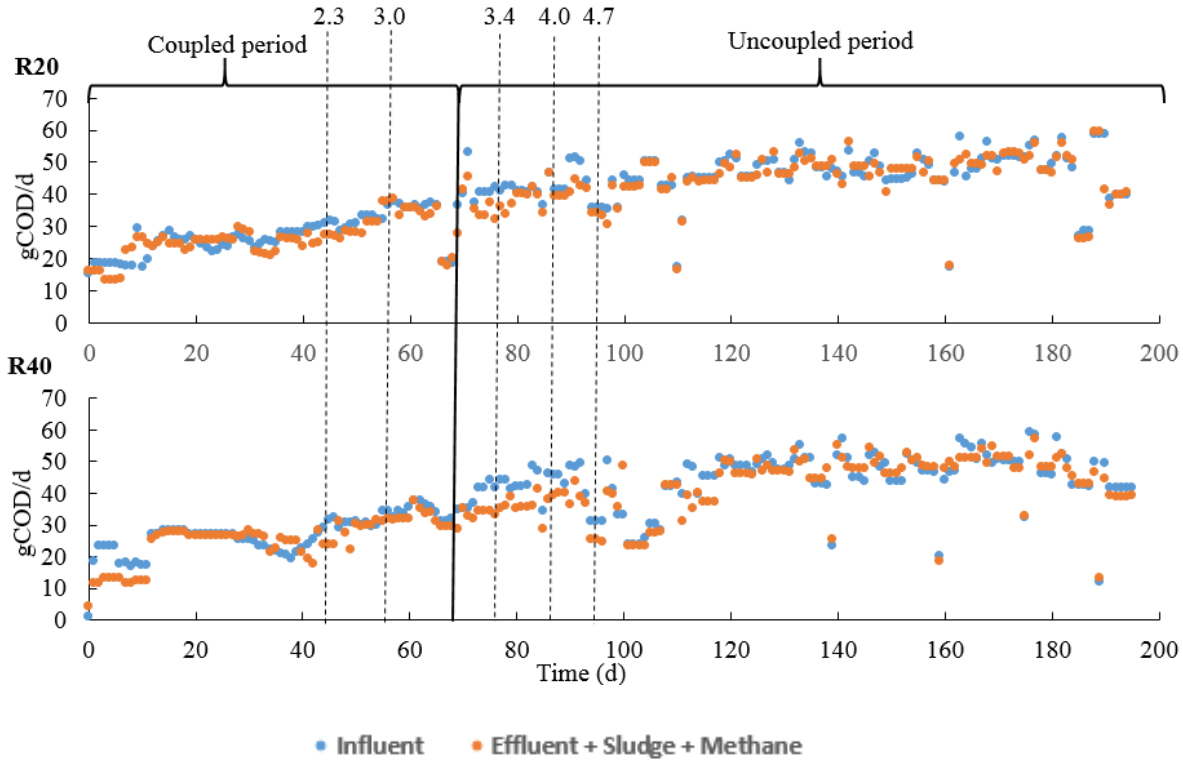

Figure 3.4 – COD mass balance

Table 3.3 – COD balance average at steady state

	R20		R40	
	g COD/ d	%	g COD /d	%
Influent	47 ± 7		47 ± 9	
Effluent	0.3 ± 0.2	0.7	0.2 ± 0.1	0.5
Sludge	5.2 ± 0.4	11.0	4 ± 0.1	8.6
Methane	42 ± 7	87.9	42 ± 8	89.8
Total		**99.6**		**98.9**

The biogas production of both reactors produced under steady conditions was very similar, i.e. (15 ± 2) NL CH_4 /d and (16 ± 1) NL CH_4 /d for the R20 and R40 reactors, respectively.

3.3.3 Total suspended solid concentration

The total suspended solid concentration (TSS) was monitored throughout the operation of the reactors. As shown in Figure 3.5, the TSS concentration decreased at the beginning of the experiment for both reactors. Throughout the coupling period, when both reactors were kept at an SRT of 30 days, the TSS concentration was constant at (7.5 ± 0.5) g TSS/L and (7.6 ± 0.3) g TSS/L for R20 and R40, respectively. When both systems were decoupled and after reaching stable operation, the TSS concentration was constant at (6.8 ± 0.2) g TSS /L and (12.4 ± 0.4) g TSS/L for R20 and R40, respectively, until the end of

the operational period. With respect to the VSS to TSS ratios, similar values were reported for both reactors of (0.93 ± 0.04) and (0.90 ± 0.02) for R20 and R40, respectively.

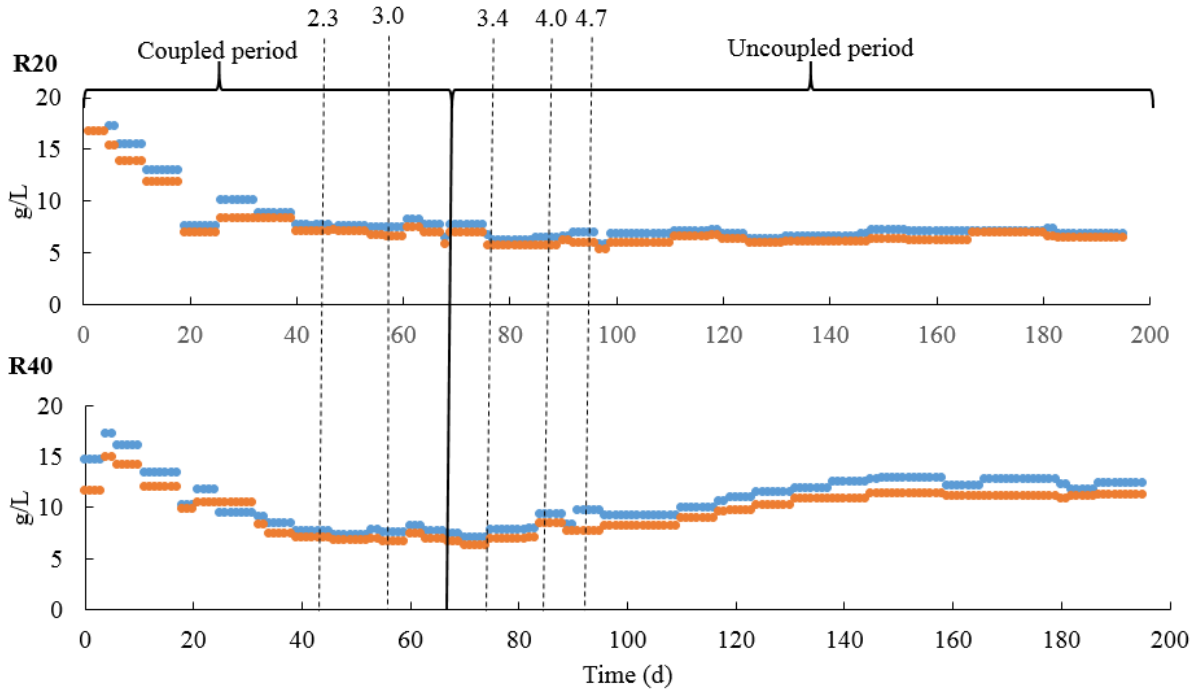

Figure 3.5 – Suspended solids concentration of both systems throughout operational time

3.3.4 Sludge lipid concentration

The lipid concentration of the sludge was determined on operational days 135 and 195 on both reactors to evaluate the potential lipid accumulation in the system; the results are presented in Table 3.4. The VSS specific lipid loading rates for the two reactors were calculated from the FOG concentration in the influent, the HRT, and the VSS concentration in each reactor for the R20 and R40 reactors; the values obtained were 0.13 ± 0.01 g lipid/ (g VSS d) and 0.073 ± 0.002 g lipid/ (g VSS d), respectively. The VSS specific lipid loading rates were relatively high, but similar to the values reported for instance by Dereli et al., (2014), i.e. 0.10 – 0.04 g lipid/ (g VSS d). Considering that the influent lipid load (g lipid/ d) to the reactors was the same for both reactors (R20 and R40) and that the VSS concentration was much higher for the R40 reactor, the R40 sludge experienced a lower VSS specific lipid loading rate.

Table 3.4 – Lipid content of the sludge per 100 g of mixed liquor (ML)

Lipid concentration	**g lipid (100 g ML)$^{-1}$**		**g lipid (g VSS)$^{-1}$**	
Operational day	**R20**	**R40**	**R20**	**R40**
132	<0.10	0.22	<0.16	0.20
195	0.10	0.15	0.16	0.13

3.3.5 Long-chain fatty acid analysis in the sludge

In both reactors the LCFA in the sludge were measured at the end of the operational period to determine a possible LCFA accumulation inside the reactors. Table 3.5 shows the detailed LCFA-COD composition determined in each reactor expressed per amount of mixed liquor (ML) and per gram of VSS in each reactor. In R40 the absolute concentrations of all LCFAs were higher than in R20 when reported as mg LCFA/(gML). However, when reported per gram of VSS, lower LCFAs concentration for R40 were compared to R20, as shown in the Table 3.5.

The most abundant LCFA types in both systems were oleic acid, i.e., 37% and 23% of the total LCFA for R40 and R20, respectively, and palmitic acid, i.e., 41% and 35% of the total LCFA for R40 and R20, respectively. The third most abundant LCFA was myristic acid, with a percentage of 19% and 13% in R40 and R20, respectively.

Table 3.5 – LCFA composition in the system expressed per amount of mixed liquor (ML) (results obtained from the 195 operational day)

LCFA concentration	**R20**		**R40**	
	mg LCFA-COD /(g ML)	mg LCFA /(g VSS)	mg LCFA-COD / (g ML)	mg LCFA /(g VSS)
Lauric acid (C12:0)	0.075	4.111	0.039	1.254
Myristic acid (C14:0)	0.370	20.398	0.547	17.115
Myristoleic acid (C14:1)	0.178	9.804	0.336	10.484
Pentadecanoic acid (C15:0)	0.023	1.265	0.043	1.344
Cis-10-Pentadecanoic acid (C15:1)	0.020	1.107	0.052	1.613
Palmitic acid (C16:0)	0.999	55.028	1.180	36.828
Palmitoleic acid (C16:1)	0.055	3.004	0.077	2.419
Stearic acid (C18:0)	0.212	11.701	0.194	6.093
Oleic acid (C18:1)	0.657	36.211	1.059	33.065
Vaccenic acid (C18:1)	0.066	3.637	0.090	2.867
Linoleic acid (C18:2)	0.026	1.423	0.047	1.523
Total LCFA **mg LCFA-COD /(g ML)**	**2.68**		**3.66**	

mg LCFA-COD /(g VSS)	**147.69**	**114.61**

3.3.6 Activity of the sludge

Throughout the entire operational period, the biomass activity was monitored for the two reactors by determining the SMA tests (Figure 3.6).

A decrease in the sludge activity was observed for both reactors, following the same trend. At the end of the operation of both reactors, the methanogenic activities on acetate, propionate, and butyrate decreased 26%, 77%, 50% for R20 and 46%, 13% and 14% for R40, showing a slightly higher decrease in the sludge activity on R20 compared to R40.

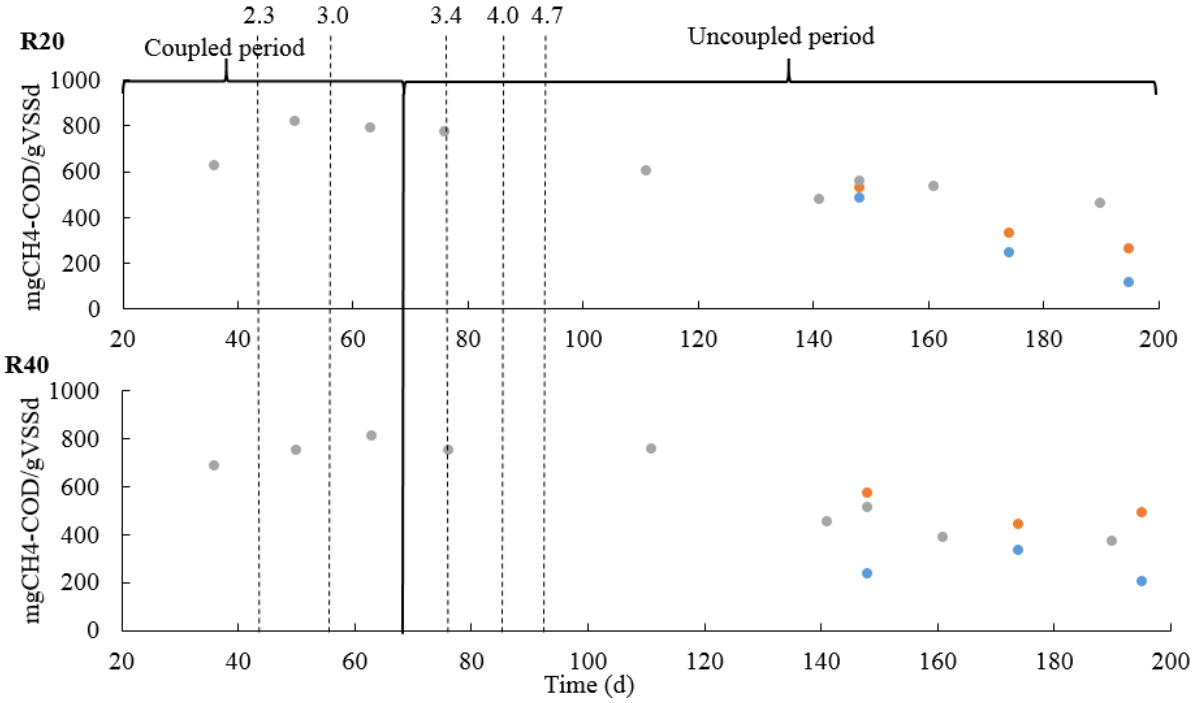

Figure 3.6 – Specific methanogenic activity for different VFA as function of the operational time of the reactors

3.4 Discussion

Both reactors operating at different sludge retention times were characterized by a stable operation indicated by both an organic matter removal of more than 99%, and by a stable biogas production; these performances were much better when compared to other studies on AnMBR treating other types of wastewater (Dereli et. al, 2014), and to other high-rate anaerobic wastewater treatment (HRAWT) systems (Alves et. al., 2009). Dairy industrial

wastewater is complex to treat using sludge bed systems or and other HRAWT systems; the presence of fats in the wastewater induces sludge flotation and washout (Perle et. al., 1195). Hawkes et al. (1995) studied the performance of a pilot scale UASB reactor treating ice-cream wastewater (lipid rich wastewater) at an OLR of 2 g COD/(Ld). The UASB system showed a poor performance with only 50% COD removal efficiency, mainly due to an unsuccessful granulation of the biomass in the system. Moreover, in the study of Rinzema et al. (1993) complete sludge flotation was reported when treating lipid rich wastewater (a solution of capric and lauric acid) with a UASB reactor. Apparently, the AnMBR may present a good alternative to treat such complex wastewater, considering that the membrane physical barrier prevents the floating sludge to be washed out of the system. Moreover, several studies treating lipid rich wastewater using HRAWT (Sayed et. al., 1987; Petruy et al., 1997) reported lipid adsorption onto the sludge surface exhibiting mass transfer limitation; therefore, reducing the conversion rate to methane. In AnMBRs, the sludge is fully suspended and thus characterized by a very high surface area. Therefore, the lipids remain in the mixed liquor fully available to the microorganisms to be converted into methane. In addition, a higher effluent quality is obtained when working with an AnMBR, i.e., very low organic matter concentrations and free of suspended solids, compared to the effluent quality obtained with other HRAWT systems (Seghezzo et. al., 1998). Such high effluent quality may introduce possibilities for water reclamation (Ersahin et. al., 2011)

The COD mass balance fits very well for both SRTs applied, i.e., 99.6% in R20 and 98.9% in R40, and the potential precipitation of lipids forming the so-called fat balls (Dereli et. al. 2014) was not identified throughout the operational time. The latter indicates that the lipids were entirely available for anaerobic degradation. Effluent VFA concentration were slightly higher for R20 compared to R40. When both systems reached steady operational conditions at an OLR of 4.7 g COD/(Ld), the VFA concentrations in the effluent were 26 mg VFA-COD/ L (16 mg acetate/L, 3 mg propionate/L) and 3.1 mg COD/L (2 mg acetate/L) for the R20 and R40 reactors, respectively. That is, the reactors properly adapted to the OLR increase in a relatively short period of time. Nonetheless, a slightly better biological performance was observed for the R40 reactor, which might be attributed to the higher biomass concentration. Overall, the observed effluent VFA values in this study for both reactors were very similar and were much lower compared to the values reported for failing anaerobic reactors, i.e., 800 mg/ L for acetic acid, propionic to acetic acid ratio 1.4, and butyric acid 5 mg/L (Hill et. al., 1988).

Slightly higher digestion efficiencies were obtained at 40 days SRT compared to 20 days SRT. This is in accordance with reported values in the literature (Huang et. al., 2013). Higher biomass concentrations resulted in a slightly higher biodegradability. Moreover, a better effluent quality, a more stable performance, and more biogas production was obtained when working at high SRT. Also, the higher the SRT as in the case of the R40 reactor, the lower the sludge wastage. In fact, the degree of sludge stabilization increases

with the applied SRT, leading to a reduction in the sludge treatment and management costs. The application of longer SRTs, such as in the study of Dereli et al. (2014) who operated the AnMBR at an SRT of 50 days, resulted in a lower applicable OLR and therefore a higher HRT, compared to applied SRTs of 20 and 30 days. In that study the worst performance was observed at an SRT of 50 days. The authors explained the better performance at the low SRTs by the formation of LCFA precipitates with cations forming fat balls, which has not been the case in our study. Very likely, by the formation of LCFA precipitates, less direct contact is experienced between LCFA and methanogenic biomass.

The applicable OLR and HRT in AnMBRs treating LCFA-rich wastewater depend on the achievable SRT and methanogenic activity of the sludge (Dereli et al., 2012). Literature data reveal that the applied HRT in AnMBRs treating lipid rich wastewater varies from 0.2 – 11 days (Dereli et. al., 2014; Christian et. al., 2011; Al-Malack et. al., 2016; Ramos et. al., 2014), all of them with COD removal efficiencies exceeding 95%. These results agree with our present results that show applicable HRTs of 2.2 days. Lipid hydrolysis proceeds relatively fast, whereas LCFA oxidation is known to be the rate limiting step in the anaerobic digestion of lipids (Masse et. al., 2002). This mismatch will result in the accumulation of LCFA in the reactor, possibly leading to perturbations. Morris et al. 1998 treated slaughterhouse wastewater (lipid rich wastewater) in an anaerobic sequential batch reactor with HRTs ranging from 0.75 to 1.5 days with a SCOD removal of 90%. When lowering the HRT, the TCOD removal decreased due to sludge flotation. The latter is a frequently observed problem in sludge bed reactors but is not apparent in AnMBRs due to the presence of an absolute membrane barrier. In our current research, results showed an excellent AnMBR performance applying OLRs and HRTs in a range similar to the discussed literature data. A further increase in OLR and/or drop in HRT is part of future studies. Taking into consideration the sludge lipid concentration, after 132 days of operation, R40 showed a higher VSS specific lipid concentration (0.20 g lipid /(gVSS)) than R20 (< 0.16 g lipid (g VSS)$^{-1}$). Possibly, the biomass in R40 was still not fully adapted for efficient lipids or LCFA conversion (Palatsi et. al., 2010). However, after 195 days of operation, R40 showed a lower VSS specific lipid concentration (0.13 g lipid/ (gVSS)) than R20 (0.16 g lipid /(gVSS)).

Regarding the LCFA profile for both reactors, palmitic and myristic acid LCFAs showed the highest concentrations. Our observations agree with the research of Lalman and Bagley (2000), who reported that palmitic acid (C16) and myristic acid (C14) are intermediates in the degradation of oleic and linoleic acids (C18). In addition, the oleic concentration was relatively high in both reactors, being higher in the R40 reactor than in the R20, which would be an indicator of an accumulation of oleic acid in the system. Oleic acid is an unsaturated LCFAs, which is considered more inhibitory for methanogens than the saturated LCFAs (Lalman and Bagley, 2000). However, apart from a slight decrease in the SMA as explained below, our study showed no significant signs of inhibition regarding the biological operation in none of the reactors. The total LCFA that

accumulated in both reactors was 2.7 and 3.7 mg LCFA-COD /(g ML) for R20 and R40, respectively. These values were much lower than the ones reported in the literature, with values of 62, 48 and 61 mg LCFA-COD /(g ML) for 20, 30 and 50 days SRT (Dereli et. al., 2014) at a similar influent lipid concentration of 1.7 g FOG/ L. According to Pereira et al., (2005), the inhibition of LCFA can be reversible between 1000 and 5000 mg LCFA-COD /(gVSS); which are much higher values compared to the values obtained in our study, i.e. 147.69 and 114.61 mg LCFA-COD /(g VSS) for R20 and R40, respectively. Very likely, the lack of mass transfer resistance in AnMBR systems results in an efficient LCFA conversion. Even though the reactor R40 was wasting less amount of lipids, the ratio LCFA-COD/VSS was the same or even lower compared to the reactor R20. Considering that the overall performance of both reactors was more or less similar, working at the highest SRT values is preferred as it add some additional advantages such as less LCFA accumulation.

According to Brockman and Seyfried (1996), one factor to consider when operating a cross-flow AnMBR is the loss of sludge activity due to the disruption of the syntrophic association between the acidogenic and the methanogenic bacteria. It has been reported that high cross-flow velocities may exert a negative effect on microbial activity and cause a disruption of syntrophic associations operating a submerged AnMBR (Lin et. al., 2009) Moreover, the use of peristaltic cross-flow pumps in lab set-ups could result in a sludge milling effect, also contributing to the destruction of the syntrophic relationships between the different trophic microbial groups. However, in the study of Jeison et al. 2009 no severe negative effect was found of the cross-flow induced shear rate on the acetogenic and methanogenic sludge activity. Results thus far, does not show evidence that the sludge methanogenic activity is negatively impacted by the applied cross-flow operation.

The results obtained from the sludge activity tests, showed a decrease in the SMA, even though the LCFA concentration measured in both reactors was lower than reported in previous studies (Alves et. al., 2005; Dereli et. al. 2014). For the R20 sludge, the highest SMA was found when using acetate as the sole substrate; whereas for the R40 sludge, the highest SMA was obtained with butyrate as the sole substrate. Results indicate an effect of the applied SRT on SMA development and/or sludge composition. However, insufficient data hampers a clear interpretation of these findings. SMA development over time is in accordance with the study performed by Dereli et al. (2014). The inhibitory effect of LCFA on methanogenic and acetogenic microorganisms has been reported before (Alves et. al., 2009) According to Pereira et al., (2005) the accumulation of LCFA in the system can lead to steric hindrance, or mass transport limitation, during substrate uptake leading to an SMA decrease. However, in our here-described studies, the decrease in the SMA was comparable for both reactors, so the applied SRT was apparently not discriminative. Regardless the results obtained concerning the SMA decrease, there was no sign of reactor perturbation, deterioration of biogas production, nor an increase in the effluent COD concentration. In addition, Vidal et al. (2000) reported SMA enhancement

in the presence of lipids when butyrate was used as the co-substrate. This observation could also explain the higher SMA using butyrate as the substrate and the slightly better conversion of lipids in reactor R40. Figure 3.6 depicts a continuous decrease in SMA of the R20 sludge throughout the operational period when compared to the R40 sludge, which remained relatively stable. These results corroborate with the slightly higher lipid/VSS ratio found in the R20 sludge, due to the LCFA accumulation inside the system, which can negatively impact the sludge SMA (Alves et. al., 2009). For the R20 sludge, the SMA continued to drop, so it would be advisable to operate the system for a prolonged period of time in order to investigate whether a further drop will be experienced or an SMA stabilization at a lower level.

Considering the obtained results, it would be advisable to operate the AnMBR at an SRT of 40 days when treating lipid-rich dairy industrial wastewater: the sludge wastage can be minimized reducing the operational costs, and both the biogas production as well as the water quality of the treated effluent can be maximized.

3.5 CONCLUSIONS

- Lipid rich wastewater simulating milk processing industry wastewater with a lipid concentration of 1.7 g FOG/ L was successfully treated in an AnMBR at different SRTs (20 and 40 days) with a stable performance regarding biogas production and COD removal efficiency during the operational time

- COD removal efficiencies over 99% and digestion efficiencies from 84% to 89% were obtained at an operational OLR of 4.7 g COD /L d and an SRT of 20 and 40 days. The VFA concentration remained low in both systems (26 mg VFA-COD/ L and 3.1 mg VFA-COD /L for the R20 and R40 reactors, respectively).

- After 195 days of operation, R40 showed lower lipid concentration (0.13 g lipid /(gVSS)) than R20 (0.16 g lipid /(g VSS)). The biomass seemed better adapted to lipids at high SRT.

