Advancing Anaerobic Membrane Bioreactors for Low Temperature Domestic Wastewater Treatment

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Environmental Engineering)
in the University of Michigan
2019

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Acknowledgements

This work was supported by numerous funding sources including a University of Michigan (UM) Department of Civil and Environmental Engineering fellowship, a U.S. National Science Foundation project CBET 1604069, a UM Predoctoral Fellowship from the Rackham Graduate School, and an Integrated Training in Microbial Systems (ITiMS) Fellowship. The ITiMS program "Instructional Program Unifying Population and Laboratory Based Sciences" at UM was funded by the Burroughs Welcome Fund.

The culmination of the work I've completed at University of Michigan would not have been possible without my extraordinary support network. For starters, I want to thank my research advisors, Lutgarde Raskin, Steven Skerlos, and Ilse Smets for providing me with the opportunity to explore my academic interests, and for the support necessary to expand upon those interests and grow as a researcher. Thank you also to my examination committee members Lise Appels, Dirk Springael, and Willy Verstraete for their constructive feedback on my dissertation. In addition to providing detailed comments and insights, I want to thank Glen Daigger and Vincent Denef for actively contributing to research discussions in my data meetings.

The staff of CEE and the infrastructure they provide to support graduate students has been instrumental in the completion of my dissertation. Thank you to Rick Burch, Ethan Kennedy, Steve Dona, and Jan Pantolin for their help with designing, constructing, maintaining, and often times fixing the various reactors I have worked with these past years. Thank you to Tom for always giving me his thoughts on which lab analyses to perform, and for his continuous

efforts in ensuring I could go ahead and perform such analyses when necessary. Thank you to the CEE purchasing team (Ingra Stimach, Sherry Brueger, Tabitha Rohn, Stephanie Ford, Christine Kropelnyckyj) for ordering the materials essential to my research and tracking lost packages whenever necessary.

I appreciate that the biotechnology research group in general is such a collaborative environment where both knowledge and tools are shared. In specific, thank you to Julie Fichefet, Bridget Vial, Juliana Huizenga, Alec Distel, Devin Pascoe, and Lindsey Hayter for helping me run and sample the various reactors we've operated over the past 5 years. Thank you to Tim, who often put time aside to help me, or spontaneously ran analyses and checked on the reactors to alleviate some of my workload. Thank you to Freddy, who consistently and proactively took on work to significantly reduce my stress through his efforts and attitude. Thank you also to Andrea and Jimmy for their help in proof-reading and editing many of my documents these past years, including this dissertation.

Finally, I want to thank the people responsible for the countless fond memories of my time spent these past 5 years. To start, I want to thank my support system in Belgium. Even though I moved across the ocean, it's so incredibly nice to be able to go back to Leuven and feel at home through the easy, warm, and welcoming nature of my family and friends. Thank you also to my surrogate family in America, the McFarlands, for giving me the same at-home feeling on the other side of the ocean. In Ann Arbor, I want to thank the following people for being the core of my support system and for consistently and unfalteringly being there for me, whether through times of struggle or times of laughter: Maggie, Xavi, Jimmy, Emily, Nicole, David, Brett, Enrique, Kate, Ted, and Raghav. I also want to thank Jeseth, Nadine, and Heather for showing me how it's done. I'll end by thanking the one person I literally have no words for: Andrea. May we forever stay connected and have each other's backs in ways I had never thought imaginable.

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Abstract

Anaerobic membrane bioreactors (AnMBRs) use anaerobic microorganisms to convert organic compounds present in waste streams to biogas, a renewable energy source. They employ a membrane to remove suspended solids from treated wastewater and ensure excellent effluent quality, which allows for water reuse. The promise of treating wastewater while producing energy and water has increased interest in AnMBRs. The domestic wastewater temperature in temperate climates is often below 20°C, with lows around 5°C. Operation at these temperatures raises economic and environmental concerns associated with membrane fouling and the loss of methane through the effluent. This dissertation research developed and evaluated novel AnMBR designs to address these concerns and advance sustainable domestic wastewater treatment.