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4

INFLUENCE OF SLUDGE RETENTION TIME ON MEMBRANE FOULING IN AN ANBR TREATING LIPID-RICH WASTEWATER

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ABSTRACT

This study evaluated the effects of the sludge retention time (SRT) on the membrane filtration performance of an anaerobic membrane bioreactor (AnMBR) fed lipid-rich synthetic dairy wastewater. The membrane filtration performance was evaluated in two AnMBR systems operated at two different SRTs, i.e., 20 and 40 days. The AnMBR operated at 40 days SRT exhibited the worse membrane filtration performance characterized by operational transmembrane pressures (TMP) exceeding the maximum allowed value and high total resistances to filtration (R_{total}). The sludge in the two reactors evaluated at the different SRTs showed similar sludge filterability properties. However, the sludge in the reactor operated at 40 days SRT was characterized by exhibiting the highest concentrations of: i) total suspended solids (TSS), ii) small-sized particles, iii) extracellular polymeric substances (EPS), iv) soluble microbial products (SMP), v) fats oils and grease (FOG), and vi) long chain fatty acids (LCFA). The cake layer resistance was the major contributor to the overall resistance to filtration. The high TSS concentration observed in the AnMBR systems apparently contributed to a less permeable cake layer introducing a negative effect on the membrane filtration performance. The lowest the SRT the better the membrane filtration performance. However, optimizing the membrane filtration operation (i.e., cleaning strategy, cross-flow velocities, and duration of cycles, among others) may eventually contribute to operate the AnMBR system at higher SRT values and TSS concentrations without negatively affecting the membrane filtration performance.

4.1 INTRODUCTION

Dairy industry wastewater is characterized by high concentrations of organic matter, suspended solids, and fat, oil, and grease (FOG) compounds (Alves et. al., 2009; Lin et. al., 2012). In addition, it contains a high concentration of lipids (Berube et. al., 2006; Cavaleiro et. al., 2008). Anaerobic wastewater treatment is a suitable option for such wastewaters, considering their capacity for processing high organic-loading rates at very low sludge production rates, while at the same time producing biogas (a gaseous energy carrier) (Berube et. al., 2006). The high FOG content in dairy wastewater can introduce operational limitations to the performance of conventional anaerobic wastewater treatment systems including floating sludge and granules disintegration, among others (Alves et. al., 2009). However, those operational challenges eventually can be overcome by using membrane filtration as the solids-liquid separation process.

An anaerobic membrane bioreactor (AnMBR) combines the advantages of anaerobic biological processes with membrane filtration. AnMBR systems provide a complete retention of the biomass and allow for slow-growing microorganisms to be retained in the system when long sludge retention times (SRTs) are applied. Moreover, AnMBRs are characterized by a high treatment performance with high organic matter removal efficiencies exceeding 98% (Cavaleiro et. al., 2008). In addition, the treated effluent has an excellent quality, i.e., low organic matter concentration and free of suspended solids, which is ideal for water reclamation applications (Pacheco-Ruiz et. al., 2017). Despite these advantages, the application of full-scale AnMBR systems is still limited due to their elevated capital and operational costs. AnMBR systems require additional equipment to operate, compared to conventional systems, such as, ultra-filtration membranes, pressure and level sensors, and chemicals for cleaning in place, among others. Moreover, the filtration process can be prone to membrane fouling, either increasing the trans-membrane pressure (TMP) of the filtration system for a given flowrate (or membrane flux) or decreasing the resulting flux for a given TMP. Thus, sustaining a certain flux and/or TMP over time requires intensive preventive and corrective membrane maintenance interventions. Despite the considerable decrease in the membrane costs in the past decades (Dereli et. al., 2015) the attainable membrane permeability, i.e., the permeate flux per unit of membrane area and applied TMP, remains the most important operational factor with a high impact on the overall capital and operational costs of AnMBR systems (Lin et. al., 2012).

Long chain fatty acids (LCFAs), i.e., the hydrolysis products of triacyl lipids, may have a negative impact on the anaerobic biomass activity. Such compounds may be adsorbed onto the biomass, eventually leading to mass transfer limitations and to sludge flotation (Alves et. al. 2009). Moreover, these compounds may also affect the membrane filtration performance on AnMBR systems (Berube et. al., 2006; Cavaleiro et. al., 2008). Only a

few studies have reported the effects of the presence of lipids and LCFA on the sludge filterability and membrane filtration in AnMBRs. Dereli et al. (2014, 2015) evaluated the filtration performance of an AnMBR treating corn-based bioethanol thin stillage wastewater (high lipids content) at different SRTs. The best membrane filtration performance was obtained at the lowest evaluated SRT of 20 days, which was attributed to the lower accumulation of lipids on the anaerobic biomass and less fine particles in the reactor. At 20 says SRT, the LCFA precipitated as fat balls and were manually scooped off from the liquid bulk Dereli et. al., 2015). Diez et al. (2012) evaluated the treatment performance of a submerged AnMBR treating a lipid-rich wastewater from a snack factory, FOG content ranging from 4.4 to 6 g/L, aiming at determining the optimum operational membrane flux and associated membrane chemical cleaning regimes. The authors reported relatively low long-term optimum operational fluxes ranging from 6.6 to 8 L/(m^2 h) at an optimum filtration cicle of 11 min, including 10 sec of pre-relaxation, 20 sec of backwash and 70 sec of post-relaxation. The addition of cleaning interventions with chemicals products and air scouring increased the membrane filtration performance. Finally, Carta-Escobar (2005) evaluated the membrane performance of an aerobic MBR system, treating lipid-rich dairy wastewater at organic loading rates from 0.24 to 0.7 g COD/ (Ld). The authors reported severe membrane fouling associated with the production of a viscous film on the surface of the membranes. There have been a few attempts to evaluate the overall impact of lipid-rich wastewater on the membrane filtration performance and membrane fouling. Most of these studies reported a reduced membrane filtration performance when high concentrations of lipids were present in the influent wastewater.

The effects of specific sludge characteristics on the membrane filtration performance and membrane fouling only have been partially reported as follows. The present description includes the most relevant literature on this issue reported over the past years. Suspended solids can accumulate on the membrane surface forming a cake layer, contributing to add resistance to the membrane filtration process. Besides, several studies have reported on the negative effects of sticky substances secreted by the microbial biomass, i.e., extracellular polymeric substances (EPS) and soluble microbial products (SMP), on the membrane filtration performance (Chang et. al., 2001; Le-clech et. al., 2006). In addition, other sludge characteristics, such as the particle size distribution (PSD), surface charge, and hydrophobicity can be linked to membrane permeability and membrane fouling (Meng et al., 2006, 2009, 2017). For instance, previous studies reported strong correlations between the PSD of the sludge and membrane fouling (Le-Clech et. al., 2006). The smaller the size of the particles present in the sludge mixture, the larger the impact on membrane fouling. It has been reported that the colloidal material can exert a significant role in membrane fouling by blocking the membrane pores (Chang et. al., 2001). Particularly, in side-stream membrane filtration, the cross-flow velocity (CFV) is a key parameter applied to control cake layer formation on the surface of the membrane

by creating a high shear stress on the sludge-membrane interface. However, such shear stress also contributes to breaking up the flocs, shifting the PSD to smaller sizes (Lousada-Ferreira et. al., 2015); thus, enhancing membrane fouling. In addition, floc disruption may also cause the release of EPS and SMP, also resulting in an increased membrane fouling (Jeison & van Lier, 2006)

There are many constituents present in the influent wastewater (e.g., LCFAs) which may influence the membrane filtration performance. In addition, sludge characteristics like the total suspended solids (TSS) concentration and PSD of suspended solids, as well as the dynamic viscosity of the sludge mixture, and the presence and concentrations of EPS and SMP, amongst others, would also influence the membrane filtration performance. However, these sludge physicochemical properties can be strongly influenced by the overall process conditions in the AnMBR including: the applied organic-loading rate, hydraulic retention time (HRT), SRT, membrane operation cycles (filtration-backwash-relaxation cycles), and membrane cross flow velocity, amongst others (Meng et. al, 209,2017; Lousada-Ferreira et. al, 2015). Eventually, the SRT is regarded as one of the most important operational parameters affecting both the biological processes and the sludge characteristics in an AnMBR system. High operational SRTs would result in high biomass concentrations inside the reactor, and possibly high biogas production rates (Dereli et. al., 2015). However, high SRTs coincide with low sludge wastage, which may lead to the accumulation of substances such as, lipids and LCFAs, inert matter, cell debris (Liao et. al., 2006) SMP and EPS, with a potential negative impact on the membrane filtration performance (Delrue et. al., 2011; Drews, 2010).

The membrane filtration performance of an AnMBR system strongly depends on the interaction between the influent wastewater characteristics, the sludge properties, and the process operational conditions. Both the process operational conditions and the influent wastewater composition, determine the sludge physicochemical properties and thus the membrane filtration performance. There is a gap in the literature on assessing the membrane filtration performance of an AnMBR when treating lipid-rich wastewater (such as dairy wastewater), considering the effects of selected process operational conditions like SRT, which had been reported one of the most important parameters in AnMBRs (Dereli et. al., 2015). The aim of this research was to evaluate the membrane filtration performance when treating lipid-rich wastewater from a dairy industry in an AnMBR at different SRTs. The effects of the dairy influent wastewater and operational SRTs on the sludge characteristics, i.e., TSS, dynamic viscosity, PSD, CST, SRF, SF, EPS, and SMP, were determined, and the impact of the sludge features on the membrane filtration performance, i.e., total resistance to filtration, flux decline, and TMP, was assessed. Other AnMBR operational parameters such as the HRT, temperature, and organic loading rate (OLR), among others remained unmodified; thus, only the effects of SRT on the membrane filtration performance could be assessed. SRT values of 20 and 40

days were chosen following the recommendations from the AnMBR manufacturer (Biothane, Veolia Water Technologies).

4.2 MATERIALS AND METHODS

4.2.1 Experimental design

The membrane filtration performance of two 10 L laboratory-scale AnMBR systems was evaluated. One reactor was operated at 20 days SRT, while the other was operated at 40 days SRT. Both reactors were fed with synthetic dairy wastewater. The OLR was gradually increased until reaching an OLR of 4.7 ± 0.8 g /(L d)for both reactors. The reactors were operated for 189 days. The impact of the sludge properties, i.e., TSS, dynamic viscosity, PSD, CST, SRF, SF, EPS, and SMP, on the membrane filtration performance was determined. The membrane filtration performance was assessed by measuring the flux decline and TMP, and by determining the total resistance to filtration defined as the ratio between the TMP and the dynamic viscosity and membrane flux.

4.2.2 Experimental setup

The experimental setup consisted of two AnMBRs. Each system consisted of a continuously mixed 10 L jacketed glass reactor with a working volume of approximately 10 L, equipped with PVDF cross-flow tubular ultrafiltration membranes (Pentair X-Flow, X-Flow BV, Enschede, The Netherlands). Each membrane had a surface area of 0.049 m^2. A PLC system was included to monitor and control the operation of the reactors. The TMP in the two systems was monitored by incorporating pressure transmitters (Series 33X, KELLER, Switzerland). The setups were provided with a permeate collection tank of 5 L made of jacketed glass. The influent wastewater was added to the AnMBR systems by a peristaltic pump (Watson-Marlow 1200s, Thermo Fisher Scientific, Sweden) from a feed vessel of 20 L. The vessel was continuously mixed using a magnetic stirrer (IKA, RCT basic, KA®-Werke GmbH & Co. KG, Deutschland). The sludge was recirculated through the membrane by a peristaltic pump (Watson-Marlow 520s, Thermo Fisher Scientific, Sweden).

The permeate was extracted by a peristaltic pump (Watson-Marlow 120s, Thermo Fisher Scientific, Sweden). The pH was controlled by the provision of a pH electrode (Model sc1000 Controller, HACH Company, Colorado, US) and two peristaltic pumps (Watson-Marlow 1200s, Thermo Fisher Scientific, Sweden) for acid and base addition. The temperature in the AnMBR system was measured by a temperature sensor Pt1000 (Metrohm, Barendrecht, The Netherlands), and controlled by using a recirculating water bath (Thermo Haake DC 10, Thermo Fischer Scientific, Sweden). The amount of biogas produced by the AnMBR system was measured with a drum-type gas meter (Ritter, Boshum, Germany). Sample ports were provided to regularly monitor and control the

sludge characteristics in the reactor. Figure 4.1 shows a schematic set-up of the AnMBR systems.

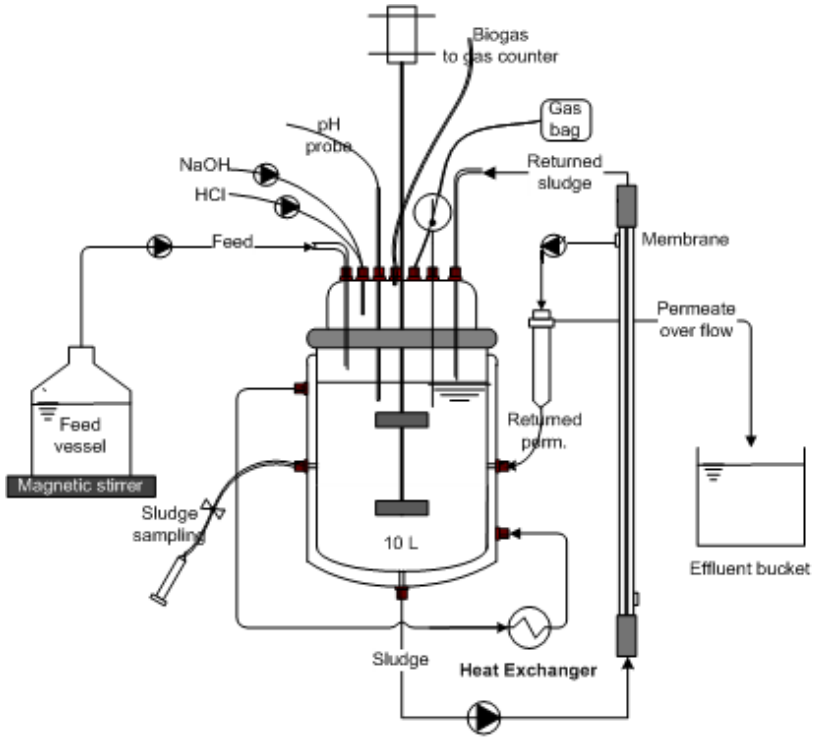

Figure 4.1 – AnMBRs experimental set-up

4.2.3 Experimental procedure

Both AnMBR systems were inoculated with crushed sieved (600 μm mesh size) granular sludge from a full-scale expanded granular sludge bed (EGSB) reactor (DSM; Delft, The Netherlands). The AnMBR systems were operated under mesophilic conditions (35 ± 1^{o} C). Both systems were fed with synthetic dairy wastewater by diluting 1/10th of whole milk. Additionally, nutrients and micronutrients were added to the system following the recipe of Zoutberg & de Been, (1997). The synthetic influent wastewater exhibited the following average characteristics: 9,988 ± 595 mg COD/ L; 1,760 mg FOG/ L with 2.87 g COD/g FOG; 2,089 ± 884 mg TSS/L; 170 ± 65 mg NH_4^+-N /L, and pH 5.8. Approximately 50% of the total COD contained in the wastewater were lipids.

During the first 69 operational days, both reactors were operated at an SRT of 30 days by wasting sludge at 330 mL/ d. This period is further to as the coupled period. Hereafter, one of the reactors was operated at an SRT of 20 days (R20) by wasting 500 mL sludge /d, and the other was operated at an SRT of 40 days (R40) by wasting 250 mL/ d. The latter period is further referred to as the uncoupled period. The OLR was gradually increased by steps of 0.5 g /(L d) every 5 days, increasing the influent flowrate with steps

of 0.5 L /d until an OLR of 4.7 ± 0.8 g /(L d) was reached for both systems. Once the final OLR was reached, the systems were operated at an influent flowrate of 4.7 ± 0.6 L /d, resulting in an HRT of 2.1 days for each reactor.

The sludge was entering the cross-flow membrane system at a flowrate of 38 L/h (912 L/d) to set a membrane cross flow velocity of 0.5 m/s. Every 890 seconds of membrane filtration, a 10 second backwash was performed. A full-scale filtration membrane module was used to simulate full-scale operational conditions. Consequently, the provided membrane area was much larger than the required membrane area to process the influent wastewater flowrate at the target operational fluxes. Therefore, to operate at the desired fluxes while sustaining a constant level in the AnMBR vessel, a fraction of the permeate was directed to a 5L permeate collection tank, and from there recirculated to the AnMBR. The remaining permeate fraction (equal to the influent wastewater flowrate) was taken out of the system via the 5L permeate tank using a peristaltic pump. The AnMBR systems were started at an operational flux of 2.5 L/(m^2 h). After one week of operation, the flux was increased to 5 L/(m^2 h); subsequently, the flux was increased by a 5 L/(m^2 h) increments until reaching an operational flux of 20 L/(m^2 h) on operational day 60. After operating the system for approximately 60 additional days at this flux, the flux was reduced to 10 L/(m^2 h); and remained at this level until the end of the experiment. Under the latter conditions, a permeate production of 11.8 L/d was obtained and approximately 4.7 L/d were taken out of the system, while the remaining 7.1 L/d were recirculated to the AnMBR vessel. Membrane chemical cleaning was performed when the TMP reached a value of 600 mbar. The chemical cleaning was started by performing a physical cleaning of the membrane to remove the cake layer; then, the membrane was soaked in a sodium hypochlorite solution (500 mg/L) for approximately one hour. After washing the membrane with clean water, the membrane was soaked in a 1% citric acid solution for approximately one hour.

4.2.4 Sludge properties determination

The TSS concentration was analyzed twice a week following the Standard Methods of APHA (Eaton, 2005). The sludge dynamic viscosity was determined twice a week with a viscometer (HAAK Viscotester 550, Thermo Fisher Scientific MA, USA) at a shear of 700 rpm (Dereli et. al., 2015). The PSD was determined every 20 days using a laser particle size analyzer (Microtrac S3500, Verder Scientific GmbH & Co. KG) with a diameter particle detection capacity range of 0.01 to 2,800 µm. The median particle size (MPS) was defined by the central point of the peak of the PSD curve. This value was calculated and reported by the software controlling the particle size analyzer.

The CST determination predicts the ability of the sludge to release water. A high CST value corresponds to a sludge difficult to dewater; thus, such sludge would be more difficult to filter through a membrane filtration system. The CST was determined once a

week using a CST apparatus (Capillary Suction Timer 304M, Triton Electronics, Essex, England) and a standard paper filter (Whatman No. 17, Merck KGaA, Darmstadt, Germany).

The SRF determination can provide information on the level of compaction of the cake layer (Jeison & van Lier, 2007). This determination involves exposing the sludge to a dead-end filtration process. The time needed to produce a given volume of filtrate (permeate) is reported as the SRF (Dereli et al., 2015). Cake layer formation has been reported as the most important fouling mechanism in AnMBRs (Jeison & van Lier, 2007); therefore, the SRF determination can provide relevant information on the membrane filtration performance when operating AnMBR system. The SRF was determined every two weeks using a dead-end filtration cell (Millipore 8050, Merck KGaA, Darmstadt, Germany) after the AnMBRs were set to their respective SRT. The samples were filtered under a constant pressure of 0.5 bar through a standard paper filter (Whatman Grade GF/F Filter 47 mm, Merck KGaA, Darmstadt, Germany) with a pore size of 0.7 µm. The SRF was calculated as described in the Equation 1 (Pollice et. al., 2008). The slope term (b) in Equation 1 was calculated as the slope of the filtration time as a function of the filtrate-volume (t/V) versus the filtrate-volume (V).

$$i. \quad SRF = \frac{2 \cdot \Delta P \cdot A^2 \cdot b}{\mu \cdot C} \qquad \text{Equation 1}$$

Where:

SRF = Specific Resistance to Filtration (m kg^{-1})

ΔP = Pressure of filtration (N m^{-2})

A= area of the filter paper (m^2)

b = slope of the filtration time (s m^{-6})

μ = viscosity (N s m^{-2})

C = TSS concentration (kg m^{-3}).

The SF determination can provide information on the membrane fouling potential due to the presence of fine particles and soluble compounds such as SMP and colloids. The SF determination evaluates the presence of such substances in the sludge supernatant. The presence of those compounds may lead to an increase in membrane fouling either due to a decrease in cake layer porosity and/or due to blocking of the membrane pores (Le-Clech et. al., 2006). The SF was determined every two weeks after the AnMBRs were set to their respective SRT by centrifuging the sludge at 17,500 g for 10 minutes. Then, the supernatant was filtered through a 0.22 µm membrane filter (Whatman membrane filters

nylon 47 mm, Merck KGaA, Darmstadt, Germany) in a stirred dead-end filtration cell (Millipore 8050, Merk Massachusetts, USA) under a constant pressure of 0.5 bar.

The EPS refers to different classes of macromolecules such as, proteins, carbohydrates, nucleic acids, and other polymeric substances secreted by the microorganisms; EPSc refers to the carbohydrate fraction, while EPSp to the protein fraction (Le-Clech et. al., 2006). The SMP consists of proteins, carbohydrates, and other soluble cellular components present in the soluble fraction of the sludge mixture; similarly, SMPc refers to the carbohydrate fraction and SMPp to the protein fraction. The EPS content was determined every two weeks after the AnMBRs were set to their respective SRT following the procedure by Dereli et. al., (2015). The EPS were extracted by thermal treatment at 100 ºC for one hour. After the thermal extraction procedure, the sample was centrifuged at 17,500 g for 10 min, and then the sample was filtered using a 0.22 µm filter (Whatman UNIFLO 25 syringe filters, Merck KGaA, Darmstadt, Germany). The SMP consists of proteins, carbohydrates, and other soluble cellular components present in the soluble fraction of the sludge mixture; similarly, SMPc refers to the carbohydrate fraction and SMPp to the protein fraction. The SMP content was determined with the same procedure as the EPS, without the thermal treatment.

4.2.5 Total resistance to filtration

The total resistance to filtration can be defined as the ratio between the TMP and the dynamic viscosity and membrane flux. The total resistance to filtration includes all specific resistances to filtration, i.e., the intrinsic membrane resistance, the cake layer resistance, and the resistance caused by pore blocking due to accumulating inorganic and/or organic compounds in the surface or the pores of the membrane. The total resistance to filtration (R_{total}) was calculated following Equation 2.

$$R_{total} = \frac{TMP}{\mu \cdot J} \qquad \text{Equation 2}$$

Where:

R_{total}: total resistance to filtration (1/m)

TMP: transmembrane pressure (Pa)

µ: dynamic viscosity of water (Pa s)

J: flux ($m^3/(m^2 s^1)$).

The different (individual) resistances to filtration as indicated in Equation 3 were determined when performing the cleaning in place (CIP) interventions according to Meng

(Meng et. al., 2006). The intrinsic membrane resistance ($R_{intrinsic}$) was determined at the very beginning of this research when using the new (unused) membrane. The cake layer resistance ($R_{removable}$), the resistance caused by inorganic and organic foulants removed by chemical cleaning ($R_{irreversible}$), and the resistance caused by foulants which cannot be removed by chemical cleaning ($R_{irrecoverable}$) were determined after performing the CIP intervention. The membrane was first rinsed with clean water, and it was later cleaned with sodium hypochlorite and citric acid. After each cleaning intervention the resistances to filtration were determined and each of the individual resistances were calculated following Equation 3.

$$R_{total} = R_{intrinsic} + R_{removable} + R_{irreversible} + R_{irrecoverable} \quad \text{Equation 3}$$

Where:

R_{total}*: total resistance to filtration (1/m)*

$R_{intrinsic}$*: intrinsic membrane resistance (1/m)*

$R_{removable}$*: cake layer resistance which can be physically removed by flushing with water (1/m)*

$R_{irreversible:}$ *caused by inorganic and organic foulants that can be removed by chemical cleaning(1/m)*

$R_{irrecoverable}$*: caused by foulants which cannot be removed by physical or chemical cleaning (1/m)*

4.3 RESULTS

The biological performance of the two reactors was thoroughly assessed and published in our previous work (Szabo-Corbacho et. al., 2019). Results showed COD removal efficiencies of up to 99% in both AnMBR systems and stable biogas production of 0.31 ± 0.02 NL CH_4 /(gCOD removed) and 0.32 ± 0.02 NL CH_4 /(gCOD removed) for R20 and R40 reactors, respectively. Moreover, an inhibitory effect of the biomass due to the presence of LCFA compounds was not observed. Apparently, at the evaluated process operational conditions, the AnMBR systems exhibited a much better biological performance treating lipid-rich dairy wastewater, compared to conventional anaerobic wastewater treatment systems (Alves et. al, 2009; Dereli et al., 2014). Comparing both AnMBRs, the R40 reactor exhibited higher COD removal efficiencies, higher biogas production, and lower biomass specific lipid concentrations compared to the R20 reactor.

4.3.1 Physicochemical properties

Total suspended solids

Figure 4.2a describes the changes in TSS concentration during the entire operational period. The reactors were inoculated with sludge at a TSS concentration of approximately 16 g/ L. During the coupled period, the two reactors were operated at the same SRT of 30 days; the TSS concentration initially decreased in both reactors until day 69 when the SRT was set to 20 and 40 days for R20 and R40, respectively. The TSS in both reactors slowly reached stable values at approximately (6.8 ± 0.3) g TSS / L and (12.4 ± 0.5) g TSS / L for the R20 and R40 reactors, respectively, meaning a steady state operation. The TSS values obtained in this study were similar to the TSS concentrations reported in the literature (Dereli et. al., 2015,2014; Diez et. al., 2012).

Dinamic viscosity

Until day 99, the dynamic viscosity remained similar in both reactors, after which an increase was observed in both reactors (Figure 4.2b). At the end of the experimental period the dynamic viscosity reached more or less 15 and 9 mPa.s in R40 and R20, respectively. In a study evaluating the rheological characteristics of anaerobic sludge, Pevere et. al., 2006 reported a dynamic viscosity of approximately 5-6 mPa.s, which is distinctly lower compared to the values found in our present research applying a similar shear rate.

Particle size distribution

The PSD shifted to lower particle size values in both reactors during the evaluated period (Figure 4.3a and b). Similar trends were observed regardless the applied SRT in the reactor. The reduction in the particle size was attributed to the applied shear forces in the cross-flow filtration unit, and the use of peristaltic pumps for the feed water recirculation. Figure 4.2c also shows the mean particle size (MPS) as a function of the exposure time for both reactors. A continuous decrease in the MPS, was observed for both systems until a similar value was reached at the end of the evaluated period. Similar findings were also reported in literature (Dereli et al., 2014).

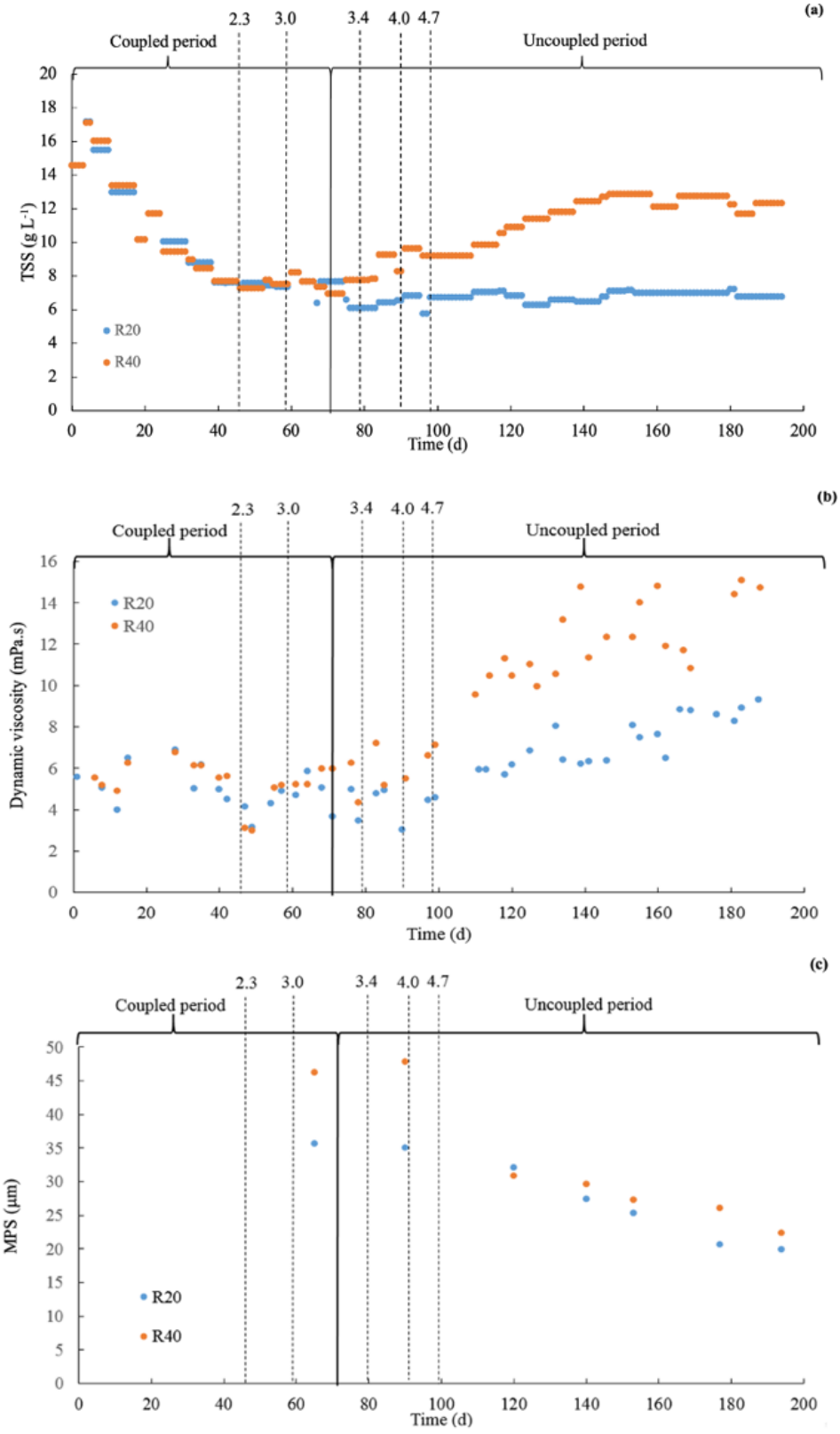

Figure 4.2 – (a) TSS concentrations, (b) Dynamic viscosity, and (c) MPS over time for the R20 and R40 reactors. The dotted lines indicate the applied OLR (g COD/ (Ld))

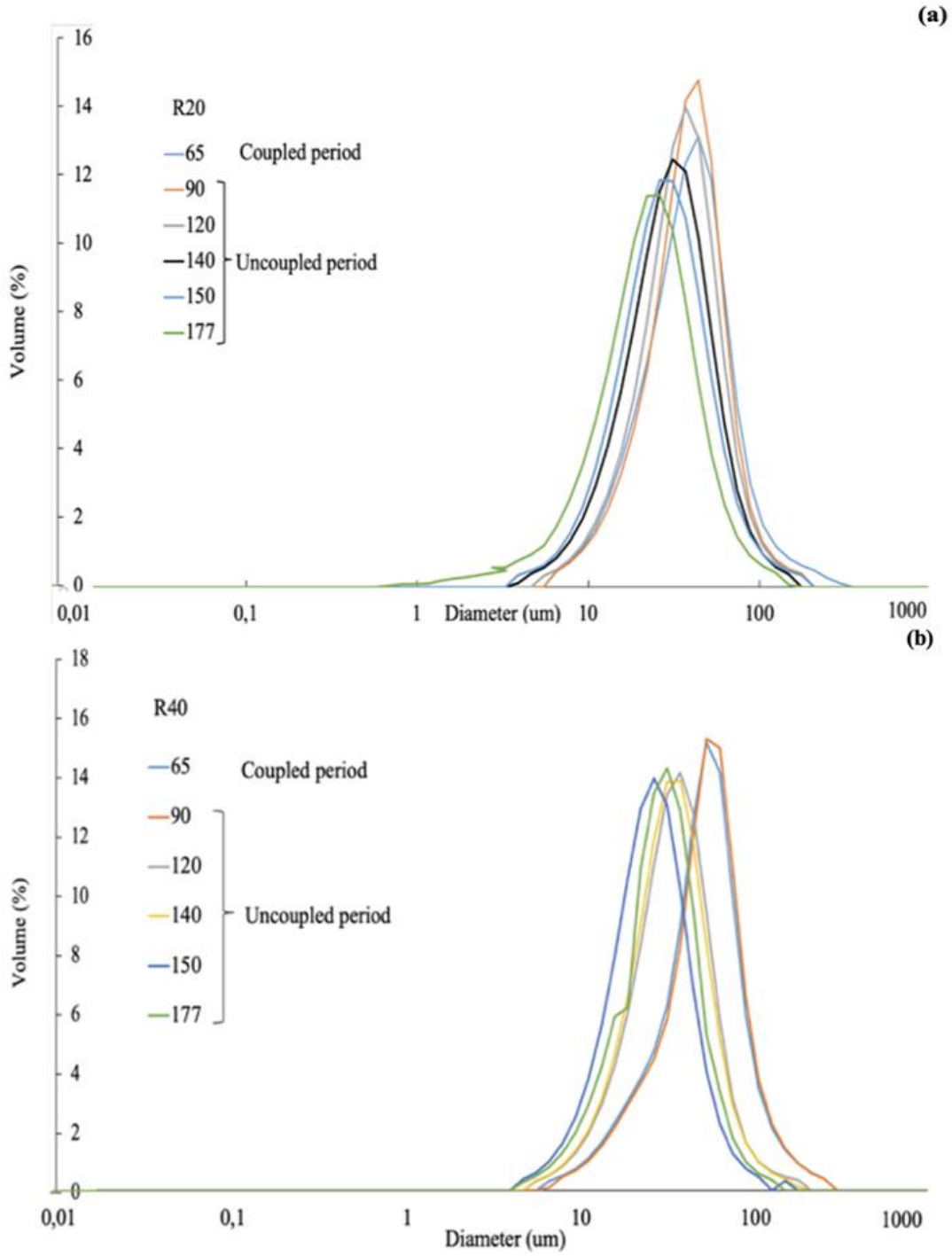

Figure 4.3 – PSD over operational time for (a) R20, and (b) R40 reactors over time.

4.3.2 Sludge filterability properties

Capilarity suction time

Figure 4.4a shows the CST of the sludge of both reactors for the entire evaluated period. An initial decrease in the CST values was noticed immediately after the inoculation of the reactors. However, from day 100 the CST values started to rapidly increase. From day 140 until the end of the experiment (day 189), average values of 956 ± 168 s and 1,321 ± 138 s were found for R20 and R40, respectively. The specific or normalized CST, i.e., the CST divided by the TSS, for the entire evaluated period was also calculated. Similar to the CST values, after an initial drop, a steep increase in the specific CST was observed from day 100, which stabilized on day 140. In the final period, specific CST values of 138 and 112 s L/ g for R20 and R40, respectively, were calculated.

Results in Figure 4.4a showed higher CST values for R40 compared to R20, indicating a better dewaterability, and suggesting better filterability for R20 sludge compared to the R40 sludge. However, very similar and even slightly higher specific CST values were calculated for the R20 sludge compared to the R40 sludge indicating the opposite trend. The specific CST values obtained for both reactors were distinctly higher than the values of 40-50 s L/ g for anaerobic sludge in conventional anaerobic wastewater treatment systems, reported in the literature (Pevere et al., 2006; Pollice et al., 2008; Wu et al., 2008).

Specific Resistance to Filtration

Figure 4.4b shows the assessed SRF values between days 69 and 160 for both reactors It is important to highlight that the SRF is normalized to the TSS concentration in the sample. Therefore, SRF values provide an indication of the specific sludge filterability, similarly to the specific CST values previously discussed. The SRF steadily increased in both reactors reaching maximum values of 12 x 10^{14} (m/kg) and 8 x 10^{14} (m/kg) on the operational day 140 for the R20 and R40 reactors, respectively. Dereli et. al., (2015) reported SRF values approximately one order of magnitude lower compared to the values shown in Figure 4.4b; thus, indicating a worse sludge filterability in our present study. The high SRF values coincided with higher TMP values compared to the TMP values reported by Dereli et. al., (2015); while working at a similar operational flux. As observed with the specific CST values, the SRF results of the R40 sludge indicated a better filterability compared to the R20 sludge. This difference was even more noticeable in the SRF assessments compared to the specific CST assessments.

Supernatant Filterability

Figure 4.4c describes the supernatant filterability for the two reactors in the experimental period from day 69 to 170.SF values of approximately 1.25 mL /min and 0.96 mL/ min were found on day 70 for the R20 and R40 reactors, when the SRT was set to 20 and 40

days, respectively. Hereafter, the SF decreased and stabilized from day 110 onwards showing similar SF values in both reactors ranging from 0.2 to 0.4 mL/ min.

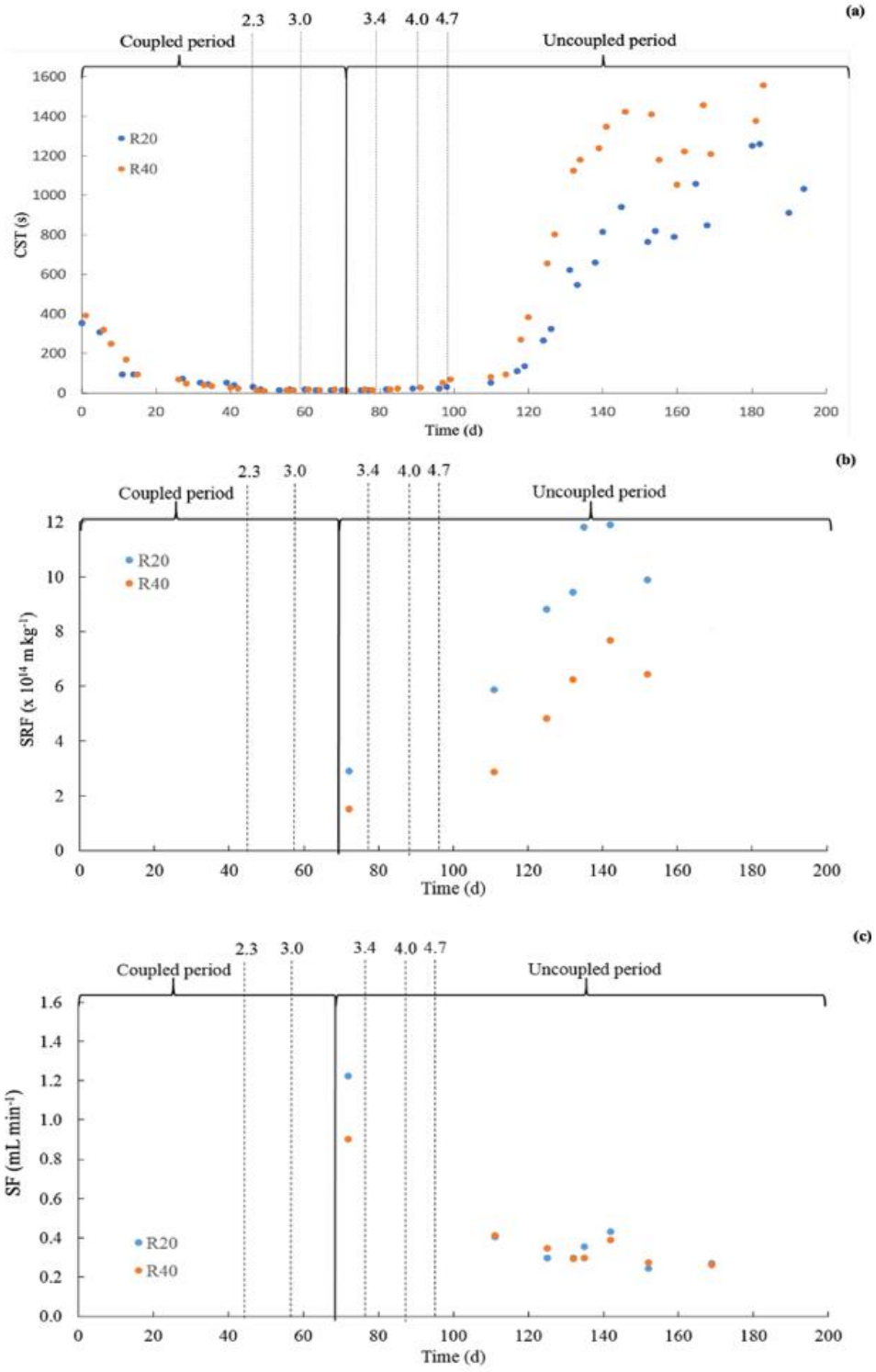

Figure 4.4 – (a) CST, (b) SRF, and (b) SF for the R20 and R40 reactors over time. The dotted lines indicate the applied OLR (g COD /(Ld))

4.3.3 Presence of soluble substances in the sludge matrix

Extra polymeric substances

Figure 4.5a presents the concentration of EPS in the sludge over time Both the protein fraction of the EPS (EPSp) as well as the carbohydrate EPS fraction (EPSc) are presented. Up to day 100, concentrations of approximately 30 and 20 mgEPSp /g SS were reported for reactors R20 and R40, respectively. Hereafter, the EPSp concentrations increased, reaching maximum values on day 145 of 110 and 85 mg EPSp / g SS for R20 and R40, respectively. Then, the EPSp concentration slightly decreased towards the end of experimental period. Lower values were reported for the EPSc compared to the EPSp for both reactors, showing EPSc concentrations below 20 mg /g SS throughout the experimental period. Results agree with the literature, showing EPSp concentrations ranging from 11 to 120 mg EPSp/ g SS and EPSc concentrations ranging from 7 to 40 mg/ g SS for aerobic MBRs and anaerobic up flow sludge bed filters (Le-Clech et al., 2006). The mentioned studies also reported an inverse relationship between the filtration performance and the concentration of EPSp and EPSc.

Soluble microbial products

Figure 4.5b shows the SMPc and SMPp concentrations in reactors R20 and R40Similar to the EPSp concentrations, the SMPp concentrations started to increase after day 100. On day 120, the SMPp concentrations reached maximum values of 425 and 300 mg SMPp/ L for R20 and R40, respectively. Hereafter, the SMPp concentrations decreased to 150 and 125 mg SMPp/ L on day 170 for R20 and R40, respectively. Le-Clech et. al., (2006), reported lower SMPp concentrations compared to our present study, ranging from 8 to 34 mg SMPp/ L for aerobic and anaerobic MBRs operated at TSS concentrations ranging from 7 to 14 g /L. In addition, the authors reported a negative correlation between the SMPp concentration and the membrane filtration performance. The SMPc concentration for both reactors remained more or less constant during the entire experimental period ranging from 50 to 100 mg SMPc /L. In addition, Le-Clech et. al., 2006 reported lower SMPc concentrations compared to our present study, i.e., ranging from 5 to 14 mg SMPc /L for aerobic and anaerobic MBR systems operated at TSS concentration from 7 to 14 g /L, which are similar TSS concentration as in our present research. Also, as observed with the SMPp, the authors reported a negative correlation between the SMPc concentration and the membrane filtration performance.

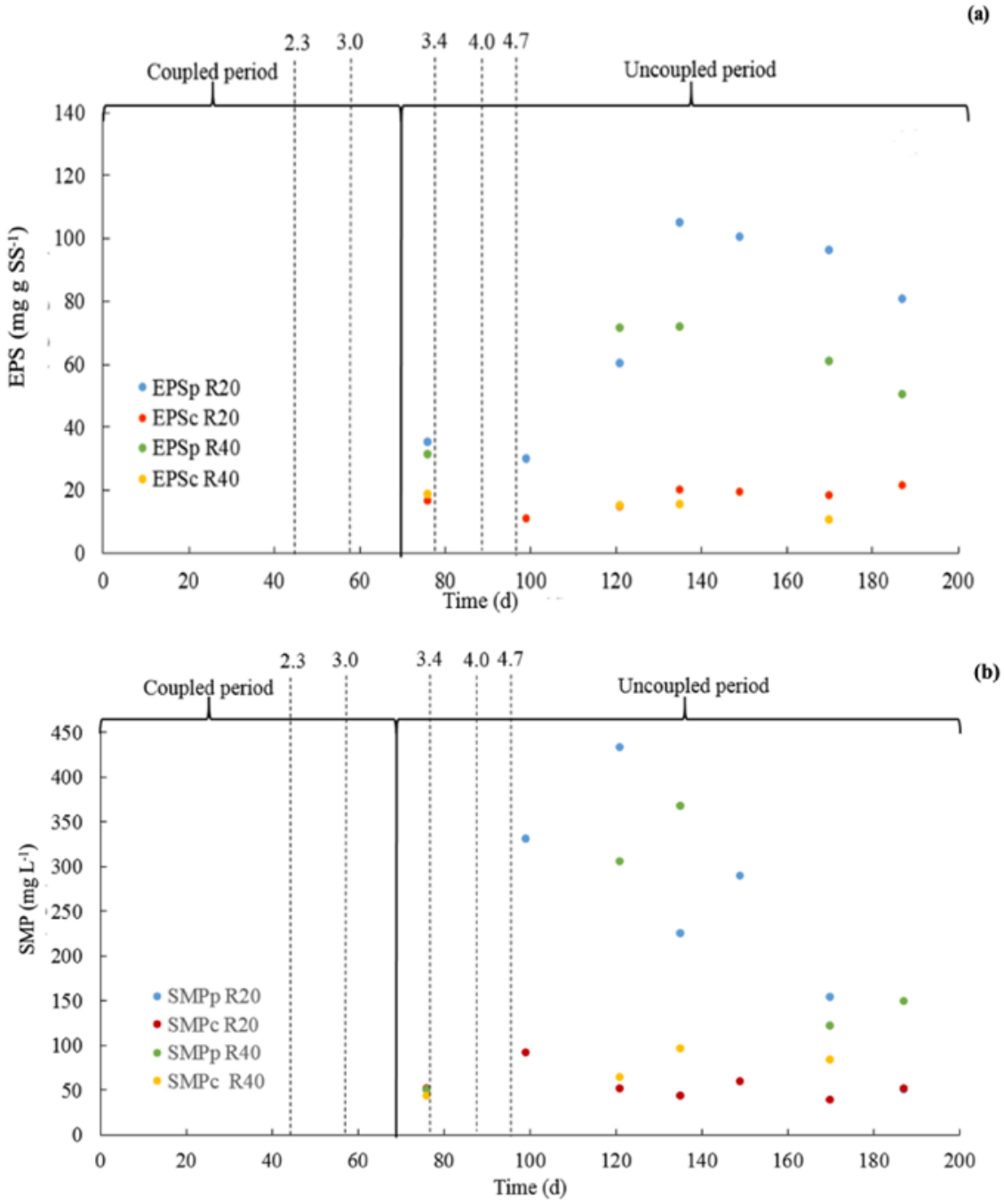

Figure 4.5 – (a) EPS fractions, and (b) SMP fractions for the R20 and R40 reactors over time. The dotted lines indicate the applied OLR (g COD/(L d))

4.3.4 Membrane Filtration Performance

Trans-membrane pressure and flux profile

Figure 4.6a shows the TMP values for both reactors at the different operational fluxes during the entire experimental period Results clearly show that the TMP for the R40 reactor was consistently higher than the TMP for the R20 reactor (with some exemptions). The flux was increased stepwise until reaching a maximum flux of 20 L / (m^2 h) on day 62, which coincided with a recorded TMP value of 71 and 107 mbar for R20 and R40, respectively, only showing a slight increase. A membrane CIP intervention was carried out on day 62 in an attempt to reduce the TMP. The CIP interventions are indicated in Figure 4.6a by a solid vertical green line. However, after the first CIP intervention the reactor operation continued to be operated at the target flux resulting in a continued increase of the TMP reaching values of 301 and 455 mbar for R20 and R40, respectively on day 100. The reported values were close to the maximum suggested operational TMP value of 500 mbar. Thus, two additional CIP interventions were carried out on days 85 and 110. In addition, the flux was reduced on day 95, first to 18 L/(m^2 h) and subsequently a few days later to 15 L /(m^2 h) expecting to reduce the observed TMP values. After the third CIP intervention on day 110, the TMP values stabilized for both reactors. However, approximately 10 days after, on day 120, the TMP values increased again reaching similar TMP values as previously observed before the third CIP intervention. Thus, on day 130, the flux was reduced to 10 L/(m^2 h) in both reactors to avoid potential damage to the membranes and to attain a stable membrane filtration performance. Two additional CIP interventions were carried out on days 140 and 180, which did not result in major improvements regarding observed TMP values. In addition, as indicated in Figure 4.6a, throughout the experimental period the OLR was increased from an initial value of 1.0 g COD /(Ld) until reaching the value of 4.7 g COD/(L d) on day 89. The increments in OLR are shown by dotted lines in Figure 4.6a. The increase on the OLR could have also impacted on the biomass characteristics, probably contributing as well to the reported TMP values. The operational flux of 10 L/(m^2 h) was slightly lower compared to the operational fluxes of 10 to 14 L/(m^2 h)for AnMBR systems reported in the literature (Dereli et. al., 2014).

Total resistance to filtration (R_{total})

Figure 4.6b shows the R_{total} for both reactors during the entire experimental period. At the start of the experiment, the R_{total} for the R20 reactor was slightly higher than for the R40 reactor, even though both reactors were operated at the same SRT of 30 days. Then, after day 62 when the SRT of 20 and 40 days was set for R20 and R40, respectively, the R_{total} increased continuously for both reactors. Since the R_{total} directly relates to the TMP, similar trends as observed in Figure 4.6a for the TMP were expected for the R_{total}. After setting the operational flux at 10 L / (m^2 h) at day 130, the R_{total} stabilized at average values of approximately (1.5 ± 0.3) x 10^{13} 1/m and (1.8 ± 0.2) x 10^{13} 1/m for R20 and

R40, respectively. The reactor R40 exhibited a higher total resistance to filtration compared to the reactor R20. Similar total resistance to filtration values is reported in the literature (Dereli et al., 2015).

The individual resistances contributing to the total resistance to filtration were calculated according to Equation 3. The results of the different individual resistances, calculated for days 140 and 188, are presented in Table 4.1 for both reactors. The cake layer resistance exhibited the highest contribution to the total resistance to filtration for both reactors at the two evaluated operational days; this observation agrees with previous findings reported in the literature on AnMBRs (Dereli et al., 201; Jeison & Lier, 2006). Results show a higher contribution of the cake layer the total resistance in R40 compared to R20, which likely can be attributed to the presence of a thicker cake layer present in R40 than in R20.

Table 4.1 – Individual resistances for the R20 and R40 reactors at operational days 140 and 188.