First, we examined patents to achieve a deeper understanding of the AnMBR innovation landscape and its technological direction. We additionally aimed to determine if environmental concerns are being addressed by the field. Our review showed that only a fraction of AnMBR inventions address membrane fouling and methane loss mitigation, two impediments to sustainable AnMBR operation as concluded by previous life cycle assessment studies.

We then evaluated methods focused on monitoring direct interspecies electron transfer (DIET) in anaerobic digesters. DIET has been suggested to enhance anaerobic digestion and we considered promoting DIET in biofilms in our novel AnMBR designs. Recent research has shown that DIET alone does not always explain observed performance enhancements. Our review indicated that a combination of methods is necessary to confirm the occurrence and expand our knowledge of DIET.

Finally, we present the design and evaluation of two novel AnMBRs: the biofilm-enhanced AnMBR (BfE-AnMBR) and the MagnaTree reactor. The bioreactor of the BfE-AnMBR is separated into three compartments using two conductive meshes to support biofilm growth and DIET. The flow in the bioreactor is regularly reversed to avoid clogging of the meshes while allowing for substrate staging and partial biomass migration between the different compartments. The bioreactor is connected to an energy efficient membrane filtration unit containing a rotating ceramic disc. The BfE-AnMBR was operated at 15°C for approximately nine months, but the anticipated substrate staging was not accomplished. The concentration of organic compounds in domestic wastewater was likely too low to achieve localized bioreactor souring. Given these unanticipated outcomes and the complexity of BfE-AnMBR design and operation, its operation was discontinued. Subsequently, a second design, the MagnaTree reactor, which primarily relies on biofilm treatment, was evaluated. Biofilm growth in the MagnaTree reactor is accomplished through biofilm development on a tree-like structure, which contains branches with openings wrapped with meshes. Similar to the BfE-AnMBR, the MagnaTree contains conductive meshes to promote DIET. Influent wastewater and biomass mixed liquor are continuously recirculated through one set of meshes to maximize biofilm treatment, while another set of meshes provides filtration for permeate production. The MagnaTree reactor achieved 86% chemical oxygen demand removal after a startup of three months at 21°C. Future work with the MagnaTree reactor will determine its performance limits at lower temperatures.

In conclusion, our work with the MagnaTree reactor confirms that biofilms can harness sufficient microbial activity to achieve adequate anaerobic treatment of domestic wastewater at 21°C. Future research is necessary to confirm if fouling and dissolved methane mitigation concerns with the MagnaTree reactor are sufficiently addressed to ensure domestic wastewater treatment with net positive energy and net greenhouse gas emission reductions.

Chapter 1. Introduction

Sustainable water management is becoming increasingly important for utilities and is driving efforts to reduce energy consumption and residuals production in domestic wastewater (DWW) treatment without compromising effluent quality (Daigger 2009). While wastewater treatment historically has mainly focused on eliminating pollutants, the wastewater treatment field is experiencing a paradigm shift (Iacob 2013, van Loosdrecht et al. 2014, Song et al. 2018) towards reusing water while recovering energy (McCarty et al. 2011) and other resources such as nutrients (Mehta et al. 2015), cellulose fibers (Ruiken et al. 2013), bioplastics (Guest et al. 2009), and biopolymers (Lin et al. 2010). Compared with conventional aerobic biological treatment, anaerobic biological treatment produces methane (a renewable energy source), generates only a fraction of the residuals, and can provide substantial energy savings (van Lier et al. 1999, Zeeman et al. 1999, Aiyuk et al. 2004, Chu et al. 2005b, van Haandel et al. 2006).