Reactor	Operational day	R intrinsic (%)	R removable (%)	R irreversible (%)	R irrecoverable (%)
R20	140	2	75	21	2
	188	3	63	28	6
R40	140	2	78	18	2
	188	3	79	16	2

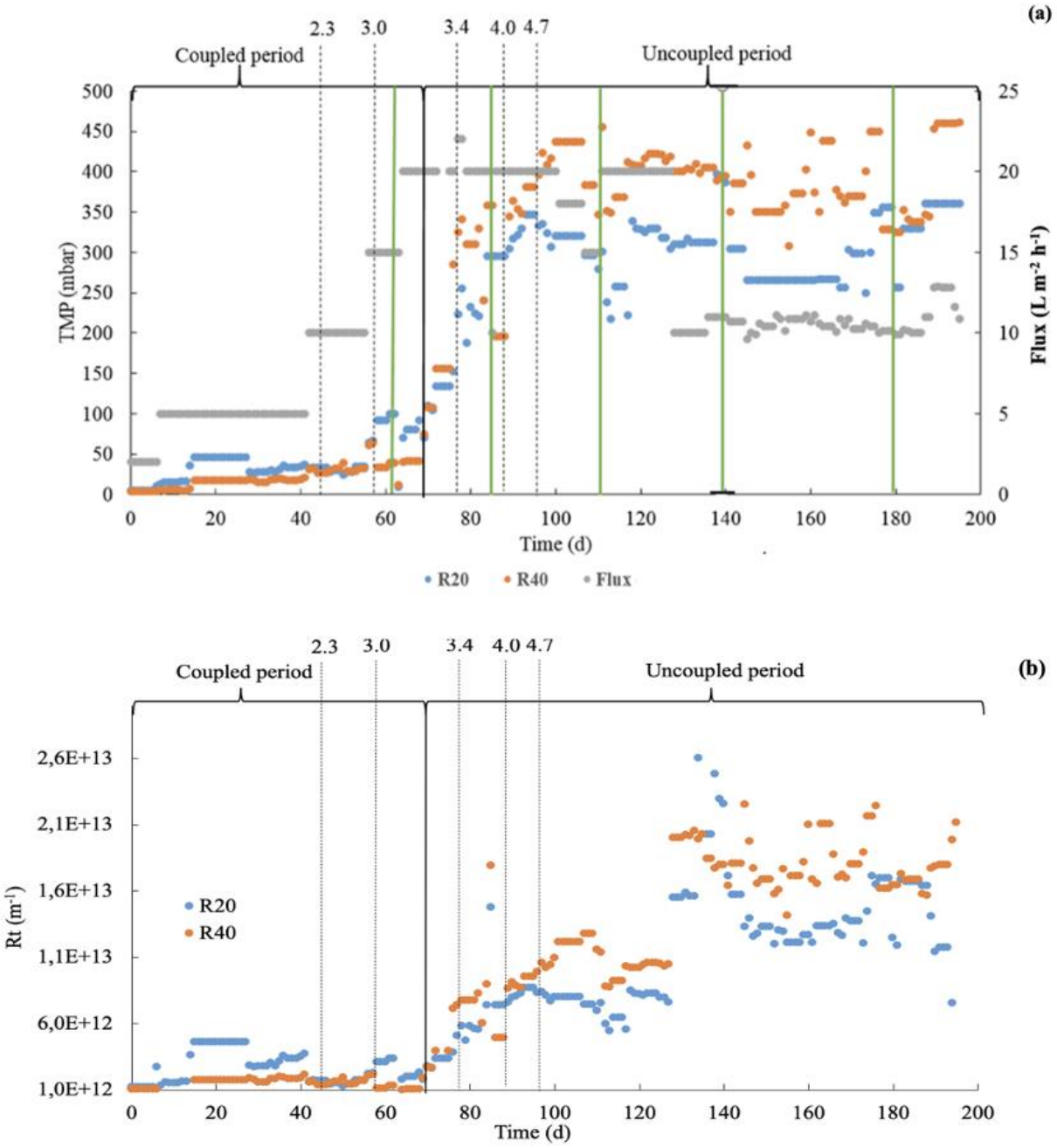

Figure 4.6 – (a) Flux L/ (m^2 h)) and TMP (mbar), and (b) Rtotal values over time for the R20 and R40 reactors. The dotted lines indicate the applied OLR (g COD /(Ld)), and the green lines indicate the CIP interventions carried out in the reactors.

4.4 DISCUSSION

4.4.1 Physicochemical sludge properties

The TSS concentration apparently exerts an important role on membrane fouling; thus, affecting the attainable membrane flux (Berube & Lei, 2006; Chang et al., 2001). A higher SRT, implies a higher TSS concentration in the reactors. The TSS concentrations in R40 and R20 were 12.4 ± 0.5 g TSS /L and 6.8 ± 0.3 g TSS / L, respectively. The higher TSS concentration in R40 coincided with higher TMP values and higher R_{total} than in to R20, indicating a worse membrane filtration performance. Lousada-Ferreira et al. (2016) reported for aerobic MBR sludge that beyond a critical TSS concentration of 10 g TSS/ L, the sludge filterability and membrane filtration performance deteriorated. The authors concluded that beyond an optimal TSS concentration, small particles, with particle diameters of approximately 20 μm and smaller, were no longer retained by the sludge cake; thus, contributing to membrane fouling. In addition, Jeison & van Lier (2007) concluded that the biomass concentration is one of the most important factors affecting the cake layer formation in AnMBR membrane filtration processes; that is, the operational flux and total membrane filtration resistance would strongly depend on the TSS concentration. In other words, a high TSS concentration results in low membrane filtration performance. Meaning, operation at high SRT conditions, such as in R40, will results in high total membrane resistance and low filtration performance. In addition, increased TSS concentrations concomitantly results in an increased presence and concentration of viscous substances in the sludge, such as carbohydrates and proteins (Meng et al., 2006), resulting in higher dynamic viscosities, as was observed in R40 (Khongnakorn et al., 2007; Pollice et al., 2008).

The PSD has an important role on membrane fouling (Bouhabila et al., 2001), largely determining membrane filtration performance. MBRs equipped with cross flow membrane filtration units are characterized by high shear stress on the sludge. Such shear forces promote sludge flocs breakage with a consequent change in the PSD of the sludge. The PSD shifts to the lower particle-size range, reducing the amount of large particles and increasing the amount of small particles, enhancing membrane fouling (Meng et al., 2009a; Wisniewski & Grasmick, 1998). In this research, the AnMBR systems were operated at a relatively low cross-flow velocity of 0.5 m/s (Dereli et al., 2015); however, it was apparently large enough to shift the PSD of the sludge to smaller particle sizes at the two evaluated conditions in R20 and R40. Although slightly higher MPS were observed for the R40 reactor compared to R20, the changes in PSD and MPS observed in this study were very similar for the two evaluated reactors; thus, the evaluated SRTs and related sludge concentration seemed not to play a significant role on promoting or avoiding such shift in PSD. Similar shear forces were applied to both reactor; thus, similar patterns regarding the PSD were expected.

The presence of fine particles contributes to form a more compact layer at the surface of the membrane, so reducing the membrane filtration performance (Choo & Lee, 1998; Jeison & van Lier, 2007; Wu et al., 2007). Jeison & van Lier (2007), also discussed the importance of compact cake layer formation on the membrane filtration performance. In the present study, the MPS decreased in both reactors (R20 and R40), while the TMP and R_{total} increased in both systems. The deterioration in the membrane filtration performance as a function of the operational time is likely due to multiple factors, including the increase in TSS concentration, increase in dynamic viscosity, sludge characteristics, amongst others.

However, the sludge particle size could have had a more pronounced impact on the membrane filtration performance in our present study compared to other causes. Various factors are presumable inter-related; for instance, the higher concentration of TSS solids in R40 may also contributed to a larger amount of fine-sized particles in R40 than in R20. Thus, both the shift on the PSD to lower particle sizes, as well as the presence of large TSS concentrations very likely increased synergistically the observed TMP and R_{total} in R40 compared to R20.

A larger concentration of TSS may directly affect the membrane filtration performance by promoting the formation of a thick cake layer at the surface of the membrane. Moreover, the TSS concentration may also impact on the other sludge properties such as the sludge dynamic viscosity, sludge PSD, CST, and presence of EPS and SMP compounds, amongst others. The impact of these properties on the membrane filtration performance is discussed below.

4.4.2 Sludge filterability properties

The CST values increased in both reactors as a function of the operational time; however, higher CST values were reported for the R40 reactor compared to the R20 reactor. High CST values indicate a poor filterability of the sludge (Dereli et al., 2014); thus, the results obtained in this study clearly indicate a better sludge filterability for the sludge in the R20 reactor. Similar to our results, Khongnakorn et al. (2007) reported that the CST exhibited a positive relation with the TSS content. In addition, Pollice et al. (2008) reported a positive correlation between the CST values and the SRT. Moreover, Wu et al. (2007) reported a positive correlation between the CST and the total resistance to filtration. However, when normalizing the CST to the TSS concentration similar specific CST values were found for both the R20 and R40 reactors; even higher specific CST were reported for the R20 sludge than for the R40. That is, both the R20 and R40 sludge exhibited very similar sludge dewaterability properties. The non-normalized CST resembles the differences in the filtration resistance in both reactors (Pan et al., 2010). The increase in the CST values as a function of the operational time were associated with an increase in TMP and R_{total}. Thus, the TSS concentration was seemingly the major cause affecting membrane filtration performance, rather than the filterability of the sludge. The

CST values observed in this evaluation were similar to the CST values reported in the literature treating similar lipid rich wastewater (Dereli et al., 2014). Dereli et al. (2014) reported similar CST values when operating an AnMBR treating lipid rich wastewater (corn-based thin stillage). The lipid-rich influent wastewater could eventually negatively affect the sludge filterability; nonetheless, the sludge concentration rather than the sludge characteristics (specific CST) seemed to pose the major negative effects on the membrane filtration performance (Szabo-Corbacho et al., 2019) under the given hydraulic conditions. As a result, even though the CST in R40 had been higher than the R20, when it was normalized the CST/TSS had similar values for both reactors.

Similar findings as for the specific CST were observed for the SRF. The SRF is also indicative for the sludge filterability (Meng et al., 2009); the SRF is normalized to the concentration of suspended solids in the sample. Higher SRF values indicate a more compact (less permeable) sludge cake layer. As previously reported in Szabo-Corbacho et al. (2019), a high SRT results in a low accumulation of lipids in the sludge; eventually, the low concentration of lipids in the sludge could lead to improved sludge filterability properties. The SRF is normalized to the TSS concentration; therefore, it is a less predictive indicator of the actual membrane filtration performance.

The specific sludge filtration characteristics for the R40 sludge were better than for the R20 sludge; however, the cake layer in the R40 is thicker, twice as thick, resulting in a worsen filtration performance. Increasing the cross-flow velocities on the membrane surface could have eventually counteracted such filtration loss, which however also could also result in an increased amount of fine particles, worsening the sludge filterability. Similar SRF values (8 to 12 x 10^{14} m/ kg) were observed in this research, as in the literature treating lipid-rich wastewater in AnMBR systems (Dereli et al., 2014). Whereas one order of magnitude lower SRF values were obtained in this study compared to AnMBR systems treating low strength synthetic wastewater (Buntner et al., 2014).

The decrease in SF values could be explained due to the presence of colloidal material remaining in the supernatant after centrifuging the sludge for carrying out the SF determination. Apparently, the differences in TSS concentrations and the possible larger fine particles concentration in the R40 reactor, did not yield different SF values under the given hydraulic conditions. The results obtained in this research were similar to other studies reported in the literature treating low strength synthetic wastewater in AnMBR systems (Buntner et al., 2014). Therefore, the type of wastewater did not exert a significant role on the SF; thus, the SF does not provide major information on the membrane filtration performance for the evaluated conditions.

4.4.3 Presence of soluble substances in the sludge matrix

According to Meng et al. (2006), the EPSc fraction contributes mostly to membrane fouling. In the present study, the EPS determinations were normalized to the TSS concentrations (sludge specific EPS concentration). Higher specific EPSp values were observed for the R20 reactor compared to the R40 reactor. Thus, the sludge in the R20 reactor may exhibit a worse filterability due to the presence of specific higher concentration of EPSp compared to the sludge in R40. As previously explained for the specific CST and SRF, it could eventually occur that the sludge in the R20 reactor produced higher specific amounts of EPSp. Even though the TSS specific EPSp concentration in R20 was higher compared to R40, the absolute concentration of EPSp was higher in R40 compared to R20, possibly explaining the worse membrane filtration performance observed in R40 compared to R20. Similar findings could be observed for the EPSc content; however, the specific EPSc concentrations were very similar for the R20 and R40 reactor. The absolute concentration of EPS in the sludge increases with the experimental period. The changes in PSD could be indicative for the release of EPS substances originally present in the sludge. Consequently, the shift in PSD may explain the increase in absolute EPS concentration, contributing to a worsened membrane filtration performance of the R40 reactor compared to the R20 reactor.

Various authors identified the SMP substances as an important parameter affecting membrane filtration performance (Berube & Lei, 2006; Meng et al., 2006; Pan et al., 2010). Similar TSS specific SMPp and SMPc concentrations were observed in both the R20 and R40 reactors as in the literature (H. Shin & Kang, 2003). A subtle increase in the SMP values was followed by stable SMP concentrations at the end of the evaluation. However, the TMP and R_{total} deteriorated as the time progressed. Therefore, the observed differences in the absolute SMP concentrations between the two reactors could have contributed to the differences in the observed membrane filtration performance between the two systems. However, Stuckey (2012) reported that there were not clear correlations between membrane fouling and the concentration of SMP in the sludge. The author reported that the differences in membrane filtration performance could be related to other sludge physicochemical characteristics being probably the TSS concentration and the cake layer formation the most relevant parameters affecting the membrane filtration performance.

4.4.4 Membrane Filtration Performance

The reactor R40 exhibited higher TMP values compared to the R20 reactor at the same operational fluxes during the entire evaluated period. The observed TMP values in this evaluation for both reactors were higher than in the literature (Dereli et al, 2014). Particularly, Dereli et al, (2014) also treating a lipid-rich wastewater (palm oil mill effluent (POME)) in an AnMBR, reported lower TMP values, from to 100 to 200 mbar.

The authors used a similar experimental set-up as in this research. However, the influent wastewater (although lipid-rich) was characterized by a different FOG content and LCFA profiles compared to the influent wastewater in our research, i.e., the percentage of palmitic acid in POME is approximately 42% of the total LCFA compared to 21% found in the whole milk used in our study (Alves et al., 2009). Therefore, the observed differences in TMP values might be attributed to the different characteristics of the obtained sludge treating different types of wastewaters, i.e., corn-based bioethanol thin stillage wastewater versus synthetic dairy wastewater. Additionally, although both wastewaters are rich in lipids, they also showed different lipid profiles and different concentrations of other substances such as divalent ions (calcium) (Dereli et al., 2019; Meng et al., 2017; Xin et al., 2016) and colloidal material (Meng et al., 2009) eventually leading to a different filtration performance.

When looking at the various sludge filterability indicators, i.e., parameters describing the inherent ability of the sludge to lose water, such as the specific CST and the SRF, no major differences were found between the R20 and R40 sludges. Moreover, in some cases, the R40 sludge showed a slightly better sludge filterability. Similar observations were made regarding the presence of fouling compounds such as EPS and SMP. Moreover, in a previous study carried out by the authors (Szabo-Corbacho et al., 2019), the sludge specific lipids and LCFA concentrations were higher in the R20 reactor compared to the R40. That is, in terms of sludge filterability, the R40 sludge exhibited lower specific lipid and LCFA concentrations, eventually showing a better inherent filterability. Therefore, the membrane performance in the two reactors seemed to be determined by other parameters beyond the inherent filterability of the sludge in each reactor. The concentration of TSS was eventually the most relevant physicochemical sludge parameter affecting the membrane filtration performance. The TSS concentration is one of the most important factors affecting the cake layer formation and its thickness. The higher the SRT, the higher the TSS concentration and the thicker the cake-layer during filtration explaining the worse membrane filtration performance of R40 compared to R20. In addition, the shift in PSD introduces larger concentrations of small particles, this was more pronounced in the R40 reactor, owing to the increased TSS concentration. A large amount of fine particles, together with the presence of a high overall TSS concentration, could have contributed to form a more thicker and possibly more compact cake layer on the surface of the membrane, reducing the membrane filtration performance. The increased TSS concentrations in R40 could additionally have led to increased the absolute concentrations of EPS and SMP substances, thus, even contributing to a more the cake layer.

In our previous work (Szabo-Corbacho et al., 2019), a better overall biological performance was reported when operating the reactor at an SRT of 40 days compared to an SRT of 20 days. Higher organic matter conversion, higher biogas production, and lower sludge wastage were observed when working at an SRT of 40 days (R40) compared

to an SRT of 20 days (R20). The results of our present research indicated that regarding the membrane filtration performance, the operation of the reactors at an SRT of 20 days is preferred. However, very likely, the filtration performance when operating at a high SRT can be further improved by introducing changes in the membrane operational strategy. Cake layer formation and consolidation is the most important parameter affecting the total membrane resistance. Therefore, changes in the membrane operational strategy can be proposed to reduce the impact of the cake layer resistance on the overall membrane filtration resistance (Wang et al., 2014). Changes in the membrane operational strategy may include changes in the backwash frequency, the addition of a membrane relaxation period, changes in the cross-flow velocities, and introducing more frequent CIP interventions, amongst others.

4.5 CONCLUSIONS

- The SRT exerted an important effect on the sludge properties including on the TSS concentration, dynamic viscosity, PSD, MPS, CST, SRT, and on the presence and concentration of EPS, SMP, lipids, and LCFA. The changes in sludge properties resulted in different membrane filtration performances. The higher the SRT, the worse the membrane filtration performance.
- The major individual contributor to the total resistance to filtration is the cake layer resistance negatively affecting the membrane filtration performance.
- The TSS concentration was the most important parameter determining the cake-layer resistance; thus, the membrane filtration performance under the given evaluated process conditions including the membrane cleaning regime.
- The changes in the SRT did not affect the specific sludge filterability.
- An SRT of 20 days resulted in a better membrane filtration performance compared to an SRT of 40 days attributed mostly to the lower sludge concentration in the reactor.

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5

INHIBITORY EFFECTS OF LONG CHAIN FATTY ACIDS ON ANAEROBIC SLUDGE TREATMENT: BIOMASS ADAPTATION AND MICROBIAL COMMUNITY ASSESSMENT

This chapter is based on: M.A. Szabo-Corbacho, P. Sharma, D. Míguez, V. de la Sovera, D. Brdjanovic, C. Etchebehere, H.A. García, J. B. van Lier. Inhibitory effects of long chain fatty acids on anaerobic sludge treatment: biomass adaptation and microbial community assessment. Accepted in Environmental Technology Innovation Journal, January 2024.

ABSTRACT

The study investigated the effects of long-chain fatty acids (LCFA) on anaerobic sludge treating lipid-rich wastewater. It involved batch experiments with three sludge samples: two acclimated to lipids and one non-acclimated. The experiments aimed to observe the degradation of LCFA, specifically oleate and palmitate, by dosing them at concentrations ranging from 50 to 600 mg/L. Measurements of the cumulative methane production and the LCFA concentration, quantified as fat, oil, and grease (FOG) were performed. To ensure the sludge was free from other biodegradable substrates, part of the samples was pre-incubated without feed. The tests were conducted with both pre-incubated and non-incubated inoculum sludge. The findings revealed that oleate was degraded more efficiently than palmitate across all sludge samples, with a greater conversion rate to methane. Sludge samples acclimated to lipids showed a superior capacity to degrade LCFA compared to non-acclimated ones. It was noted that at concentrations above 400 mg/L, the conversion of LCFAs to intermediate compounds was inhibited, although this did not affect the subsequent methane production. The study concludes with a recommendation for sludge adaptation strategies to boost the efficiency of anaerobic wastewater treatment systems dealing with lipid-rich waste. The presence of LCFA-degrading bacteria families like *Kosmotogaceae, Petrotogaceae*, and *Synergistaceae* in the acclimated sludge samples underscores the adaptation and potential for improved degradation performance.

5.1 INTRODUCTION

Anaerobic wastewater treatment exhibits distinct advantages compared to aerobic treatment, particularly, when treating wastewater with high concentrations of organic matter, as in food processing wastewater (van Lier, 2008). Wastewater produced by dairy, meat processing, and oil processing industries are characterized by high concentrations of both organic matter, including lipids (Perle et al., 1995; Sayed & de Zeeuw, 1988; Beccari et al., 1996). The largest group of lipids consists of triacyl glycerides, which are long chain fatty acids (LCFA) esterified to glycerol (Alves et al., 2009). Lipids are commonly measured using their biochemical properties, i.e., hydrophobicity, and are then identified as fats, oils, and grease (FOG). According to Hwu et al. (1998), the most commons LCFAs found in lipid rich wastewaters are palmitic acid (C16:0), a saturated LCFA; and oleic acid (C18:1), an unsaturated LCFA.

In the anaerobic treatment of wastewater with a high FOG content, the hydrolysis (by extracellular or membrane-bound lipases) of FOG occurs relatively fast resulting primarily in LCFAs and glycerol, when the FOG consists of primarily triacyl glycerides. However, the subsequent degradation of LCFA to acetate and hydrogen occurs at a low pace, potentially leading to the accumulation of LCFAs in the reactors (Pavlostathis & Giraldo-Gomez, 1991). The LCFA (saturated and unsaturated) conversion to acetate and hydrogen occurs via the β-oxidation reaction, in which an acetyl group is subsequently split off from the long aliphatic carbon chain of the LCFA. In the β - oxidation pathway, initially, the LCFA are transported into the bacterial cells (Mackie et al., 1991), converted into acyl-CoA thioesters by acyl-CoA synthetase, and then undergo β - oxidation. This process, detailed by DiRusso et al. (1999), cyclically shortens the acyl-CoA, producing acetyl-CoA and hydrogen (Sousa et. al., 2009).

However, there are differences in how the different LCFAs are degraded. Saturated LCFAs undergo immediate degradation via the conventional β - oxidation pathway. In contrast, the breakdown of unsaturated LCFAs may necessitate an initial step of hydrogenation or may follow a different degradation route, as indicated by research from Weng & Jeris (1976) and Roy et al. (1986). The degradation of unsaturated LCFAs involves two steps: hydrogenation to the saturated LCFA with the same chain length, like oleate (18:1) conversion to stearate (C18:0), and then followed by β -oxidation. The hydrogenation step, converting oleate to stearate, is often the limiting factor in this process, while the subsequent β - oxidation of stearate to palmitate (C16:0) typically proceeds more rapidly (Pereira et al., 2005). There is uncertainty whether these steps are carried out by a single microorganism or multiple species (Sousa et al., 2009). Observations from anaerobic bioreactors treating oleate-based effluents (Pereira et al., 2002) show palmitate accumulation outside cells, suggesting a bottleneck after one β - oxidation cycle. This could indicate that bacteria responsible for degrading oleate also

handle saturated fatty acids like palmitate, but the reverse is not always true (Sousa et al., 2010).

Alves et al. (2009), Becker et al. (1999), and Broughton et al. (1998) reported that a high concentration of LCFAs in anaerobic reactors may lead to inhibition of the microorganisms involved in the β-oxidation pathway; thus, limiting the complete conversion of LCFA to acetate and possibly propionate. Notably, evenly numbered LCFAs only generate acetate, while non-evenly numbered LCFAs also produce propionate (Pereira et al., 2001). Potential inhibition reduces the treatment performance, as well as the potential amount of biogas that can be obtained by the subsequent conversion of acetate to methane. LCFA accumulation, therefore, limits the maximum amount of methane that can be obtained when treating lipid rich wastewater (Alves et al., 2009). Pereira et al. (2005) mention that inhibitory effects already can be visible at concentrations as low as 50 mg/L. However, this inhibition is not permanent; biomass can adapt, overcoming what is termed as "reversible or temporary inhibition." (Pereira et al., 2001). Bactericidal toxicity, on the other hand, may cause cell lysis due to interactions between LCFAs and microbial membranes (Rinzema et al., 1993). Studies have demonstrated that LCFA-laden biomass can still degrade LCFAs to methane when the mass transfer limitations are removed, thus maintaining the integrity and activity of the microbial cells (Pereira et al., 2005).

Anaerobic treatment of lipid-rich wastewater also poses challenges due to the adsorption of lipids onto biomass, which can cause issues like biomass flotation, biomass washout, and mass transport limitations (Hawkes et al., 1995; Hwu et al., 1998; Rinzema et al., 1989; Singh, 2019). Adsorption and accumulation of long-chain fatty acids (LCFA) on the biomass may result in prolonged lag phases in sludge batch experiments (Pereira et al., 2005).

The successful anaerobic degradation of lipid-rich wastewater, as studied by Silva et al. (2014), depends on a balance between LCFA-degrading bacteria and methane-producing archaea. Schink (1997) and Stams et al. (2006) described how certain bacteria break down LCFAs into acetate and hydrogen/formate, which are then converted into methane by archaea. This process is critically dependent on hydrogen-transfer rate between microbes, highlighting the essential nature of these microbial partnerships. The Gibbs' free energy change of acetogenic reactions becomes sufficiently negative at low partial pressure of hydrogen, maintained by a synergistic relationship between acetogenic bacteria and hydrogenotrophic methanogens. The partial pressure of hydrogen needs to be below 10 Pa for maintaining high conversion rates (Schink, 1997; Lalman & Bagley, 2002).

Research indicates enhanced degradation of LCFAs like oleic and palmitic acids, in the anaerobic treatment of lipid-rich wastewater using pre-acclimated biomass (Silva et al., 2014). Commonly, such wastewaters are characterized by concentrations ranges of oleic

and palmitic acids from 100 to 900 mg/L (Cavaleiro et al., 2008). While most studies indeed use anaerobic sludge pre-acclimated to these common wastewater LCFAs, the impact of varying oleate and palmitate concentrations on LCFA degradation is less explored (Hanaki & Nagase, 1981). Notably, high concentrations (above 700 mg/L) of oleic and palmitic acids might inhibit their degradation in a different manner; an effect potentially influenced by their respective unsaturated and saturated characteristics (Pereira et al., 2005). Our present study aimed to evaluate the degradation of oleate and palmitate at varying concentrations using acclimated and non-acclimated sludge from different dairy wastewater treatment reactors. The research included methanogenic activity assays and microbial population analysis through 16S rRNA gene sequencing to understand the roles of different microbes in the LCFA degradation pathway.

5.2 MATERIALS AND METHODS

5.2.1 Analytical methods

Chemical oxygen demand (COD) was determined using Spectroquant Kits (Merck Sharp & Dohme Corp., NJ, USA). Total suspended solids (TSS) and volatile suspended solids (VSS) were determined using gravimetric analysis (Eaton et al., 2005). The lipid content of the sludge was determined following the norm ISO 1443. A COD/ FOG theoretical ratio of 2.88 gCOD/gFOG for palmitic acid and 2.89 gCOD/gFOG was used for converting oleic acid into COD.

5.2.2 Sludge sources

This study utilized three distinct sludge samples. S1 came from a full-scale digester at a dairy plant where lipids are pre-removed (DAF unit), serving as a non-lipid-exposed baseline. Sludge sample S2, derived from a lab-scale AnMBR, was initially inoculated with S1, and later adapted by treating lipid-rich ice-cream wastewater for 635 days. Sludge sample S3, which was sourced from a full-scale anaerobic flotation reactor (AFR) (Biopaq®AFR, Hellendoorn, The Netherlands) treating FOG-rich dairy wastewater for over eight years, provided a second example of lipid-acclimated sludge. The S3 sample would have a potentially different microbial community due to its origin, coming from a brewery wastewater treating anaerobic high-rate reactor.

5.2.3 Assessment specific methanogenic activity

A modified specific methanogenic activity (SMA) test was conducted to assess the effects of selected LCFAs on the anaerobic sludge activity. Oleate and palmitate were selected as the representative LCFAs, considering both their abundance in industrial dairy wastewater and their potential toxic effects on the sludge (Karadag et al., 2015; Pereira et

al., 2002). Different concentrations of synthetic reagent grade commercially available oleic acid and palmitic acid were added as substrates to the test vials.

Biodegradable substances possibly present in the original sludge samples could interfere during the SMA assessment carried out in batch assays. To determine such possible interferences, SMA assays were conducted using both non-incubated (NI) and pre-incubated (PI) sludge samples as inoculum. The PI sludge was incubated at 37°C until no biogas production was observed, which lasted 15 days. The batch assays using the PI and NI sludge samples were carried simultaneously, at identical operational conditions.

For both oleate and palmitate, five batch experiments were carried out using PI sludge, applying initial concentrations of LCFA of 50, 100, 250, 450, and 600 mg /L. Using NI sludge, two batch experiments were carried out at initial concentrations of 250 and 600 mg/L of either oleate or palmitate.

Prior to the batch experiments, the sludge samples were characterized by determining the following parameters: TSS, VSS, COD, and FOG content. At the end of each batch test, the FOG content in the sludge in every batch test was again determined. In addition, the SMAs of the PI sludge samples were assessed (without adding any LCFA) by dosing acetate at an initial acetate concentration of 1.2 g COD/L. These experiments were carried out to assess the maximum methanogenic capacity of the sludge samples. For properly evaluating the LCFA degrading capacity by measuring the methane production rate, methanogenesis should not be the rate limiting step.

The batch experiments were conducted in 120 mL sealed serum bottles with a working volume of 65 mL. Buffer was provided by adding sodium bicarbonate at a concentration of 3.5 g/L in the bottles and the initial biomass concentration was 2.0 g VSS/L. The headspace of the bottles was flushed with nitrogen gas (99.99% N_2, Linde Uruguay LTDA, Montevideo, Uruguay) to create anaerobic conditions. The bottles were incubated at 37°C (Memmert S25, Memmert GmbH + Co. KG) and shaken at 150 rpm in an orbital shaker (MaxQ Orbital shaker, Thermo Fisher Scientific). The batch experiments extended over a duration of approximately 36 days, while the SMA tests on the PI samples were conducted over a 5-hour period, utilizing 15-minute measurement intervals. The amount of produced biogas was determined using a pressure transducer at regular intervals (Colleran et al., 1991). A digital manometer (Flus, ET-922) was used to monitor the pressure increase. The methane content in the biogas produced was measured by liquid displacement, passing the biogas through a 2 M NaOH solution (Casallas-Ojeda et al., 2021). The methane content was corrected considering the non-standard temperature and pressure (STP) conditions in the test vials. The VSS specific methane production rate was calculated by linear regression, using the slope of the recorded methane production curve as suggested by Colleran et al. (1991) and was expressed in mg CH_4-COD/g VSS.d. The

batch experiments were conducted in triplicate; and blanks were carried out in all experiments.

5.2.4 Microbial community analysis

Biomass samples were taken both from the sludge inoculum (S1, S2, and S3), as well as from each batch test carried out at 50 mg/L, 250 mg/L, and 600 mg/L of oleate and palmitate. Sludge sample S1 at 250 mg/L oleate, and sludge sample S2 at 250 mg/L palmitate were not taken for microbial community analysis. The samples were stored at -20^{o}C until performing the DNA extraction. For conducting the DNA extraction, the biomass was first separated by centrifugation (5,000 rpm for 10 min).

The DNA extraction was conducted using the ZR Soil Microbe DNA MiniPrepTM kit (Zymo Research, CA, USA). 16S rRNA (16S ribosomal RNA) gene amplicons were obtained by PCR from the extracted DNA using adapters, barcodes, and the V4 Universal primers set 520F 5´-AYTGGGYDTAAAGNG-3´ and 802R 5'-TACNNGGGTATCTAATCC-3'), as in Claesson et al. (2009). A specific primer set, (340F 5´-CCCTAHGGGGYGCASCA-3´ and 787R 5´-GGACTACVSGGGTATCTAAT -3´), targeting the archaeal 16S rRNA gene region was also used to improve the recovery of methanogens (Pinto & Raskin, 2012; Yu et al., 2005). The PCR products were visualized on a 1% agarose gel electrophoresis. The amplicons were purified using a commercial kit (ZR Zymoclean™ Gel DNA Recovery Kit, USA). 16S rRNA gene amplicon libraries were sequenced on an Ion Torrent PGM (Life Technologies, Thermo Fisher Scientific Inc., MA USA).

Bioinformatic processing was done using the QIIME Pipeline Version 1.9.1. Low quality reads were filtered (criteria: coefficient greater than 25) and sequences were trimmed to remove primers, barcodes, and adapters. Effective reads were obtained, which were further processed to remove chimera and noise. Operational taxonomic units (OTU) were made using the Uclust algorithm (Edgar, 2010) with a 97% identity threshold. The Silva database, release 132, was used for classification with a confidence threshold of 80%.

Principal coordinate analysis (PcoA) was performed to determine differences and similarities of the microbial communities from the following samples: (i) sludge samples taken as inoculum (S1, S2, and S3); and (ii) the sludge samples taken at the end of the different experiments as previously described. The PCoA was performed using the Bray-Curtis similarity index with the PAST software (Hammer et al., 2001).

5.3 RESULTS

5.3.1 Physicochemical characterization and acetoclastic methanogenic activity of the inoculum sludge samples.

The physicochemical characteristics of the inoculum sludge samples (S1, S2, and S3) were determined before (NI) and after (PI) incubation. The results are presented in Table 5.1. The incubation of the sludge samples resulted in a decreased VSS and COD content.

Activated sludge biomass ($C_5H_7O_2N$) typically has a COD/VSS ratio of 1.42 (Hoover & Porges, 1952). This ratio increases to 1.53 with anaerobic biomass ($C_5H_9O_2N$) (Batstone et al., 2000). Lipid/FOG accumulation can raise this ratio up to 2.0 - 2.9 (Ahnert & Krebs, 2021). Sludges S2 and S3 showed increased lipid content, which declined after pre-incubation, with S3 retaining more lipids than S2. The S1 COD/VSS ratio also reduced post-incubation, more than expected.

The maximum SMA values of the three PI sludge samples were determined using acetate as the substrate. The results showed that S1 and S2 exhibited similar SMA values of 1.11 ± 0.12 and 1.17 ± 0.15 g CH_4-COD/gVSS.d, respectively, while S3 showed a higher value of 1.65 ± 0.14 g CH_4-COD/gVSS.d. All the PI sludge samples showed a high methanogenic activity towards acetate, indicating that the three sludge samples were appropriate for testing the methanogenic activity using oleate and palmitate as the substrate (Pereira et al., 2005).

Table 5.1 – Physicochemical characterization of the inoculum sludge samples. The average values are presented.

Sludge	TSS (g/L)	VSS (g/L)	COD (g/L)	COD/VSS
S1-NI	41.8 ± 0.2	16.8 ± 0.1	23.1 ± 0.2	1.39
S1-PI	36.4 ± 0.2	15.3 ± 0.4	18.5 ± 0.2	1.21
S2 -NI	8.5 ± 0.1	6.5 ± 0.2	11.2 ± 0.4	1.72
S2-PI	6.6 ± 0.3	4.6 ± 0.2	7.0 ± 0.1	1.52
S3-NI	4.5 ± 0.1	3.2 ± 0.3	6.8 ± 0.2	2.13
S3-PI	4.1 ± 0.1	2.7 ± 0.3	5.4 ± 0.2	2.00

5.3.2 Methanogenic activity evaluations

Pre-incubated sludge evaluations

Figure 5.1 compares cumulative methane production (CMP) from sludges when fed oleate versus palmitate. Higher methane yields and production rates were seen with oleate for all sludges, consistent with prior studies. All samples experienced a tree to four days lag phase before biogas production began, with peak methane rates in the first ten days, then plateauing. With palmitate, increased initial concentrations up to 250 mg/L enhanced methane production, which then declined at higher concentrations.

Figure 5.2a presents maximum methane production from pre-incubated sludge, and Figure 5.2b shows the corresponding production rates. Observed methane yields were significantly lower than theoretical maximums, particularly at LCFA concentrations above 250 mg/L. Methane yields were lower with palmitate except at 250 mg/L, where near-theoretical yields were seen. Sludge S3 showed better adaptation to LCFAs but still experienced inhibition at concentrations above 450 mg/L.

The methane production rates (Figure 5.2b) indicated that oleate conversion rates increased with concentrations up to 450 mg/L, while palmitate did not show this trend and had overall lower rates. Early oleate inhibitory effects were less pronounced, while palmitate results were more variable.

Non-incubated sludge evaluations

Batch experiments using non-pre-incubated sludge were performed to evaluate the interferences of residual substrates present in the raw sludge, when dosing the LCFAs. Only two concentrations of oleate and palmitate were evaluated, i.e., 250 and 600 mg/L.

The results from the batch experiments shown in Figure 5.3 indicated that the assessed CMP for the three evaluated sludge samples were always higher than the CMP observed in the PI sludge samples (Figure 5.2). Strikingly, high CMP values were found for the blank, non-fed incubations.

5.3.1 FOG mass balances in PI sludge samples

Table 5.2 and Figure 5.4 present the changes in FOG content in the sludge samples. After pre-incubating the sludge samples, residual FOG concentrations were still present (Table 1), likely consisting of non-biodegradable FOG under the prevailing pre-incubation conditions. At the start of the batch incubations, a known dose of LCFAs were added to each sludge sample, and the lipids content were determined again at the end of the batch experiments for assessing an FOG mass balance.

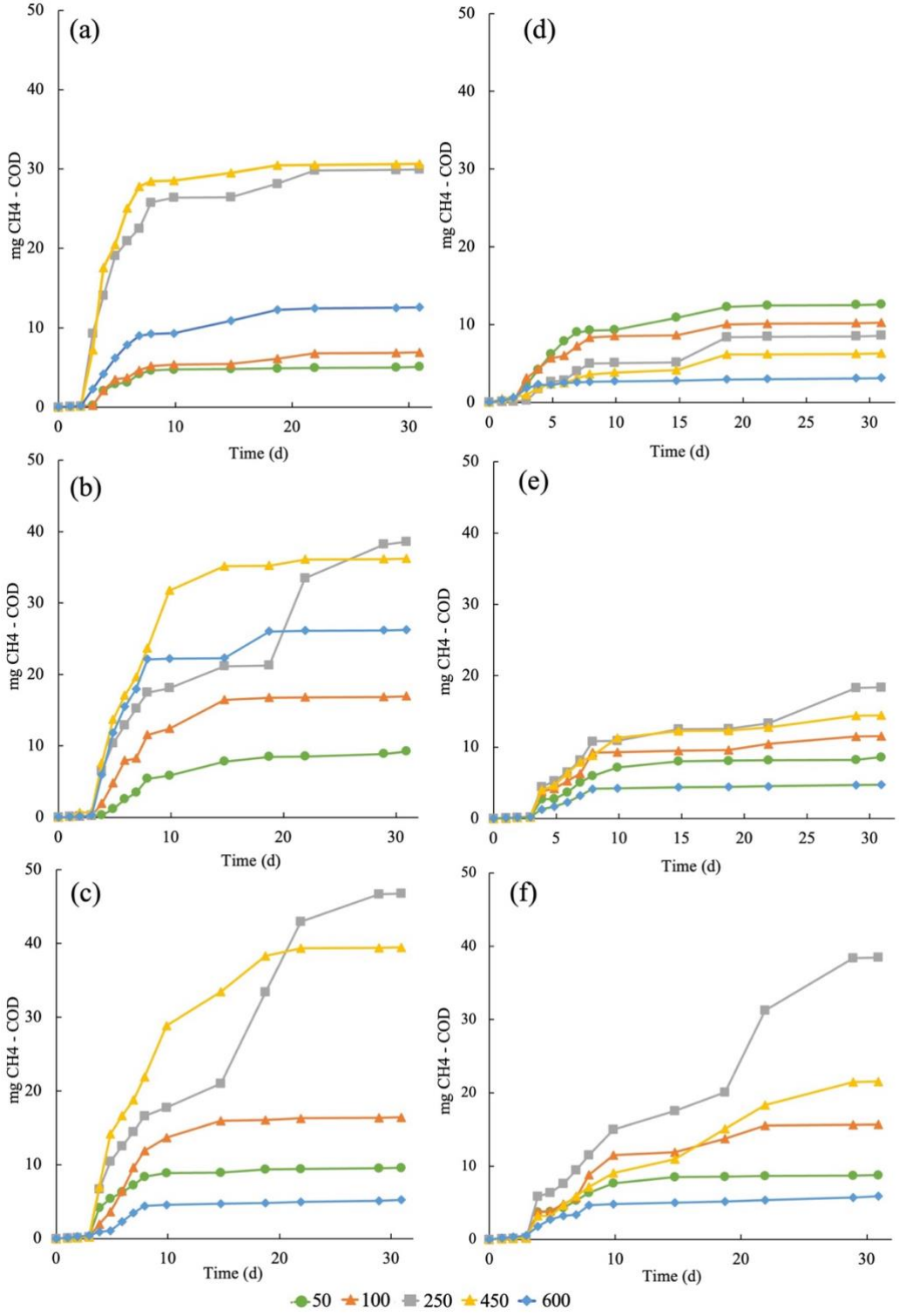

Figure 5.1 – Cumulative methane production exhibited by the PI sludge samples S1 (a, d), S2 (b, e) and S3 (c, f) at different oleate (a, b, c) and palmitate (d, e, f) concentrations. Average values are presented (maximum standard deviation of 10% were obtained – not shown in the figures); The PI blanks did not produce any methane.

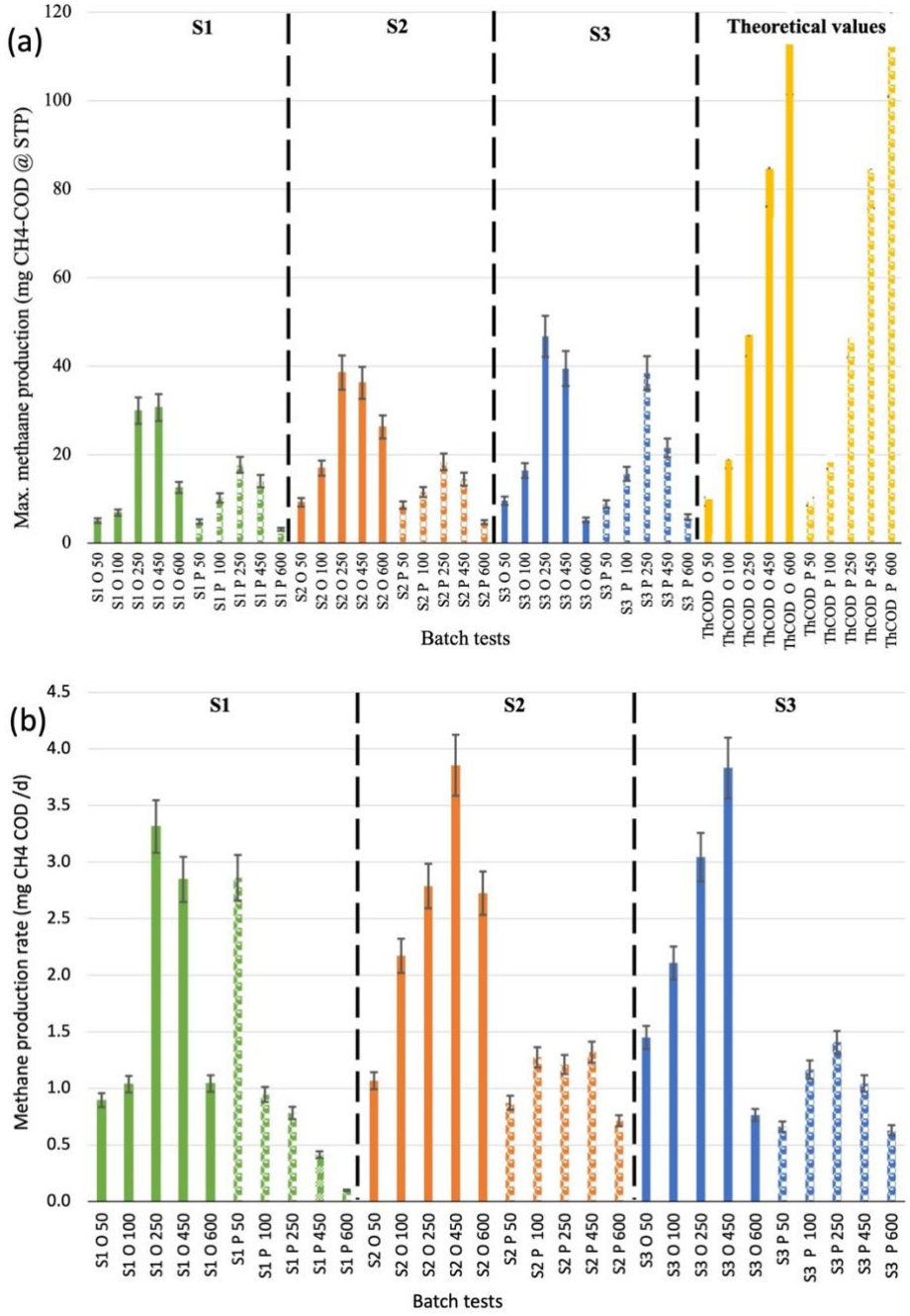

Figure 5.2 – (a) Maximum cumulative methane productions assessed with the PI sludge samples. S1: green; S2: orange; S3: blue. The solid color shows the experiments carried out with oleate, while the dashed color with palmitate. In yellow, the theoretical maximum cumulative methane productions based on the COD balance are shown; (b) Methane production rates for the PI sludge samples. S1: green; S2: orange; S3: blue. The solid color indicates oleate as the substrate, while the dashed color indicates palmitate. Average values are presented; the maximum standard deviation was 10%.

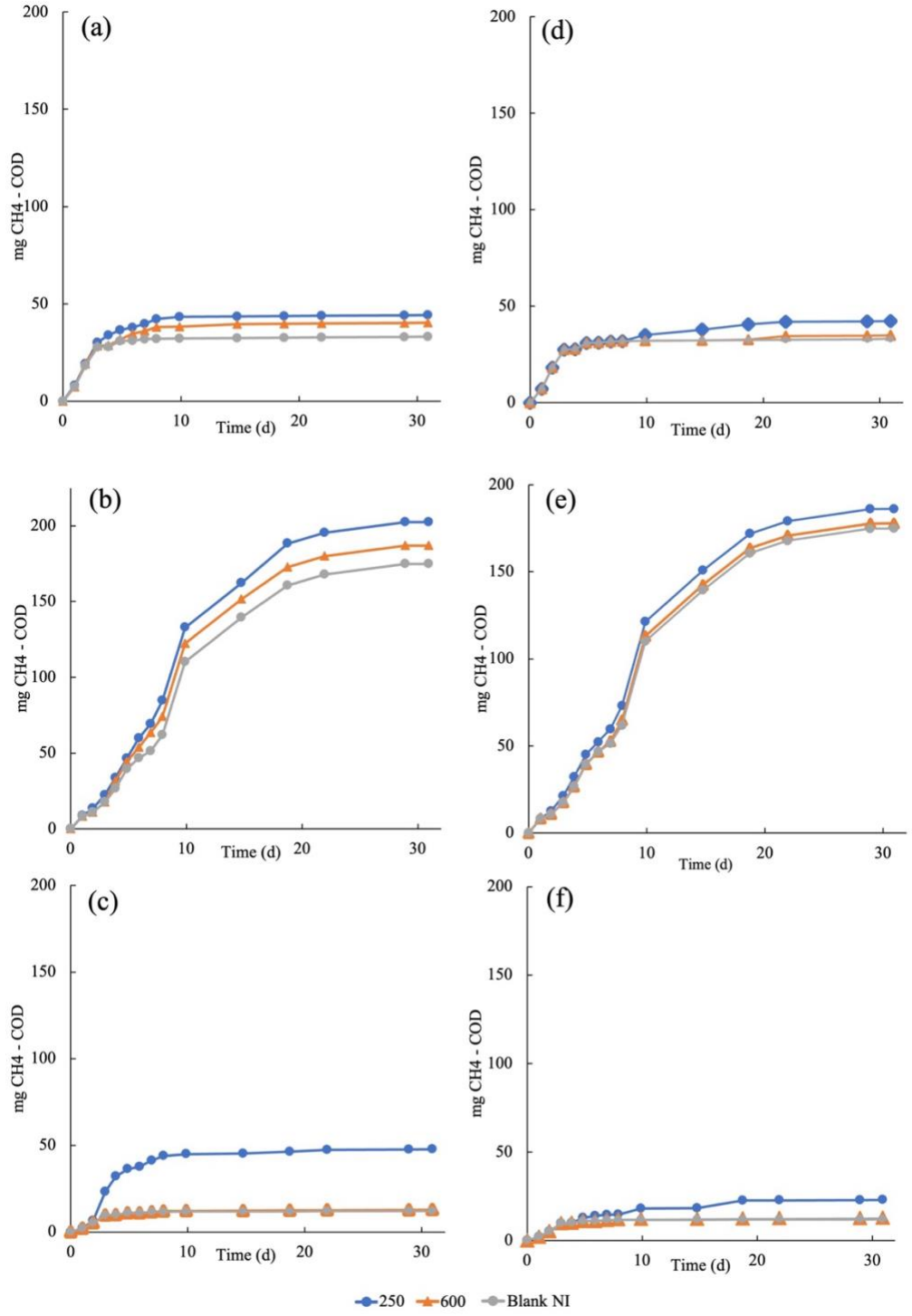

Figure 5.3 – Cumulative methane production exhibited by the NI sludge samples (S1, S2 and S3) at different oleate (a, b, c) and palmitate (d, e, f) concentrations. Average values are presented (maximum standard deviation of 10% were obtained – not shown in the figures). The NI blanks are shown in the figures.