Several full-scale systems that anaerobically treat DWW exist (Heffernan et al. 2011); however, their use is often restricted to warm climates (Van Haandel et al. 1994, Dev et al. 2019). Although anaerobic processes are conventionally operated at 35-37 °C (Gomec 2010), substantial energy gains can be made by not heating wastewaters from their original temperatures to 35-37 °C (Kettunen et al. 1997, Lettinga et al. 1999). Unfortunately, anaerobic biochemical reactions are slower at DWW temperatures typical for cold to temperate climates, which are generally below 20°C and can drop to below 5°C during the coldest days of the year (Tchobanoglous et al. 2004), relative to those at 35-37 °C (de Man et al. 1988, Van de Last et al. 1992, Lettinga et al. 2001, Bowen et al. 2014, Schalk et al. 2019). Slow anaerobic degradation rates and impaired microbial activity at low temperatures result in reduced biogas production and low quality effluents (de Man et al. 1988, Matsushige et al. 1990, Van de Last et al. 1992, Schalk et al. 2019, Schmidt et al. 2019). Research has focused on addressing these problems using a variety of anaerobic technologies, including the upflow anaerobic sludge blanket reactor (UASB) (Singh et al. 1996, Turkdogan-Aydinol et al. 2011), the expanded granular sludge bed (EGSB) (Rebac et al. 1999, Dong et al. 2013), and the anaerobic membrane bioreactor (AnMBR) (Chu et al. 2005a, Ho et al. 2009). While AnMBRs separate biomass and other suspended solids from the treated wastewater using different types of membranes, UASBs and EGSBs solely rely on settling of granular biomass for solids/liquid separation (Lin et al. 1991). The settleability of granular sludge is known to deteriorate with decreasing temperatures, which results in insufficient biomass retention and negatively affects effluent quality when temperatures fall below 20°C (Uemura et al. 2000, Chong et al. 2012). The use of membranes in AnMBRs ensures biomass retention even at low temperatures. Additionally, membranes produce a higher quality effluent, broadening the application potential of AnMBR technology from recovering energy to recovering water suitable for water reuse applications (Daigger 2009, Maaz et al. 2019).

While helpful in improving effluent quality, membranes are costly and require considerable energy for permeation and to mitigate the fouling layer that unavoidably develops on the membrane surface (Aslam et al. 2018, Maaz et al. 2019, Petropoulos et al. 2019). Membrane fouling intensifies at low temperatures (Gao et al. 2014, Smith et al. 2015b, Ding et al. 2019), thus requiring higher pressures to maintain wastewater flow and resulting in increased operational costs. Ozgun et al. (2015) concluded that treatment of DWW with membrane-coupled UASBs was not feasible at 15°C due to exacerbated membrane fouling. Although the occurrence of membrane fouling has its distinct drawbacks, several AnMBR studies indicated that the fouling layer serves as an active biofilm that contributes to the removal of organic compounds (Ho et al. 2010, Vyrides et al. 2011, Smith et al. 2012). Smith et al. (2015a) illustrated how the membrane biofilm increasingly contributed to organics removal with decreasing temperatures, providing over 50% of total organics removal at temperatures below 12°C.

Concomitant with an increase in membrane fouling at decreasing temperatures, the degree of methane oversaturation has been shown to gradually increase (Smith et al. 2015a, Smith et al. 2015b). Several studies have noted that methane concentrations in the permeate can be substantially higher than predicted by equilibrium calculations (Giménez et al. 2012, Smith et al. 2015b, Smith et al. 2015a, Crone et al. 2016). The increasing methane oversaturation observed for decreasing temperatures was attributed to an increasing dependence on the membrane biofilm for treatment, which resulted in greater methane production at the membrane surface and its entrainment in the permeate. This dependence on the membrane biofilm for treatment was also observed in a study by Alibardi et al. (2014), where it resulted in the loss of 62% of produced biogas through the effluent. Finally, while Ozgun et al. (2015) and Yoo et al. (2014) observed no decrease in chemical oxygen demand removal when the temperature decreased from 25 to 10°C, Gao et al. (2014) reported a decrease from 74.0 ± 3.7% at 35°C to 67.1 ± 2.9% at 25°C and even 51.1 ± 2.6% at 15°C. Even though these hurdles are not yet sufficiently mitigated to facilitate sustainable implementation of AnMBR technologies (Lei et al. 2018, Ding et al. 2019, Petropoulos et al. 2019), recent research shows that low temperature AnMBRs can successfully treat DWW (Smith et al. 2012, Gouveia et al. 2015, Smith et al. 2015a, Seib et al. 2016a, Seib et al. 2016b).

The use of AnMBRs for DWW treatment has the potential to enhance the sustainability of current water management practices. To achieve this goal, a deeper understanding of challenges, including poor organics removal, loss of methane through the permeate, and membrane fouling when operated at low temperatures, becomes necessary. By identifying novel design and operating characteristics suited for low temperature AnMBR treatment of DWW, this dissertation aims to move the anaerobic wastewater treatment field forward towards net positive energy operation with minimal environmental impacts.

The overarching goal of this dissertation is to identify AnMBR design characteristics that best enhance DWW treatment in temperate climates. As illustrated in Figure 1.1, this dissertation commences with a review of AnMBR designs as presented in patents to ensure the novelty of our design ideas (Chapter 2). The aim of this chapter is to understand the AnMBR innovation landscape and identify the technological direction of the field. To do so, a collection of AnMBR patent documents were studied to derive historical, regional, and institutional activity trends. Since technologies that focus on enabling low temperature AnMBR treatment can also enhance performance at higher temperatures, patents often do not specify the temperature at which they aim to operate. This study therefore includes all AnMBR patents as opposed to exclusively including patents focused on low temperature treatment. To further study the technological direction of the AnMBR field and understand whether it addresses pressing environmental concerns, Chapter 2 includes a broad but critical overview of the designs presented in the collected AnMBR patents.

In Chapter 3, we investigate how to evaluate the occurrence of direct interspecies electron transfer (DIET) in anaerobic digesters to help inform when to exploit this phenomenon to boost anaerobic digestion performance. DIET is a recently discovered electron transfer pathway widely claimed to enhance anaerobic digestion. One novel AnMBR design characteristic evaluated in this dissertation is the promotion of biofilm growth inside AnMBR bioreactors. We hypothesize that such biofilm growth will counter the decline of organics removal previously observed with low temperature AnMBRs. To support the development of an active microbial community inside these biofilms, we considered promoting DIET. Chapter 3 consists of a critical literature review and identifies advantages and pitfalls of methods used to monitor DIET. Next, we designed, constructed, and evaluated two novel AnMBRs for low temperature DWW treatment (Chapter 4). Design decisions were motivated by challenges identified in previous work in our laboratory and described in the literature. After constructing the novel AnMBRs, we evaluated the performance of these systems for DWW treatment at a temperature consistent with the average DWW temperature observed in temperate climates. Finally, Chapter 5 presents overarching conclusions and future research perspectives.