Table 5.2 *– FOG mass balances, expressed as COD, for the PI sludge samples when dosing oleic acid (O) and palmitic acid (P). Average values are presented with a maximum standard deviation of 10%.*

Batch test	Initial FOG - COD (mg/L)	Oleic/Palmitic acid addition COD (mg/L)	Initial Batch test FOG - COD (mg/L)	Final FOG - COD (mg/L)	Degradation COD (mg/L)	Oleic/Palmitic acid degradation (%)
S1 O50	144	144	288	201	87	60
S1 O250	144	720	864	420	444	62
S1 O600	144	1728	1872	1694	178	10
S2 O50	1134	144	1278	1080	144	100
S2 O250	1134	720	1854	1280	574	80
S2 O600	1134	1728	2862	2450	412	24
S3 O50	459	144	603	405	144	100
S3 O250	459	720	1179	400	720	100
S3 O600	459	1728	2187	2100	87	5
S1 P50	144	144	287	220	68	47
S1 P250	144	720	864	630	234	32
S1 P600	144	1728	1872	1820	52	3
S2 P50	1134	144	1278	1100	144	100
S2 P250	1134	720	1854	1580	273	38
S2 P600	1134	1728	2862	2790	72	4
S3 P50	459	144	603	460	143	100
S3 P250	459	720	1179	585	594	83
S3 P600	459	1728	2187	2100	87	5

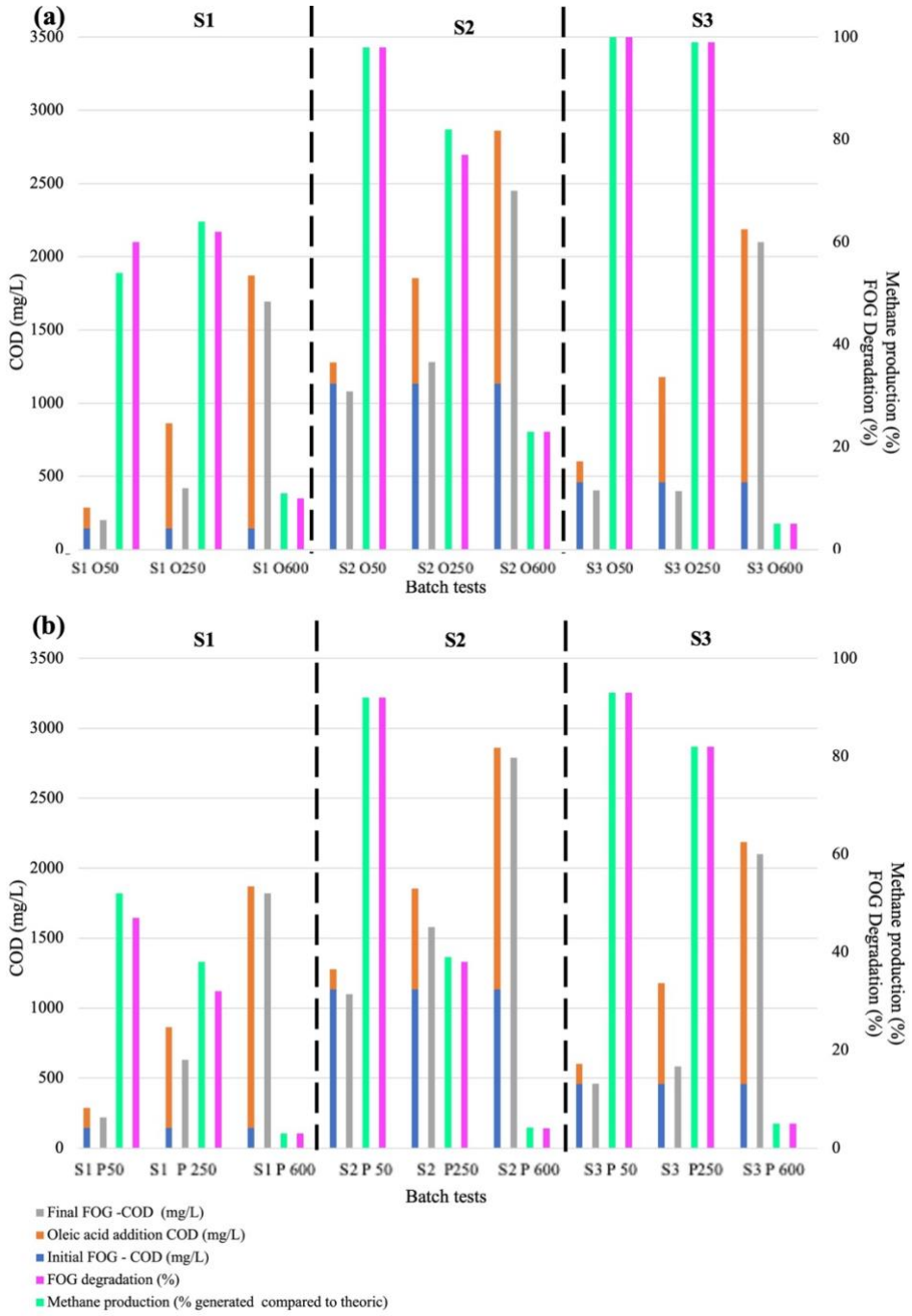

Figure 5.4 – FOG-COD mass balance for the PI sludge samples dosing (a) oleate; and (b) palmitate. From left to right bars: initial FOG-COD (blue) stacked with oleate/palmitate addition (orange); final FOG-COD (grey); methane production as % from theoretical amount (green); FOG degradation % (pink). X- axis: batch tests; sludge samples (S1, S2 and S3) at different oleate (O) and palmitate (P) at different concentrations (50, 250, 600 mgLCFA/L). Left y-axis: COD concentration in mg/L; right y-axis: Methane production in % and FOG degradation in %.

5.3.2 Microbial community analysis

Community analysis - Bacteria

The microbial community composition was quite different at the phylum level for the three evaluated sludge samples. As shown in Figure 5a, the *Thermotogae* phylum was dominant in the sludge samples S2 and S3 with 60% and 71% relative abundance, respectively. In the sludge sample S1, this phylum was practically absent at a relative abundance of only 3%. The sludge S1 exhibited other dominant phyla such as the *Proteobacteria*, *Acetothermia*, and the predominant phylum *Chloroflexi* with relative abundances of 15%, 30%, and 30%, respectively. Overall, the sludge S1 showed a broader variety of phyla compared to S2 and S3. Sludge samples S2 and S3 were acclimated to lipids, while S1 was not.

The relative abundance at the family level is shown in Figure 5b. Also at this level, a more diverse microbial community was observed for the sludge S1 compared to the sludge S2 and S3. *Aquaspirillaceae* and *Burkholderlaceae* were the dominant families in the sludge S1, exhibiting a relative abundance of approximately 20% each. *Petrotogaceae* and *Synergistaceae* were the dominant families in the sludge S2 with a relative abundance of approximately 40% each. *Kosmotogaceae* was the predominant family in the sludge S3 at a relative abundance of approximately 80%.

Community analysis – Archaea at genus level

The microbial populations were also determined for archaea. The universal primer used for bacterial analysis was not very effective detecting methanogens; hence, an archaeal primer was used instead (Fischer et al., 2016).

The *Methanosaeta* archaea were the dominant species in sludge samples S1 and S3 at a relative abundance of 80%, while the *Methanosarcina* archaea were the dominant species in the sludge S2 at a relative abundance of 60% (Figure 5.5c). The *Methanosaeta* relative abundance increased in the sludge S1 at high concentrations of oleate and palmitate (600 mg/L) along with a decrease in the *Methanolinea* abundance.

Regarding the sludge S2, the *Methanosarcina* relative abundance decreased when dosing either oleate or palmitate at 600 mg/L, while the *Methanosaeta* and *Methanobacterium* relative abundance increased.

For the S3 sludge, the inoculum microbial community showed the highest relative abundance of *Methanosaeta* (about 80%) and *Methanobacterium* (20%). After the addition of the oleate and palmitate at 600 mg/L, a modest increase in relative abundance of *Methanobacterium* was observed. The genus *Methanobacterium* is commonly the predominant hydrogenotrophic methanogenic found in anaerobic digesters (Siegert et. al., 2015).

The presence of *Methanobacterium* might be related to the ubiquitous presence of an electron sink in the syntrophic consortia, consisting of acetogens and methanogens, for the required β -oxidation reactions during LCFA degradation.

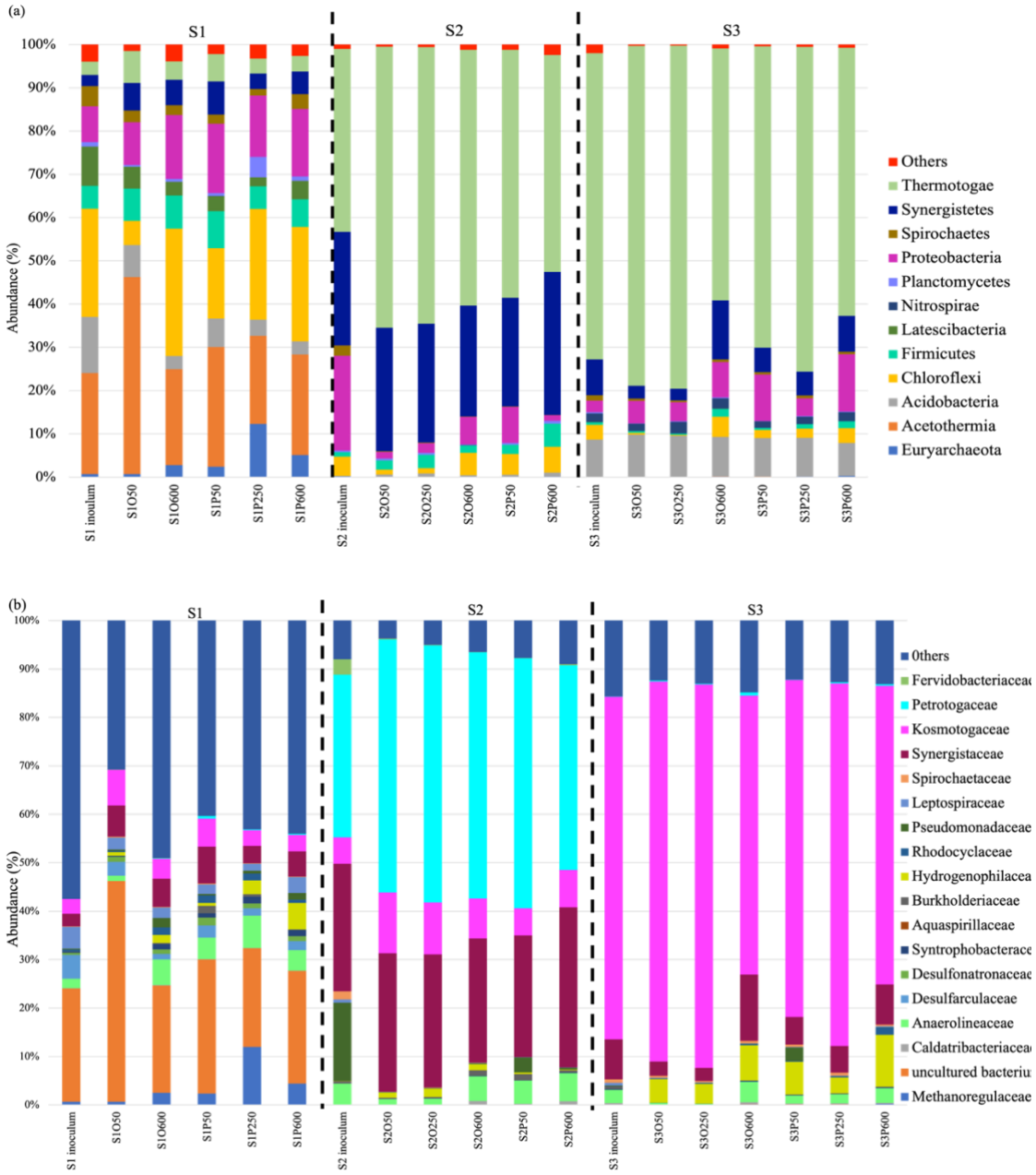

Figure 5.5 - Microbial community composition according to the 16S rRNA gene analysis using the Universal primers in the samples taken from the three inoculum and from the different experiments. The different colors represented the relative abundance at: (a) Phylum level; and (b) Family level. X-axis: inoculum for each sludge sample; batch tests; sludge samples (S1, S2 and S3) at different oleate (O) and palmitate (P) concentrations (50, 250, 600 mgLCFA/L). Y-axis: Abundance (%)

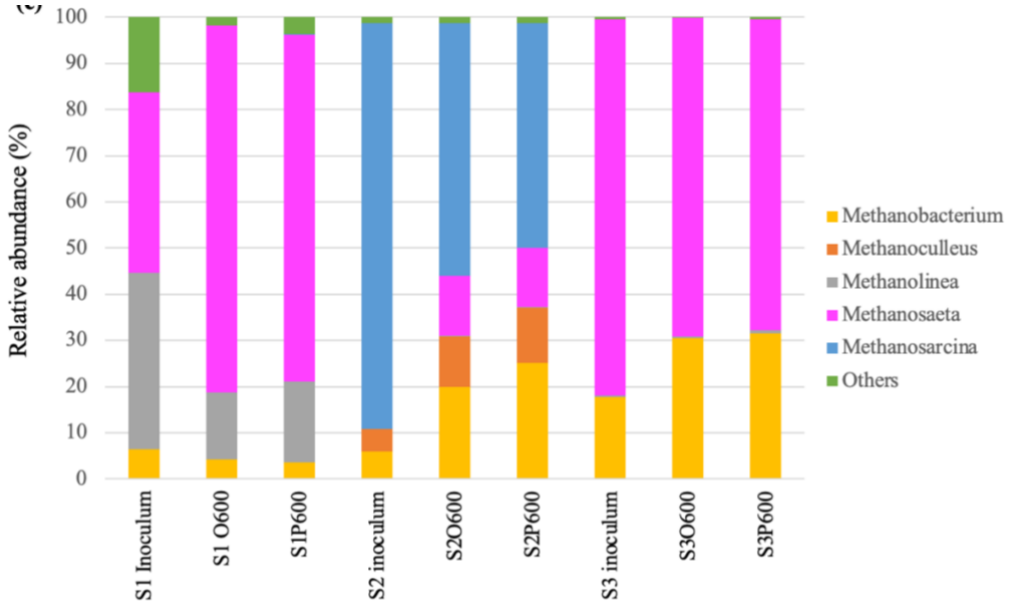

Figure 5.6 – Microbial community composition according to the 16S rRNA gene analysis using archaeal primers in the samples taken from the three inoculum and from the different experiments. The different colors represented the relative abundance at archaeal genera. X-axis: inoculum for each sludge sample; batch tests; sludge samples (S1, S2 and S3) at different oleate (O) and palmitate (P) concentration of 600 mgLCFA/L. Y-axis: Abundance (%)

Principal coordinate analysis (PCoA) for bacteria

The PCoA used the Bray-Curtis index to evaluate differences and similarities in bacterial communities of sludge samples, based on 16S rRNA gene data at the family level. Analysis across all samples revealed three distinct clusters (S1, S2, and S3) representing different sludges and their reactions to various LCFA concentrations. Separate analyses for each sludge (S2, S3, S1) showed groups corresponding to the original sludge, and those incubated with oleate and palmitate, highlighting shifts in microbial composition due to LCFA exposure.

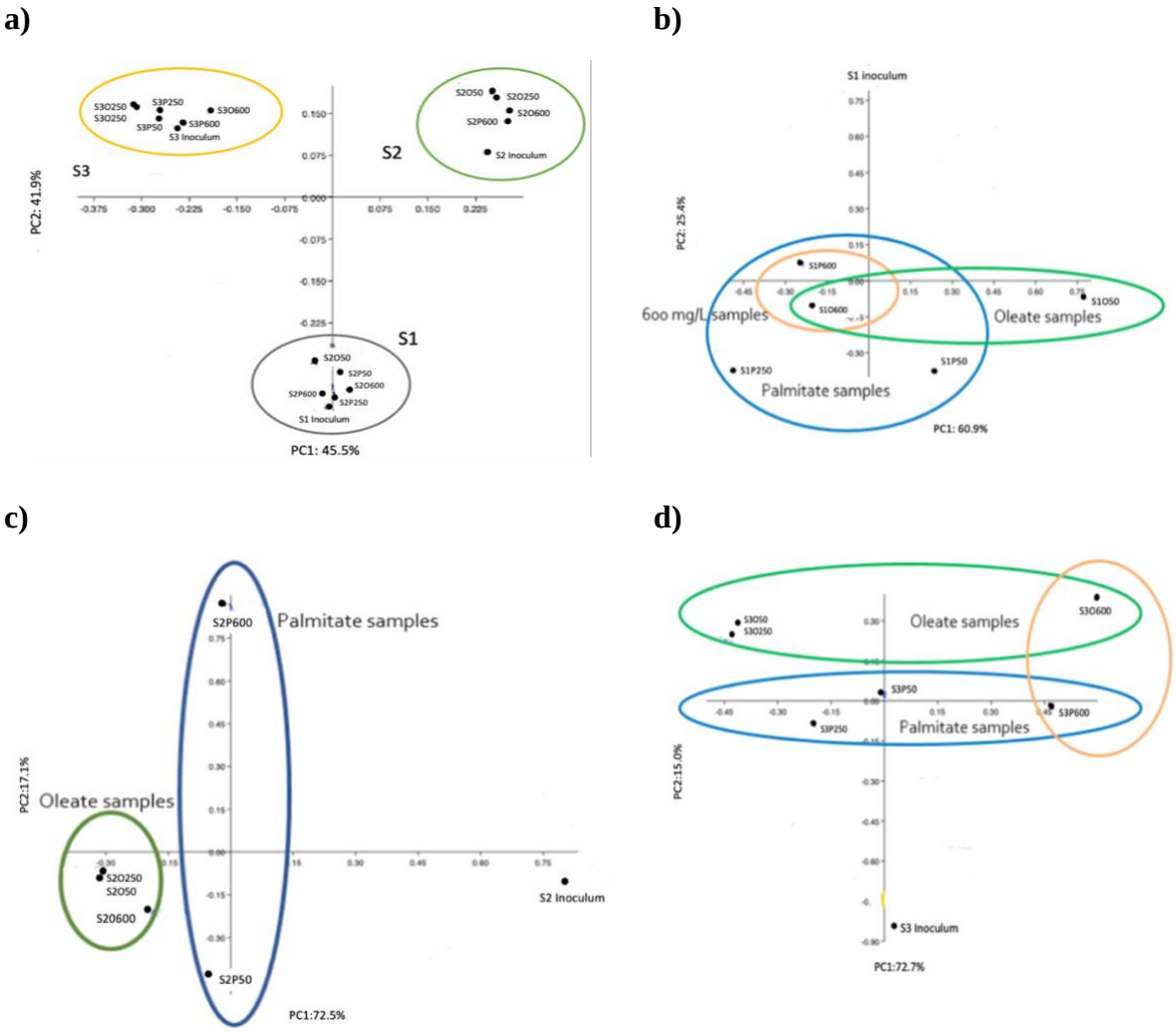

Figure 5.7 – PCoA performed using the bacterial composition classified at family level a) PCoA performed using all the samples; b) PCoA performed using the results from the samples S1; c) S2 PCoA; d) S3 PCoA. Note: The orange circle in figures c and d indicates the sludge samples S1 and S3 at the highest dose of oleate and palmitate of 600 mg/L. The dots indicate the inoculum for each sludge sample; batch tests; sludge samples (S1, S2 and S3) at different oleate (O) and palmitate (P) at concentrations (50, 250, 600 mgLCFA/L)

5.4 DISCUSSION

5.4.1 Physicochemical characterization and acetoclastic methanogenic activity of the inoculum sludge samples.

Physicochemical characterization of the sludge samples showed differences in the characteristics of each inoculum, attributable to their different origins (Table 5.1). Sludge S1 derived from an anaerobic digester, not acclimated to lipids, was characterized by high TSS and COD values. Sludge samples S2 and S3, obtained from high-rate anaerobic treatment (HRAT) reactors, showed lower TSS and COD values compared to S1; COD/VSS ratios were according to expectations.

The incubation period was effective in reducing both the volatile solids and the COD content of the sludge in the three evaluated samples regardless their origin (Table 5.1). Some of the organic matter present in the sludge consisted of FOG (Table 5.2, Figure 5.4). The pre-incubation period lasted until no methane production was observed. At the end of the pre-incubation period, the sludge samples still contained residual organic matter, which likely was non-biodegradable FOG-COD. The pre-incubation period was effective in conditioning the sludge samples for conducting the subsequent experiments. Regarding the assessed SMA values of the sludge samples, S3 showed the highest SMA compared to S1 and S2. Results indicated that all three sludge samples exhibited appropriate SMA values to be used as inoculums for further evaluating the LCFA degradation capacity (Alves et al., 2001; Cavaleiro et al., 2010; Silva et al., 2014).

5.4.2 Methanogenic activity using LCFA as substrate

Cumulative methane production tests using pre-incubated sludge

The batch incubations using oleate as the substrate produced more methane than the tests using palmitate. Alves et al. (2009) reported similar observations. Moreover, the same authors observed that unsaturated fatty acids (oleate) could be degraded by a wider range of bacteria compared to saturated fatty acids (palmitate). Recently, it has been shown that the biochemical pathways involved in the degradation of saturated fatty acids, such as palmitate, requires an additional step for degrading the fatty acids, compared to unsaturated fatty acids, such as oleate (Holohan et al., 2022; Pereira et al., 2001). Following current hypothesis, prior to the β - oxidation pathway, the saturated fatty acid needs to pass a preliminary dehydrogenation and hydration step at the alpha and beta carbons, eventually making the degradation process more complex. Such a dehydrogenation step is not observed for the unsaturated fatty acids, which explains the increased CMP when degrading oleic acid compared to palmitic acid.

At the lowest oleic acid and palmitic acid concentration range, i.e., from 50 to 250 mg/L, the three sludge samples performed much better, resulting in a higher CMP, than at LCFA concentrations of 450 and 600 mg/L. The S2 and S3 sludge samples, acclimated to lipids conversion, achieved full conversion of the low LCFA concentrations to methane, in agreement with the theoretically calculated values. Sludge sample S1, which was not acclimated to lipids, only partially converted LCFA, even at 50 mg/L. Several authors reported similar findings regarding the role of sludge acclimation in LCFA degradation (Kougias et al., 2016; Silva et al., 2014; Ziels et al., 2016). Although sludge samples S1 and S2 were originally from the same source, sample S2 was exposed to lipid rich substrate in an AnMBR during a period of 500 days.

Sludge sample S3 showed highest LCFA conversion to methane compared to S1 and S2 sludge, likely attributable to the inoculum origin. The S3 sludge was obtained from an industrial scale AFR, treating dairy ice cream wastewater with a COD concentration of 10 - 25 g/L consisting of 50% FOG (Frijters et al., 2014). Apparently, the S3 sludge was well adapted to oleate and to a lesser extent palmitate.

In the study by Cavaleiro et al. (2008), sludges acclimated to oleate and palmitate individually showed complete methane conversion at concentrations ranging from 100 to 900 mg/L, with no observed inhibition. In contrast, our study, which utilized sludges (S2 and S3) previously acclimated to a mix of lipids and long-chain fatty acids (LCFAs), demonstrated a decline in maximum methane production at concentrations exceeding 250 mg/L for both oleate and palmitate. Complete conversion to methane was only achieved at concentrations lower than 100 mg/L for each LCFA. These observations suggested that the prior acclimation to a complex mixture of wastewater components, despite pre-incubation, impacts subsequent degradation efficiency. The sludge in our study, which was exposed to a diverse range of lipids and LCFAs, developed a microbial community that, while more versatile, might lack the specialized efficiency for degrading high concentrations of specific fatty acids like oleate and palmitate (Ziels et. al., 2016).

In our study, a decline in oleate conversion rates was observed, leading to reduced methane production beyond a certain oleate concentration. This trend was also seen with palmitate. Unsaturated LCFAs, with their lower melting points and higher fluidity, transfer more easily to microorganisms (Wu et al., 2017), impacting both inhibition and degradation rates (Zonta et al., 2013). As a result, high concentrations of LCFA, especially oleate, inhibit both hydrogenotrophic and aceticlastic methanogenic activity and can limit the kinetics of syntrophic β-oxidizing bacteria (Hanaki et al., 1981; Hwu and Lettinga, 1996; Silva et al., 2016; Sousa et al., 2013).

The assessed methane production rates (Figure 2b) were lower than reported by Cavaleiro et al., (2008), who also used oleate and palmitate as the substrate in concentrations ranging from 100 to 900 mg/L. The authors acclimated their sludge with oleate and

palmitate for approximately 100 days before dosing oleate and palmitate. In our study, the inoculum sludges S2 and S3 that showed best performance, were acclimated to lipid-rich wastewater with a variety of LCFAs present as well as skimmed milk.

5.4.3 FOG mass balances on PI sludge samples

Table 5.2 and Figure 5.4 show the disappearance of LCFAs next to the methane production, relative to the theoretically expected value. Assuming that the initial FOG fraction of the PI sludge samples was non-biodegradable, then, only the added LCFA would be potentially biodegradable during the tests. With oleic acid as the substrate, the observed FOG removal agreed with the observed methane production, shown in Figures 2a, b, and c. The S2 and S3 sludge samples exhibited higher FOG removal compared to the S1 sludge. In addition, FOG removals using S2 and S3 sludge samples were high, applying oleate concentrations of 50 and 250 mg/L, whereas very low removals were observed at 600 mg/L. Likewise, when dosing palmitate, observed FOG removal agreed with the observed methane production, being high at low concentrations and low at high concentrations.

The S1 sludge seemed less effective in degrading LCFAs compared to the S2 and S3 sludge samples. In addition, high concentrations of LCFAs (up to 600 mg/L), inhibited LCFA conversion. Results showed a clear correlation between LCFA removal expressed as FOG-COD, with the methane production expressed as relative value of the expected maximum theoretical methane production value (Figure 4). For instance, for S1 sludge, FOG-COD removal efficiencies were 60, 62 and 10% for oleate concentrations of 50, 250, and 600 mg/L, respectively. For the same concentrations, results in Figure 2a showed relative methane productions of 54%, 63%, and 11%, respectively.