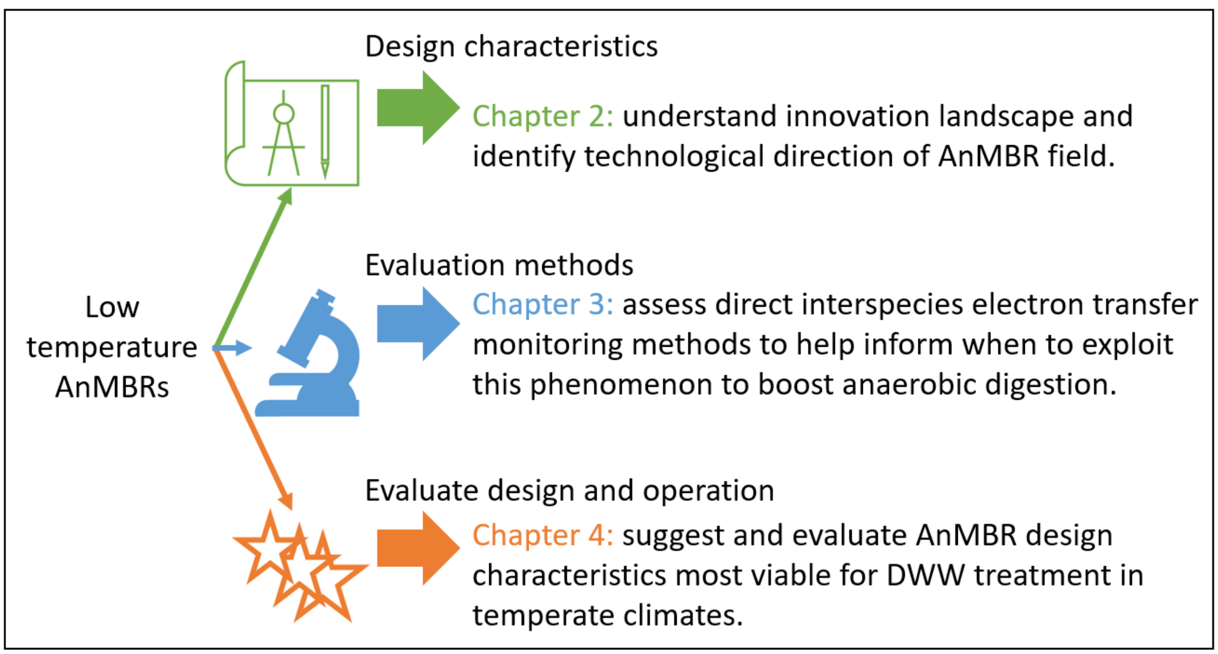

Figure 1.1: Schematic representation of this dissertation's objectives, organized in three main chapters.

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Chapter 2. Evaluation of Anaerobic Membrane Bioreactor Design Field Through a Patent Review

2.1 Abstract

Anaerobic membrane bioreactors (AnMBRs) are increasingly researched and applied to treat a variety of waste streams, however, previous life cycle analyses have shown that these AnMBR applications are coupled with environmental concerns associated with membrane fouling mitigation techniques and permeate dissolved methane oversaturation. This study examined patents to achieve a deeper understanding of the AnMBR innovation landscape and its technological direction, as well as to determine if environmental concerns are being addressed by the field. A broad keyword search resulted in a collection of 4007 patent documents, of which 688 AnMBR patents remained after removing duplicate patents and patents not targeting volatile fatty acid or methane production, typical products of AnMBRs. Since 2009, AnMBR patents have been dramatically increasing in number. While most applicants are Chinese and most patents are filed in China, the relative contribution of other countries increases when specifically looking at patents targeting fouling and dissolved methane. Many patents within the AnMBR patent collection comprise of an extensive treatment train including anaerobic and anoxic processes as well as aerobic membrane bioreactors, making these innovations irrelevant to AnMBR designs. Most inventions on the reactor side aim to enhance anaerobic degradation as well as minimize fouling in the subsequent membrane unit, but none focus on avoiding or eliminating permeate dissolved methane. Furthermore, even though only a low percentage of all AnMBR patents focus on fouling, the patents specifically discussing membrane improvements most often aim to mitigate fouling or enhance membrane flux. Finally, while a handful of post

treatment processes does focus on dissolved methane removal, the majority focus on removing nutrients. This work highlights the need for increased R&D efforts towards finding solutions for fouling, but especially dissolved methane mitigation, to ensure sustainable implementation of AnMBRs.