The FOG mass balances matched the CMP values obtained in the PI sludge incubations, indicating that the biodegradable LCFAs were indeed degraded and further converted into methane. The different pre-incubated sludge samples contained different initial concentrations of non-biodegradable FOG-COD after the pre-incubation step (Table 5.1). The S2 sludge exhibited the highest initial FOG-COD concentration after pre-incubating the sludge. However, S2 performed as good as the sludge sample S3 in degrading the LCFAs. S3 contained approximately half of the FOG-COD concentration after pre-incubation.

Sludge sample S1 contained the lowest concentration of the non-biodegradable FOG-COD after the pre-incubation period and exhibited the worse performance among the three evaluated sludge samples regarding the breakdown of the added LCFAs. Apparently, the remaining non-biodegradable FOG-COD content after the pre-incubation period did not exhibit any inhibitory effects on the breakdown of the added LCFAs and on their final conversion to methane.

5.4.4 Microbial community analysis

In our study, the three sludge samples exhibited distinct microbial compositions, influenced by their source. The sludges S1 and S3 originated from a full-scale system, whereas sample S2 was derived from a lab-scale AnMBR setup. Understanding the differences in these community compositions and their dynamics is crucial for enhancing wastewater treatment processes, as highlighted in Matsuda et al. (2010). The exposure to LCFAs in full-scale systems, as in lab-scale systems, causes notable shifts in microbial communities, marked by an increase in syntrophic LCFA-degrading bacteria like *Syntrophomonadaceae* (Sousa et al., 2009). These shifts affect the functional stability and adaptability of syntrophic and methanogenic populations, which are vital for anaerobic wastewater treatment (Ziels et al., 2017). Studies show methanogens' resilience to high LCFA levels, underlining the importance of monitoring microbial dynamics for optimal treatment performance (Salvador et al., 2013).

Community analysis - Bacteria

The sludge samples S2 and S3, acclimated to degrade lipids, exhibited the presence of *Thermotogae, Synergistetes* and *Firmicutes* phyla at much higher relative abundances, compared to the non-acclimated sludge sample S1. The presence of those phyla was confirmed both in the sludge used as inoculum and in the samples taken after the incubation with different types and concentrations of LCFAs. The *Thermotogae* phylum, both thermophiles and mesophiles, are able to degrade a large variety of substrates producing hydrogen gas as a by-product (Gupta et al., 2014). Some *Thermotogae* species were found in the microbiota of animal gut, significantly increasing their abundance when the animals were exposed to lipid-rich/high-fat substrates (Ni et al., 2014). Similar observations were also reported for the *Synergistetes* phylum, which includes a group of 20 gram-negative anaerobic bacteria (Roquetto et al., 2015). Kurade et al., (2019) reported a fivefold increase in the S*ynergistetes* population in an anaerobic digester treating municipal wastewater sludge (primary sludge and aerobic secondary sludge, as well as anaerobically digested sludge) after the addition of FOGs. Similar findings were also reported by Callejas et al., (2019). Thus, the presence of these families could be related to the adaptation of the sludge to degrade LCFAs. On the other hand, the *Proteobacteria* phylum was clearly more abundant in the S1 sludge, which much less relative abundances in the S2 and S3 lipids-acclimated sludge samples. Previous studies associated the presence of the *Syntrophomonadace*ae and *Syntrophaceae* families, within the *Firmicutes* phylum, to the FOG digesting properties of the sludge (Palatsi et al., 2010; Sousa et al., 2007). At least fourteen acetogenic bacteria degrading LCFA in syntrophy with hydrogen scavengers have been reported to belong to those two families (Alves et al., 2009; Baserba et al., 2012; Callejas et al., 2019). Surprisingly, species of this phylum were barely found in the lipid-acclimated sludge samples S2 and S3 (not shown in Figure 5a); the community size was more abundant in the non-acclimated sludge S1 (not shown

in Figure 5.5b). These types of bacteria are syntrophic, working in partnership with hydrogen scavengers.

The inoculum sludge S1 was taken from a digester equipped with an upstream preliminary treatment for removing FOG; so, only little FOG reached the anaerobic digester. Nonetheless, a small amount of fats could have passed to the digester promoting the development and presence of the *Syntrophomonadaceae* family. The S2 and S3 raw sludge samples did not exhibit the presence of this phylum. The composition of a specific microbial community in a particular acclimated sludge sample would be strongly influenced by the microbial composition of the inoculum sludge. Still, the degradation of LCFAs would be possible due to the capacity of numerous species of acetogens to switch to the β - oxidation pathway when necessary (Kougias et al., 2016).

The *Petrotogaceae* and *Kosmotogaceae* families, both belonging to the *Thermotogae* phylum (Bhandari & Gupta, 2014), were the dominant families in the S2 and S3 sludge samples, respectively. The *Synergistaceae* family was also reported in these sludge samples. The inoculum sludge S2 also exhibited communities from the *Pseudomonaceae* family (*Proteobacteria* phylum). The relative abundance of this family seemed to decrease after dosing the LCFAs as also reported in previous studies (Baserba et al., 2012; Kurade et al., 2019).

The *Petrotogaceae*, *Kosmotogaceae*, and *Synergistaceae* families were not found, or found at a very low relative abundance, in the non-acclimated S1sludge. In the S3 sludge, at the lowest LCFAs concentrations of 50 and 250 mg/L, the *Kosmotogaceae* seemed to dominate. However, when dosing 600 mg/L of either oleate or palmitate, the *Synergistaceae* family dominated. Moreover, as the dosage of the LCFAs increased, the relative abundance of the family *Hydrogenophilaceae* (*Chloroflexi)* also increased. These findings were also reported in other studies when exposing anaerobic sludge to lipid-rich wastewater (Ntougias et al., 2013). Likely, high LCFA concentrations exert different degrees of bactericidal effects leading to the observed differences in relative abundance.

In sludge S2, no major changes in the relative abundance were observed due to the addition of LCFAs; the most abundant families were *Petrotogaceae* and *Synergistaceae*. The occurrence and abundance of these two families were reported already in other studies when treating lipid rich wastewater (Bhandari & Gupta, 2014; Hatamoto, et al., 2007a).

As reported in previous studies treating lipid-rich wastewater, this study also confirmed the presence of a relative high abundances of the *Kosmotogaceae*, *Petrotogaceae* (*Thermotogae* phylum), and *Synergistaceae* families in the lipid-acclimated sludge samples S2 and S3 (Baserba et al., 2012; Bhandari & Gupta, 2014; Hatamoto et al., 2007b; Palatsi et al., 2010). The *Anaerolineaceae* family within the *Chloroflexi* bacteria phylum, and the *Pseudomonadaceae* family within the phylum Proteobacteria also have been

previously reported in sludge treating lipid-rich wastewater. However, these populations were not found in our studies at a high relative abundance (Nakasaki et al., 2020; Bialek et al., 2010; Shigematsu et al., 2006; Sousa et al., 2008).

The degradation of long-chain fatty acids (LCFA) involves a transition from acetogenic oxidation to β - oxidation, which is influenced by specific conditions. Saturated and unsaturated LCFA are degraded to acetate and hydrogen via β-oxidation (Sousa et al., 2009). However, the inhibitory effects of long-chain fatty acids on volatile fatty acid (VFA) degradation and β-oxidation can impact the process (Shin et al., 2003). Additionally, the kinetics of LCFA inhibition on acetoclastic methanogenesis, propionate degradation, and β-oxidation are crucial in determining the transition (Kim et al., 2004). Under methanogenic conditions, LCFA degradation requires a syntrophic association of LCFA-degrading anaerobes and hydrogenotrophic methanogens (Sousa et al. 2009). The oxidation of LCFA is thermodynamically unfavorable in such environments unless the consumption of reducing equivalents (hydrogen and formate) is coupled with this oxidation (Hatamoto et al., 2007a). LCFA feeding frequency also has been identified as an essential parameter for kinetics and microbial stability during anaerobic degradation of LCFA, where pulse feeding may trigger the activity of β - oxidizing bacteria and improve LCFA degradation (Ziels et al., 2016).

Community analysis - Archaea at genus Level

The dominant groups in the raw sludge S1 included mostly *Methanosaeta* (39%) and *Methanolinea* (29%); a small fraction of *Methanobacterium* was also reported. The addition of the LCFAs led to an increase in the relative abundance of the *Methanosaeta* population.

In sludge samples S2 and S3, the samples containing the largest concentrations of the added LCFAs, exhibited an increase in the relative abundance of the hydrogenotrophic *Methanobacterium*. Still the dominant species in S2 and S3 were *Methanosarcina* and *Methanosaeta*, respectively. Duarte et al. (2018) also reported the dominance of the hydrogenotrophic *Methanobacterium* over the acetoclastic *Methanosaeta* when anaerobically treating LCFA-rich wastewater under mesophilic conditions. That is, the more acclimated the sludge to LCFAs, the higher the relative abundance of *Methanobacterium.*

In the S2 sludge, the hydrogenotrophic *Methanobacterium* exhibited a higher relative abundance over the acetoclastic *Methanosaeta*. However, in the S3 sludge the *Methanosaeta* exhibited the highest relative abundance (81%) followed by the *Methanobacterium* (18%). In agreement, Raskin et al. (1994) reported that the acetoclastic *Methanosaeta* still exhibited an important relative abundance in the LCFA adapted sludge microbial community.

No major diversification in archaea genus level was observed for the sludge S3 after the LCFAs addition. However, the relative abundance of the *Methanobacteria* increased when dosing the LCFAs at concentrations of 600 mg/L. Likely, the *Methanobacteria* genus is less susceptible for high LCFA concentrations. *Methanobacteria* are the major hydrogenotrophic methanogenic genus commonly found in anaerobic digesters. In addition, their presence is crucial for the development of syntrophic relationships with acetogens to promote the β - oxidation process.

Principal coordinate analysis (PCoA) for bacteria

The PCoA analysis shown in Figure 5.7a indicated the diverse nature of the different sludge types used in this research. It should be noted that the batch tests were conducted over a period of only 36 days following a single substrate dose at day 0. Likely, the short incubation time and the single substrate dose was insufficient to observe pronounced changes in the microbial community that can be ascribed to bacterial growth. However, high LCFA doses may exert different levels of bactericidal effects to bacteria and archaea. When observing the PCoA of the acclimated sludge samples S2 and S3 in Figures 5.7c and 5.7d, respectively, three clusters were clearly observed corresponding to the inoculum and to the samples taken after dosing oleate and palmitate.

On the other hand, when observing the PCoA for the S1 sludge (Figure 5.7b), some overlapping among the different communities were observed. Still, the sludge samples containing the LCFA addition in sludge S1 differed from the raw sludge. Another interesting observation was the grouping of the microbial populations when dosing the LCFA at the highest concentrations in the sludge samples S3 and S1 (Figure 5.7d and b – orange circles). The sludge samples that were exposed to 600 mg/L of oleate and palmitate exhibited similarities in the microbial community dynamics. Results indicated that the high LCFA dose negatively impacted specific species, having led to distinct changes in the microbial population.

5.4.5 Treatment Implications

This study was part of a large research program on enhancing anaerobic wastewater treatment in the presence of high concentrations of FOG. Current results showed that the degradation of LCFAs is impeded when their concentration surpasses approximately 250 mg LCFA/L, equivalent to 720 mg COD/L, or 125 mg LCFA/g VSS. Retarded LCFA conversion may lead to LCFA accumulation in continuous-flow systems, causing issues like toxicity and operational challenges in high-rate anaerobic treatment (HRAT) systems, including biomass flotation and sludge degranulation. To mitigate these effects, process design modifications for anaerobic treatment are recommended. For HRAT systems, it's advisable to adjust the fat, oil, and grease (FOG) feed concentrations, keeping LCFA concentrations below 360 mg LCFA-COD/g VSS, in agreement with recommendations of Rinzema et al. (1989). Completely mixed reactor systems, although having lower

substrate conversion rates than plug-flow reactors, can prevent high LCFA concentrations in the reactor's bulk liquid, while operating at reduced LCFA loading rates. AnMBRs are regarded a suitable treatment alternative for high-lipid wastewaters. They combine complete mixing and relatively high sludge concentrations, akin to HRAT, with ultrafiltration membranes for high-quality effluent.

Monitoring lipid degrading species *(Syntrophomonas and Syntrophus),* as well as integrating 16S rRNA gene sequencing with multi-omics approaches, are considered useful tools for evaluating LCFA degradation effectiveness (Hollohan et al., 2022). However, extended genomic databases are needed for comprehensive analysis.

5.5 CONCLUSIONS

In this study, oleate was found to degrade more efficiently than palmitate in all sludge samples, a critical observation for the treatment of LCFA. The disappearance of LCFAs, quantified as FOG, closely corresponded with methane production capacity across various sludge concentrations, highlighting LCFA conversion as the rate-limiting step. When LCFA concentrations exceeded 250 mg/L, inhibitory effects emerged, compromising their conversion to methane. This threshold is considered pivotal for managing LCFA levels in wastewater treatment. The research also revealed the importance of sludge adaptation strategies. Pre-acclimated sludge samples, S2 and S3, were more efficient in methane generation from LCFAs than the non-acclimated S1 sample, emphasizing the need for sludge adaptation in treating lipid-rich wastewater. Furthermore, the detection of bacterial families like *Kosmotogaceae, Petrotogaceae, and Synergistaceae* in these acclimated sludge samples indicated a biological adaptation to LCFA degradation, crucial for optimizing the anaerobic digestion process in high-lipid wastewater treatment.

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6

REFLECTIONS AND OUTLOOK

6.1 REFLECTIONS

This thesis research addressed several gaps in the anaerobic digestion of industrial lipid-rich wastewater. In the following paragraphs, a reflection is made on the progress considering the main hypotheses from the chapter 1:

i. Applying anaerobic digestion to lipids presents promising prospects for sustainable and efficient lipid valorization, enabling the long-term operation of stable systems.
ii. A high SRT will result into a high sludge concentration, a better substrate removal efficiency, and less production of waste sludge. An increased substrate conversion rate is expected at high SRT due to a low F/M ratio, corresponding to a better bioconversion of organic matter and lipids to methane.
iii. Sludge filterability is negatively affected by an increase in SRT, resulting in an increase in transmembrane pressure (TMP). At high SRT, the SMP and EPS are expected to increase due to a higher biomass concentration and cell lysis in the AnMBR. High SMP concentrations might result into cake compaction and pore blocking of the membrane, which would lead to an increase in the TMP when working at a constant flux.
iv. The acclimation of sludge to dairy lipid-rich wastewater is critical for achieving a proper methane production, mainly when the feed contains oleic and palmitic acids.

The sections provided below delineate findings from the four key areas of this research.

6.1.1 Anaerobic digestion of lipids: limitations and its valorization (Chapter 2)

Lipids in wastewater are challenging for anaerobic treatment, but advancements in anaerobic reactor technology and microbial understanding have improved the treatability of lipid-rich wastewater.

The first hypothesis of this study addressed the complexity of anaerobic digestion of lipid-rich waste(water), next to the potentials of lipids conversion into methane in anaerobic reactors. The literature research specifically focused on the current state of the art as well as on existing literature gaps regarding novel strategies for improving anaerobic lipids conversion, the involved microorganisms, and the collaboration between industry and academia to address this topic.

Waste lipids in industrial waste(water) are considered substrates of high interest for anaerobic digestion, because of the high amount of potential methane production per weight of lipids compared to other substrates, i.e., 1.425 L biogas/g lipids (Alves et. al., 2009). However, treating lipid-rich wastewater with HRAT technology is challenging

since the lipids and long-chain fatty acids adsorb to the sludge, leading to sludge flotation, washout, and potentially may cause microbial inhibition. Recent insights and progress in fundamental knowledge in microbiology and biochemical pathways, allowed us to address the classical problems of microbial inhibition and sludge washout, proposing new approaches with technological alternatives for improving the anaerobic digestion of lipids.

However, following the critical review, some issues in high-rate anaerobic treatment of LCFA and lipid-rich wastewaters remain challenging for the wider implementation of this technology. First, the relation between LCFA accumulation and biodegradation to methane is yet to be fully understood. High sludge loadings of fats, oil, and grease (FOG) result in LCFA accumulation, posing challenges for the effective bioconversion process. Several studies developed novel strategies to increment LCFA biodegradation, such as micro aeration (Duarte et. al., 2018), or conductive materials addition to boost the process (Martins et. al., 2018).

Moreover, for a profound understanding, it is imperative to conduct thorough research on various aspects of LCFA conversion and the microorganisms involved, including floc formation, spatial organization within microbial aggregates, and the formation of exopolysaccharides.

Chapter 2 discusses the up-to-date knowledge regarding the anaerobic treatment of lipid-rich wastewater, the metabolic pathways and microbiology, and presents a summary of the applied HRAT technologies, including the second-generation technologies (developed in the last decades) for treating lipid-rich wastewater, such as the AnMBR. These reactors use membranes to ensure total biomass retention (van Lier et. Al., 2020). Yet, the essential physical separation device adds a significant cost to the anaerobic bioreactor. Hence, optimizing the process to reduce the filtration area in membrane units is crucial.

Prior studies indicate that sludge filterability is influenced by its characteristics (Dereli et al. 2012), which in turn are affected by the operational SRT. In the subsequent sections, the research topics are discussed constituting the experimental work of this PhD thesis, tackling the challenges highlighted in the critical review.

6.1.2 Effects of solids retention time on biological performance (Chapter 3)

The findings of this study demonstrated that operating the AnMBR at a solids retention time (SRT) of 40 days resulted in a significantly enhanced and more stable reactor performance compared to an SRT of 20 days.

In continuous flow experiments, the role of SRT was investigated, using two AnMBR systems treating synthetic lipid-rich wastewater, simulating dairy effluent from a milk processing industry. The SRT affects the degree of sludge stabilization and, therefore, the extent of lipid conversion, as well as the scavenging of LCFA from the liquid broth.

Therefore, the biological performance of an AnMBR treating synthetic dairy wastewater at different SRTs was evaluated while examining the impact of LCFA presence and accumulation.

Results demonstrated that both reactors, operated at different SRTs, achieved stable process performance with organic matter removal exceeding 99% and consistent biogas production. These results outperformed previous studies on AnMBR treating various wastewater types, as well as other HRAT systems.

When both systems reached stable operational performance at an organic loading rate (OLR) of 4.7 g/ (L.d), they performed similarly, with a slightly better biological performance observed in the reactor operating at an SRT of 40 days. This can be attributed to the higher biomass concentration in the system, potentially enhancing adaptation to lipids at longer SRTs.

The applied AnMBR systems, characterized by fully suspended sludge with a high surface area, enabled efficient conversion of lipids into methane by providing ample availability of lipids to the microbial surface area. Furthermore, AnMBR systems exhibit superior effluent quality, characterized by low organic matter concentrations and the absence of suspended solids, surpassing the performance of other HRAWT systems. Such high-quality effluent opens possibilities for water reclamation and reuse in the industrial production process, making AnMBR a promising solution for treating lipid-rich dairy wastewater and potential water reuse.

Based on the obtained results, it was suggested to operate the AnMBR at an SRT of 40 days, while treating lipid-rich dairy wastewater. An SRT of 40 days instead of 20 days offered several advantages, including reduced sludge wastage and therefore lowering the operational costs. Additionally, an SRT of 40 days promoted maximized biogas production and improved the water quality of the treated effluent. Therefore, selecting an SRT of 40 days seemingly optimizes the economic and environmental aspects of the AnMBR system.

In addition to the bioconversion performance, further insight in filtration performance is required in dependence to the applied SRT. Optimized filtration performance will minimize the required filtration area and thus membrane units, which are considered a crucial cost factor. Previous research indicated that sludge filterability is influenced by the prevailing characteristics of the sludge, which, in turn, are affected by the operational SRT (Dereli et. al., 2013). In section 6.1.3. research on filtration performance, applying an SRT of 20 and 40 days in the AnMBR, is further discussed.

6.1.3 Solid retention time effect on membrane filtration performance (Chapter 4)

The higher TSS concentration observed in the reactor operated at an SRT of 40 days contributed to a less permeable cake layer, which introduced a negative effect on the membrane filtration performance compared to the reactor operated at an SRT of 20 days.

At the start of the research, it was postulated that the SRT will affect sludge filterability and cake layer density, increasing the transmembrane pressure TMP negatively, reducing the membrane performance. To test this hypothesis, comprehensive analyses of sludge properties and a thorough assessment of total resistance to filtration were conducted at the two different SRTs applied. The aim was to understand the relationship between SRT, TMP, sludge characteristics, and the overall performance of the membrane filtration process.

The results provided clear evidence that increasing the operational SRT had a detrimental effect on membrane filtration performance. This effect can be attributed to the influence of SRT on various sludge properties, including total suspended solids (TSS) concentration, dynamic viscosity, particle size distribution (PSD), mean particle size (MPS), capillary suction time (CST), and the presence and concentration of extracellular polymeric substances (EPS), soluble microbial products (SMP), lipids, and long-chain fatty acids (LCFA). These changes in sludge properties directly impacted the membrane performance of reactors operating at different SRTs.

Regarding sludge filterability, it was observed that high-SRT sludge exhibited lower concentrations of specific lipids and LCFA, resulting in improved filterability. However, other physicochemical sludge parameters, such as TSS concentration, emerged as the most significant determinant affecting membrane filtration performance. High SRT values increased TSS concentration, resulting in a thicker cake layer during filtration. The latter could explain the inferior membrane filtration performance observed at an SRT of 40 days compared to 20 days at the applied cross flow velocity of 0.5 m/s.

The total resistance to filtration tests revealed that the cake-layer resistance was the primary factor contributing to overall resistance, negatively impacting membrane performance. Consequently, the concentration of TSS was identified as the critical sludge parameter determining cake-layer resistance and, therefore, membrane filtration performance. It should be noted that cake layer resistance can be lowered by amending the membrane filtration process parameters, such as crossflow velocity. An increased crossflow velocity will increase the membrane shear, minimizing the cake layer thickness at the expense of a higher energy consumption. The latter was not further tested in our experimental set-up. Nonetheless, at the applied crossflow velocity of 0.5 m/s an SRT below 40 days is recommended.

Although improved biological performance was observed at an SRT of 40 days, the reduced membrane filtration performance constrained the overall process. The formation

and consolidation of the cake layer were identified as critical factors influencing total membrane resistance. Next to increased crossflow velocity, proposed modifications may include adjustment of the backwash cycle and frequency, incorporating a membrane relaxation period, and implementing more frequent cleaning-in-place (CIP) interventions, among other potential measures.

It should be noted that the effects of changing membrane operational parameters on membrane filtration performance were hardly investigated in this study. Therefore, additional research would be necessary to validate and confirm the hypothesis regarding the impact of such modifications.

6.1.4 LCFA inhibitory effect in the anaerobic degradation of palmitic and oleic acid

The study revealed inhibitory effects during anaerobic degradation when dosing LCFA concentrations higher than 250 mg/L. Furthermore, the results demonstrated the significance of sludge acclimation to lipids in improving the conversion of LCFA to methane.

The primary focus of this part of the research was to investigate the inhibitory effects of two different LCFAs on anaerobic digestion, namely palmitate and oleate, at various concentrations, using three distinct sludge samples. The findings from the study showed evident inhibition at concentrations exceeding 250 mg/L. Additionally, a strong correlation was observed between the disappearance of LCFA in the batch reactors and the cumulative methane production, indicating a direct relationship between LCFA conversion and methanogenesis.

One notable discovery was the significant role of sludge acclimation to lipids. Results evidenced that acclimated sludge samples exhibited superior performance compared to non-acclimated sludge. This suggested that the acclimation process enabled the sludge to develop a higher tolerance and enhanced conversion of LCFA during anaerobic digestion.

Furthermore, a comprehensive microbial community assessment was conducted to investigate the microbial population composition in the different sludge samples and to observe possible changes occurring during batch incubation of the sludges. Analysis revealed the presence of specific microbial families in the acclimated sludge, including *Kosmotogaceae, Petrotogaceae, and Synergistace*ae. These families are known to be of crucial importance in anaerobic sludge and their predominant presence further emphasized their importance in the breakdown of LCFA during anaerobic digestion.

In summary, this part of the study provided valuable insights into the inhibitory effects of LCFA at elevated concentrations, highlighting the beneficial impact of sludge acclimation to lipids on overall performance, and shed light on the dynamics of microbial communities associated with high LCFA concentrations in anaerobic systems.

6.2 OUTLOOK AND RECOMMENDATIONS

This thesis addressed critical issues concerning the treatment of lipid-rich wastewater using anaerobic digestion technologies. Conventional anaerobic treatment plants, like UASB and EGSB, typically remove lipids at the beginning of the process using, e.g., DAF technology, resulting in the loss of significant biochemical energy that could otherwise be utilized for biogas production. Therefore, lipid-rich wastewaters present an intriguing substrate for anaerobic digestion. However, several challenges have been reported in their treatment, including issues such as lipid adsorption onto biomass, sludge flotation and washout, and inhibition of the anaerobic process.

To overcome these challenges, appropriate selection and proper operation of reactor technology are crucial. Among the various options, anaerobic membrane bioreactors (AnMBRs) have emerged as a technology of interest for the treatment of this challenging wastewater. This thesis introduces AnMBRs as a viable solution for the treatment of lipid-rich dairy industrial wastewater, highlighting their potential benefits and suitability for addressing the unique challenges associated with this type of wastewater.