2.2 Introduction

Anaerobic membrane bioreactors (AnMBRs) are increasingly being researched to achieve small physical footprints, energy recovery, and stable effluent quality for the treatment of a variety of waste streams (Lin et al. 2013, Dvořák et al. 2016, Massara et al. 2017). However, the plethora of challenges associated with membrane fouling (Krzeminski et al. 2017, Meng et al. 2017) and loss of methane through the permeate (Smith et al. 2014, Pretel et al. 2015) raise economic and environmental concerns for AnMBRs. Even though there are distinct differences between aerobic membrane bioreactors (MBRs) and AnMBRs, many issues associated with MBRs are similar regardless of whether they rely on aerobic or anaerobic microbial processes. Therefore, the more widely available background information on aerobic MBRs can be used to help review the membrane-related problems for AnMBRs.

The economic and environmental costs associated with membrane fouling are the main bottlenecks impeding widespread MBR implementation (Krzeminski et al. 2017, Meng et al. 2017). Membrane fouling limits flux, which can be counteracted by providing more membrane surface area, resulting in increased capital costs. Fouling mitigation techniques (e.g., gas sparging) require a substantial amount of energy, and the associated cost often dominates the total operating costs and has substantial environmental impacts. Although membrane costs have decreased and fouling mitigation techniques have improved, MBRs still entail higher capital and operating costs than conventional activated sludge without tertiary treatment (Xiao et al. 2019).

The presence of dissolved methane in effluents or permeates is a concern specifically associated with anaerobic treatment systems, including AnMBRs (Shin et al. 2016). Crone et al. (2016) estimate that AnMBRs treating domestic wastewater can lose between 19 and 63% of the total generated methane through their permeates, contributing to substantial greenhouse gas emissions when not removed. Methane emissions associated with wastewater (including collection, treatment, and disposal) account for over 8% of all anthropogenic methane emissions (Karakurt et al. 2012). Eliminating dissolved methane from AnMBR permeates is crucial to make this technology environmentally competitive with conventional water treatment techniques (Smith et al. 2014, Pretel et al. 2015). While the permeate methane can be recovered via a variety of techniques to reduce its environmental impact (Crone et al. 2016), avoiding this oversaturation is, evidently, the most sustainable avenue.

A recent review on pilot-scale AnMBRs treating domestic wastewater mentions the lack of nutrient removal as a third concern associated with AnMBRs (Shin et al. 2018). Organic compounds degraded during anaerobic treatment are no longer available for conventional biological nutrient removal, thus requiring alternative approaches. Nutrient-rich anaerobic effluents can be treated with processes that require low concentrations of organic compounds such as anaerobic ammonia oxidation (anammox) for nitrogen removal (Delgado Vela et al. 2015) or with processes relying on chemical precipitation for phosphorus removal. Life cycle assessments often consider the use of nutrient-rich AnMBR effluents for irrigation, and therefore do not consider nutrient removal a main sustainability challenge for AnMBRs (Smith et al. 2014, Pretel et al. 2015, Becker et al. 2017).

Irrespective of these environmental concerns, research into and implementation of AnMBRs continues to expand. The number of peer-reviewed AnMBR publications as found in Scopus, both for industrial and domestic wastewater, increased steadily from 2000 to 2015 (Dvořák et al. 2016). Additionally, AnMBRs comprise 1% of the current MBR market (Krzeminski et al. 2017), which has increased over sixfold from 2010 to 2017, reaching a global cumulative wastewater treatment capacity of almost 7,000,000 m^3/d in 2017 (Xiao et al. 2019).

The abovementioned sustainable challenges seem inconsistent with the increased interest in AnMBRs, at least from an environmental point of view. We decided to study patents to help provide insights into the innovation landscape and understand where AnMBR technologies are heading. Not only can numerical analysis of international patent documents be a valuable tool for corporate technology analysis and planning (Mogee 1991, Abraham et al. 2001, Ernst 2003), patent analyses can also inform the general public regarding innovation activity across a field (Griliches 1998). Mogee (1991) discusses how such an analysis can reveal technological directions of the field, of specific firms, and of important world markets. Patent citations (when given sufficient time for citations to accumulate (Hall et al. 2005) and number of patents (Griliches 1981) correlate positively to the market value of a firm. A comprehensive review on patent-based research furthermore concluded that patent documents form a unique resource for the analysis of the process of technical change and can be used as a substitute for R&D data (Griliches 1998). In recent years, the insights that can be gained from patent studies are expanding due to the growing availability of global patent data (Nagaoka et al. 2010). Hence, we conducted a patent review to evaluate technological directions of AnMBRs and assess if AnMBR technologies are addressing sustainability issues raised by recent life-cycle assessments (Smith et al. 2014, Pretel et al. 2015).