Based on the existing literature, the operational SRT has been identified as a crucial factor in AnMBRs. Consequently, this parameter was thoroughly studied to evaluate its impact on both the biological and membrane performance. The findings regarding the biological performance suggested that a higher SRT promotes better adaptation to lipids compared to a lower SRT, leading to improved overall performance. However, when considering the effect of SRT on membrane filtration performance, the results indicated that a lower SRT is more favorable for achieving superior filtration. Apparently, there is a trade-off between the desired biological performance and membrane filtration performance for selecting the most appropriate SRT, which will be linked to the rate limiting step in the overall treatment process. However, literature results indicated that the filtration performance can be enhanced by optimizing the filtration step, e.g., by increased cross flow velocities, improved backwash cycle, or membrane cleaning procedures. Notably, such changes will result in an increased energy (and/or chemicals) demand, negatively impacting the economics of the system. Process optimization requires further research and knowledge to determine the optimal SRT that can simultaneously deliver outstanding biological and membrane filtration performance at the lowest operational costs. This knowledge gap necessitates a deeper understanding of the interplay between SRT, biological processes, and membrane filtration dynamics to achieve optimal outcomes in AnMBR systems treating lipid-rich wastewaters.

6.2.1 Resource recovery

Linking wastewater management to sustainability, the anaerobic treatment of lipid-rich wastewater contributes to the circular economy. As the global scarcity of essential resources such as water, minerals, and metal ores intensify, entities like the United Nations (2021) are increasingly advocating for sustainable development and comprehensive resource recovery across various sectors. Wastewater treatment holds significant potential for resource recovery, especially in sectors like the dairy industry, known for its high-water consumption. Membrane treatments, like the here researched AnMBR treatment process, offer pathways to reduce water consumption in dairy plants (Vourch et al., 2008). The resultant purified permeate can be recycled within the industry for various operations, such as temperature regulation, boiler water replenishment, and cleaning (Vourch et al., 2008).

Historically, wastewater treatment processes primarily aimed to eliminate contaminants, releasing treated water back to the environment. This method represents a linear, resource-consuming approach. Contemporary views on wastewater treatment have shifted, viewing these systems not just as protective measures but as resource generators producing clean water, renewable energy, and essential nutrients. Processes encompassing biological, physical, and chemical methods facilitate this resource recovery. In addition to water and biogas, essential elements like ammonia (nitrogen) and struvite (phosphorus) can be effectively harvested from wastewater processes (van Lier et al., 2020). These developments reflect a paradigm shift towards maximizing the value and sustainability of wastewater treatment processes by harnessing the potential for resource recovery.

Water reclamation

The utilization of treated reclaimed wastewater must be carefully considered, considering each industry's specific needs, and intended applications. In the context of the dairy industry, where the handling of food products requires utmost caution, it is crucial to restrict the use of treated reclaimed wastewater to processes that do not involve direct contact with edible products. This precautionary measure is essential to mitigate the risk of potential contamination (Andrade et al., 2014). By implementing strict guidelines and adhering to stringent safety protocols, the dairy industry can maintain the highest standards of product quality and consumer safety, while benefiting from the sustainable practice of reclaimed water usage. In fact, there are several areas within the industry where water reuse can be effectively implemented as a viable alternative. For instance, reclaimed wastewater can be safely utilized for non-food contact purposes, including heating, cooling, sanitation (flushing toilets), and various good manufacturing processes. These processes include washing external areas, cleaning floors, and sanitizing delivery trucks (Andrade et al., 2014). The dairy industry can strike a balance between sustainable water management practices and ensuring the utmost safety and quality standards for its products. Careful consideration and appropriate application of reclaimed water can

contribute to resource conservation, while upholding the industry's commitment to food safety and hygiene.

The intended use of reclaimed water plays a crucial role in determining the necessary treatment and post-treatment processes. When reclaimed water is intended for applications that align with good manufacturing practices, such as general cleaning, it is essential to achieve water quality parameters that closely resemble those of drinking water standards. These parameters include the absence of E. coli bacteria (0 CFU/100 mL), total coliforms (0 CFU/100 mL), low turbidity (less than 1.0 NTU), a pH range between 6.9 and 9.2, electrical conductivity (EC) levels between 300 and 400 µS/cm, and total dissolved solids (TDS) ranging from 500 to 1500 mg/L (Heydari et al., 2013; WHO, 2017). Considering these aspects, membrane separation is the most suitable post-treatment technology due to its high efficiency in solids-liquid separation. Membrane separation operates based on a driving force, which can be either a concentration difference or pressure difference. Pressure-driven membranes are classified based on pore size, including microfiltration (MF) with a pore size of 0.1–1 µm, ultrafiltration (UF) with a pore size of 5–100 nm, nanofiltration (NF) with a pore size of 0.5–2 nm, and reverse osmosis (RO) membranes.

To meet the reuse standards, it is crucial for any treatment process to efficiently eliminate the critical parameters from the reclaimed wastewater prior to its utilization. For applications like feedwater in cooling systems or boilers, minimizing water hardness and ensuring that the pH remains below 11, while keeping the total dissolved solids (TDS) level below 1000 ppm is of high importance (NBBI, 2015). Consequently, the treatment process should focus on adequately removing hardness salts, such as calcium and magnesium carbonates, as well as dissolved solids, while ensuring the reclaimed wastewater remains within the required pH range. This is crucial to ensure the suitability of the reclaimed wastewater for reuse in heating or cooling systems (Andrade et al., 2014).

Implementing membrane treatment contributes to water conservation and brings about cost savings and environmental benefits for the dairy industry. By optimizing water management through membrane processes, the industry can enhance its sustainability, reduce its environmental footprint, and foster more efficient resource utilization.

Nutrient recovery

Although AnMBRs have demonstrated high COD removal efficiencies, nutrients like nitrogen and phosphorus are hardly removed and solubilized as ammonium and orthophosphate in the permeate. Grundestam and Hellström (2007) reported nitrogen and phosphorus removal rates of only 9%, when investigating domestic wastewater treatment using AnMBR. Experiments described in Chapter 3 also showed high concentrations of NH_4-N and PO_4-P in AnMBR permeate, requiring suitable post-treatment technologies to meet stringent water quality requirements, particularly for reuse in the dairy industry (Andrade et al., 2014). However, since the permeate contains relatively high concentrations of valuable nutrients in the form of NH_4-N and PO_4-P, post-treating the

permeate offers an opportunity for nutrient recovery. Hence, the selected technology may not only facilitate water reclamation but also enable nutrient recovery.

Nitrogen recovery

Global trends indicate an unprecedented demand for nitrogen, particularly as fertilizers, shifting the focus from nitrogen removal to nitrogen recovery. The demand for chemical fertilizers, including ammonium-nitrogen as a key component, is on a steady rise and is projected to continue increasing in the coming decades. This growth is driven by factors such as population growth and economic welfare, and thus, the subsequent increase in food demand. (FAO, 2019).

Commercial ammonia fertilizer production primarily relies on the energy-intensive Haber process, which converts hydrogen and nitrogen into ammonia. By considering natural gas as energy and hydrogen source, this process is associated with high energy requirements (28-30 MJ/kgN) and significant carbon emissions (approximately 1.6 - 2.86 tons of carbon dioxide emitted per ton of ammonia produced) (Beckinghausen et al., 2020; Yan et al., 2018). Alternative sources of ammonia nitrogen need to be explored, especially for local use, to ensure affordable and readily available fertilizers for low-cost, sustainable, and eco-friendly food production.

According to Beckinghausen et al. (2020), nitrogen recovery processes can be categorized as biological, physical, chemical, or hybrid. Chemical nitrogen recovery processes may involve the precipitation of insoluble crystalline ammonium salts, such as magnesium ammonium phosphate (MAP or struvite), which contains equal amounts of magnesium, phosphate, and ammonium ions. Physical processes for nitrogen recovery comprise adsorption, membrane filtration, and stripping (thermal or vacuum) (El-Bourawi, et al., 2007; Gerardo et al.,20013 Ukawani et. al., 2016). Zeolite is frequently utilized for ammonia adsorption because of its notable selectivity and cation exchange capacity. Zeolites, once loaded, can be directly applied as a soil additive (Smith, D., & Smith, N., 2015). Recently, electrochemical nitrogen recovery was developed and commercialized using bipolar membrane electrodialysis (Deng et al., 2022, van Linden et al., 2020, 2022)

Biological nitrogen recovery may include bio-electrochemical systems (BES) that employ electrochemically active microorganisms, which catalyze reactions to convert chemical energy in organic substrates into electrical energy. Common types of BES include microbial fuel cells (MFCs), microbial desalination cells (MDCs), and microbial electrolytic cells (MECs). One notable advantage of utilizing BES is the simultaneous generation of power in the form of hydrogen or electricity during the nitrogen recovery process (Beckinghausen et al., 2020). Thus far, these systems are not commercialized.

In this context, a significant shift from conventional nitrogen removal to nitrogen recovery from wastewater holds promising potential. This approach would allow for the extraction and utilization of ammonia nitrogen from wastewater, presenting a sustainable solution for fertilizer production. By capturing and reusing ammonia nitrogen from

wastewater, we can contribute to the realization of environmentally friendly and likely economically viable food production practices.

Phosphorous recovery

In recent years, concerns have risen regarding the depletion of global phosphorus reserves. This worrisome situation stems from phosphorus being only available in limited natural deposits worldwide, with no viable synthetic alternative discovered to date (Yan et al., 2018). Most active phosphorus is lost to sewerage systems through residues from detergents and fertilizers and finally ends up diluted in the oceans. To mitigate the impending acute global shortage, projected to occur as early as 2050, while still meeting the growing demand, it is essential to identify a sustainable and renewable source of phosphorus. Wastewater represents a promising potential source (Yan et al., 2018).

Wastewater, both from municipal and industrial sources, is an abundant and readily available source of phosphorus. It primarily originates from residues in food, cleaning detergents, and runoff from fertilizers that eventually make their way into the sewerage system (Andrade et al., 2014). Similar to nitrogen, there are global policies that require the concentrations of phosphorus in the final effluent to be discharged into a receiving body, such as a river or lake, to be as low as possible (in some countries like in the Netherlands, as low as <1 mg/L, with projected values of 0.1 mg/L in the near future) (Chen et al., 2020; von Sperling et al., 2005). This stringent standard is necessary because phosphorus can harm the environment and aquatic life, leading to algae blooms and eutrophication. Consequently, many wastewater treatment plants incorporate a phosphorus removal step through biological or chemical precipitation to meet these strict phosphorus standards (Chen et al., 2020).

However, there is a growing recognition of the importance of shifting from phosphorus removal to phosphorus recovery to promote circularity in phosphorus sourcing and usage. Phosphorus can be recovered in various forms, including hydroxyapatite (HAP) ($Ca_5(PO_4)_3(OH)$), struvite (MAP) ($MgNH_4PO_4 \cdot 6H_2O$), phosphoric acid, or nitrogen-phosphorus-potassium (NPK) pellets. Among these forms, struvite and hydroxyapatite are particularly promising as slow-release fertilizers. The struvite precipitation is most effective at slight alkaline pH of around 8.5, and the reaction can be completed within approximately 10 minutes per batch. In struvite recovery, the feedwater is first characterized to determine the concentrations of magnesium (Mg) and phosphorus (P). The amount of additional Mg is calculated to achieve successful struvite precipitation with an ideal Mg:P ratio of about 1-1.3:1. Similarly, hydroxyapatite recovery involves characterizing the feedwater to establish the concentrations of calcium (Ca) and phosphorus. The calculated additional Ca ensures a successful hydroxyapatite recovery with a Ca:P ratio of 1.67:1 (Vourch et. al., 2008).

6.2.2 Membrane operation

Based on the findings of this research, it is evident that membrane filtration and cleaning are crucial factors influencing the performance of the AnMBR system (Le-Clech et. al., 2006; Drews,2010; Meng et. al., 2017). Furthermore, optimizing the membrane filtration process by fine-tuning parameters such as crossflow velocity, flux and transmembrane pressure (TMP) and implementing effective cleaning procedures can significantly enhance the overall system performance, resulting in reduced capital expenditure (CAPEX) and operational expenditure (OPEX) costs.

The results of this study highlight the importance of understanding and controlling the membrane operation within the AnMBR system. By carefully managing the membrane permeate flux and membrane shear (crossflow velocity), operators can strike a balance between achieving optimal system performance and minimizing fouling issues. Adjusting the flux can prevent excessive fouling, maintain consistent permeate quality, and improve the overall efficiency of the AnMBR system at the expense of a larger membrane surface area. Moreover, the research emphasized the significance of controlling the TMP during membrane filtration. By monitoring and optimizing the TMP, operators can ensure that the pressure exerted on the membrane is within the desired range, preventing membrane damage and prolonging its lifespan. Proper TMP management also maintains stable filtration performance and avoids potential operational issues.

In addition to optimizing membrane operation, the results of this study highlights the importance of implementing effective cleaning procedures. Membrane fouling occurs when unwanted substances accumulate on the membrane surface, a common challenge in membrane filtration systems. Operators can mitigate fouling and extend the intervals between major maintenance activities by applying increased crossflow velocities and/or by developing and employing suitable cleaning protocols, such as chemical cleaning, backwashing, or biogas scouring in AnMBR. Proposed measures will improve the system's performance and longevity and reduces the associated costs.

Overall, this research demonstrates that a comprehensive optimization of membrane operation and cleaning procedures is key for enhancing the performance of the AnMBR system. By fine-tuning parameters like flux, TMP, crossflow velocity, and implementing effective cleaning protocols, operators can maximize system efficiency, reduce capital and operational costs, and achieve a more sustainable and economical wastewater treatment solution.

In our study, we observed that increasing the operational SRT positively impacted the system's biological performance. However, it was noted that the membrane filtration was adversely affected and deteriorated compared to low SRT conditions. As a result, it is crucial to conduct further research to optimize the membrane performance to achieve sustainable operations at high SRT levels. This optimization can be achieved through collaboration with membrane suppliers and conducting additional studies to ensure the successful operation of the entire system. To address this challenge, it is recommended to

collaborate with membrane suppliers who can provide valuable insights and guidance. The expertise and knowledge of membrane suppliers can help to identify suitable membranes less prone to fouling. Additionally, working closely with membrane suppliers can facilitate the development of customized cleaning protocols that are specifically tailored to address the challenges faced at high SRT levels. Furthermore, it is essential to conduct further studies to explore and understand the underlying factors contributing to the decline in membrane performance at high SRT. These studies can explore fouling mechanisms and innovative approaches to mitigate fouling issues. By understanding the membrane fouling mechanisms and their relation to high SRT, it will be possible to devise effective strategies for sustaining membrane performance under these conditions.

The design and operation of (An)MBR systems involve various parameters that can influence membrane fouling, including feed characteristics, biomass characteristics, membrane characteristics, and operational conditions. The control and optimization of these parameters are essential for achieving better performance in both AnMBR and MBR technology (Judd, 2011). Nonetheless, membrane fouling remains a prevalent issue in AnMBR technologies (Meng et al., 2017), and its complexity necessitates further understanding and exploration, particularly concerning membrane cleaning protocols suitable for high operational SRTs (Wang et al., 2014).

Membrane cleaning plays a crucial role in the operation of (An)MBRs and significantly impacts membrane performance. It is widely acknowledged that membrane cleaning can be categorized into physical and chemical cleaning. Sustainable (An)MBR operation relies on the combination of physical cleaning methods, such as application of proper membrane shear (cross flow velocities), membrane relaxation or backflushing, complemented by periodic chemical cleaning in place (CIP). Physical cleaning primarily targets the removal of loosely attached materials on membrane surfaces, commonly known as "reversible fouling." In contrast, chemical cleaning is employed to eliminate more tenacious materials, referred to as "irreversible" fouling (Wang et al., 2014).

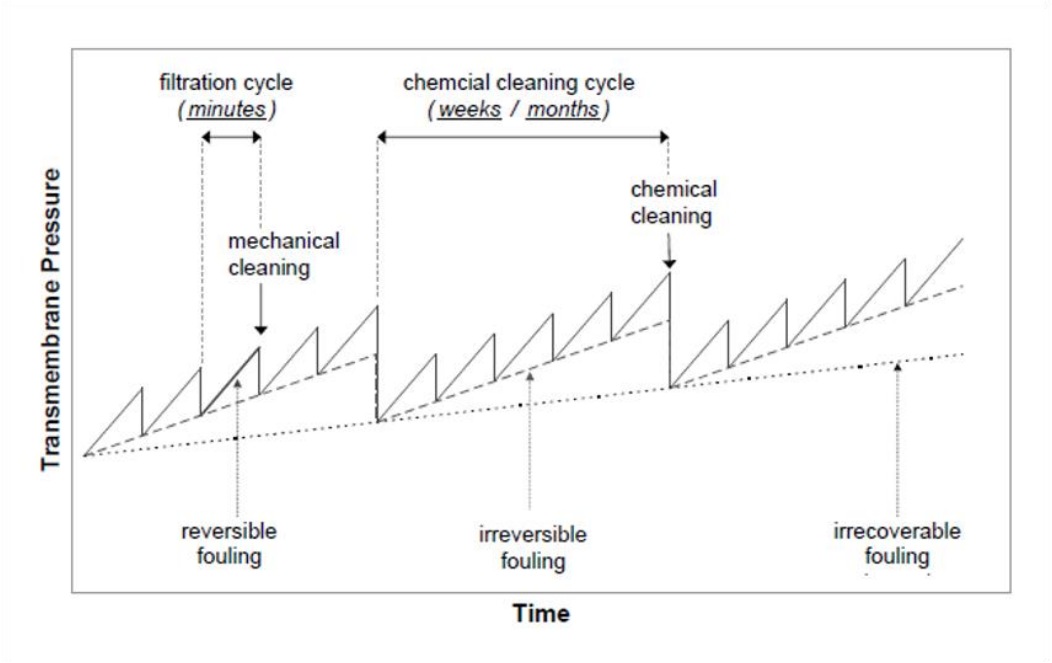

Figure 6.1 – Description of different types of fouling rates (Adapted from Drews, 2010)

Different types of fouling, including reversible, irreversible and irrecoverable fouling, exhibit varying fouling rates (dP/dt). Reversible fouling typically exhibits the highest fouling rate, followed by irreversible fouling. Irrecoverable fouling accumulates over an extended period and has the lowest fouling rate, ultimately determining the membrane's lifespan. The rate of dP/dt is closely linked to the interval between cleanings. High dP/dt values result in reaching the threshold pressure (P_{max}) more rapidly, necessitating more frequent cleaning cycles at Pmax, thereby shortening the cleaning cycle time (Wang et al., 2014). The maintenance cleaning methods are more effective than backwashing or relaxation, as they can restore membrane permeability close to the original baseline value (P_0). After several cycles of maintenance cleaning, recovery (intensive) cleaning or ex-situ cleaning becomes necessary to eliminate residual fouling and restore membrane permeability to a level similar to P_0.

Optimizing membrane cleaning protocols is essential for mitigating fouling in (An)MBRs. Understanding the different fouling types and associated cleaning requirements is crucial for maintaining membrane performance and prolonging membrane lifetime. By combining physical and chemical cleaning methods, (An)MBR operators can effectively manage fouling and restore the membrane permeability. Furthermore, by investigating the nature of membrane foulants, understanding their effects, and exploring fouling mitigation strategies, we can enhance our knowledge of fouling mechanisms and develop improved cleaning protocols and strategies. Such research is essential for ensuring the long-term, efficient operation of the membrane filtration system.

In membrane filtration processes, operation below the critical flux will guarantee a long-term filtration process without disturbing fouling events. For assessing the critical flux,

typically, a stepwise increase in flux is applied at a set membrane shear (crossflow velocity in our set up), while monitoring the transmembrane pressure (TMP) at each step. When the TMP is no longer stable and rapidly increases during the filtration step, indicating a rapid accumulation of foulants, the critical flux in filtration is reached. This, so-called, weak definition of the critical flux, assures no decline in total permeability, while operating at a membrane flux below this critical flux, whereas detrimental fouling is only observed at membrane fluxes above the critical flux (Le-Clech et al., 2006). In continuous operation, the critical flux will depend on the prevailing shear force applied on the membrane's surface. When a high shear force is applied, e.g., in crossflow filtration, the critical flux is redefined as sustainable flux.

When planning fouling experiments, it is vital to consider several key factors, including the duration of filtration cycle (short-term versus long-term), the operational mode (constant flux versus constant transmembrane pressure), the initial state of the membrane (new versus cleaned), operating conditions, and the cleaning protocol. Above mentioned factors should be carefully selected, reported, and analyzed, related to the obtained results. The concept of critical flux and its determination through flux-step experiments remains an interesting tool for assessing fouling propensity under specific operating conditions (Le-Clech et al., 2006). However, for full scale (An)MBR systems, the concept of sustainable flux is commonly considered, which allows filtration to be maintained over an extended period, at the expense of applying a high shear force (such as crossflow velocity).

Regarding the physical and chemical cleaning methods, there are still ample opportunities to align cleaning protocols with the types of fouling substances encountered. It is imperative to conduct further research on the characteristics of membrane foulants, their impact, and strategies to mitigate fouling. This will lead to a deeper comprehension of fouling mechanisms and the development of enhanced cleaning protocols and strategies.

In conclusion, for sustained operation it is important to consider operating the membrane unit below the critical or sustainable flux to prevent fouling and optimize membrane performance. The selection and reporting of experimental parameters and the determination of critical flux or sustainable flux are essential. Furthermore, there is a need for further research into the nature of foulants, their effects, and the development of effective fouling mitigation strategies. By addressing these aspects, we can enhance our understanding and control of fouling phenomena and improve the overall performance of membrane filtration systems.

6.3 FINAL REMARKS

The thesis provides compelling evidence for the successful treatment of dairy industrial wastewater with high lipid concentrations using an AnMBR. The study specifically focused on one of the most critical operational parameters, the SRT, which significantly affects both the biological and membrane performance of the system. Moreover, the thesis investigated the impact of the two most prominent LCFAs, namely, oleate, and palmitate, on anaerobic digestion. The findings reveal a clear inhibitory effect of these LCFAs, which are commonly found in lipid-rich wastewaters. Additionally, the study highlights the crucial role of the inoculum, demonstrating that an acclimated inoculum outperforms a non-acclimated one in the anaerobic digestion of this type of wastewater.

To advance the understanding and application of AnMBR systems for dairy industrial wastewater treatment, further research is recommended in several key areas.

- Firstly, there is a need to investigate and optimize the filtration performance and membrane operations to enhance the overall efficiency and reliability of the AnMBR process
- Secondly, exploring opportunities for resource recovery from the permeate of the AnMBR is of great importance. Identifying and developing suitable methods for the recovery of valuable resources from the treated wastewater can contribute to the sustainability and economic viability of the treatment process.
- Thirdly, it is crucial to conduct in-depth studies on the combined effects of different LCFAs on the inhibition of the anaerobic digestion process. Understanding the interactions and synergistic impacts of various LCFAs will enable the development of more effective mitigation strategies and process improvements.

In conclusion, the thesis convincingly demonstrates the feasibility of using AnMBR for the treatment of dairy industrial wastewater with high lipid concentrations. However, there are significant opportunities for further research to optimize membrane operations, explore resource recovery options, and deepen our understanding of the combined effects of LCFAs on anaerobic digestion inhibition. Addressing these areas will contribute to the advancement and wider adoption of AnMBR technology in treating lipid-rich wastewaters.

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Maria Alejandra Szabo Corbacho completed her bachelor's degree in chemical engineering at the University of the Republic (UdelaR), Uruguay in 2012.

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She is currently working as an Associate Proffesor in the Water Engineering and Sustainable Development program at UTEC-Universidad Tecnologica del Uruguay and is part of the national system of researchers (SNI-ANII) in Uruguay.

LIST OF PUBLICATIONS

Journal Articles

Holohan, B. C., Duarte, M. S., Szabo-Corbacho, M. A., Cavaleiro, A. J., Salvador, A. F., Pereira, M. A., ... & Alves, M. M. (2022). Principles, advances, and perspectives of anaerobic digestion of lipids. Environmental Science & Technology, 56(8), 4749-4775.

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- Energy production through microbial processes, Brazilian Argentinian School of Biotechnology (2019)
- Research data and code skills – Online Genomics Workshop, TU Delft (2020)
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The global population's continuous growth has led to increased resource consumption, particularly water resources, resulting in potential shortages and environmental concerns. Industrialization has further exacerbated this issue by intensifying the demand for freshwater and contaminating water sources. To address this issue industry-led initiatives for wastewater reduction and treatment, and technological advancements are crucial. Within wastewater systems, lipids present both opportunities and challenges. They can be converted into bioenergy but can disrupt anaerobic wastewater treatment processes. Anaerobic digestion (AD) plays a key role in sustainable development, producing renewable energy, recycling nutrients, and minimizing sludge production. New technologies like anaerobic membrane bioreactors (AnMBR) have emerged to address lipid-intensive wastewater treatment.

This research focused on the role of solids retention time (SRT) in AnMBR operation, sludge filterability, and membrane filtration performance. Different SRT configurations had a significant impact on LCFA conversion, with a 40-day SRT showing slightly enhanced biological conversion. The study also highlighted the influence of LCFA on anaerobic sludge processes, with lipid-acclimated sludges demonstrating better LCFA degradation potential. In summary, this doctoral research emphasized the importance of SRT and the role of LCFAs in anaerobic digestion processes, shedding light on prospects and challenges in treating lipid-rich dairy wastewater using AnMBR technology.

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