To date, no patent review has been performed for AnMBR designs. Furthermore, patent review studies in areas of anaerobic or membrane waste treatment are scarce. The most relevant patent reviews focused on membrane aerated biofilm reactors (Li et al. 2008), immobilized microorganism technologies in wastewater treatment (An et al. 2008), and nanofiltration systems (Hussain et al. 2009). These reviews provide general context and an overview of technologies studied in line with 'literature reviews' (further referred to as 'critical reviews') as opposed to 'systematic reviews', which aim to answer specific research questions (Robinson et al. 2015). Only a handful of systematic patent reviews have been performed on topics relevant to AnMBRs including reviews on membrane technologies (Zhai et al. 2014, Woo 2018) and wastewater treatment, such as the wastewater treatment field in general (around the world (González-Cabrera et al. 2014); focused on Japan (Hara et al. 2016); focused on China (Yuan et al. 2009)) or specific wastewater treatment technologies (Alvarez-Pugliese et al. 2014). These studies each used keywords or technology classes to build a patent collection (ranging from 74 patents (Alvarez-Pugliese et al. 2014) to 169,312 patents (Yuan et al. 2009)) to assess the target technology or field.

We combined systematic and critical review approaches to determine where AnMBR designs are heading and if environmental sustainability concerns are addressed by the field. Our patent review used a systematic review approach based on keywords searches to find patents relevant to AnMBR technology and to quantify trends within this patent collection. A first impression of the commitment and progress towards environmental sustainability challenges was obtained by numerically analyzing the metadata (e.g., field of activity, region or country, industrial or academic activity) for relevant patents through an additional keyword search. Finally, we performed an iterative search to provide a broad but critical overview of the different designs presented in AnMBR patents and analyzed technological directions of the field to evaluate whether they addressed environmental concerns.

2.3 Methods

InnovationQ (Version 4.6, 2019) was used to find and analyze AnMBR patents. InnovationQ is an intellectual property analytics tool powered by a semantic search engine (Semantic Gist) that contains more than 100 million patents (InnovationQ). A search was conducted on May 13th 2019, using a combination of keywords 'anaerobic, membrane, and bioreactor' or 'anaerobic and MBR' in the title, abstract, or claims, which resulted in 4,007 patent documents. In general, MBRs associated with anaerobic processes are used for a variety of applications, including MBRs not intended solely for anaerobic wastewater treatment. For example, they include MBR processes for microbially mediated nitrogen or phosphorus removal, which require bioreactor configurations that include anaerobic steps. As AnMBRs of interest in the current study aim to convert organic compounds in wastewater into volatile fatty acids (VFAs) or methane (CH_4), an additional keyword search was performed to find patents relevant to AnMBRs. Of the 4,007 patents originally identified, 1,232 mentioned "methane", "CH_4", "volatile fatty acid", "volatile fatty acids", "VFA", or "VFAs" in their title, abstract, claims, or description. Of these 1,232 patent documents, 688 remained upon removal of replicates. InnovationQ groups identical patent documents submitted to multiple authorities (both application and granted documents) as "simple family members", and as such, these simple family members were assumed to be replicates. This set of 688 patents is referred to as the "AnMBR patent collection". Our systematic patent selection process is illustrated in Figure 2.1.

The front page of patent documents discloses filing characteristics of each patent, which are typically used by economists for innovation research (Mogee 1991, Nagaoka et al. 2010).

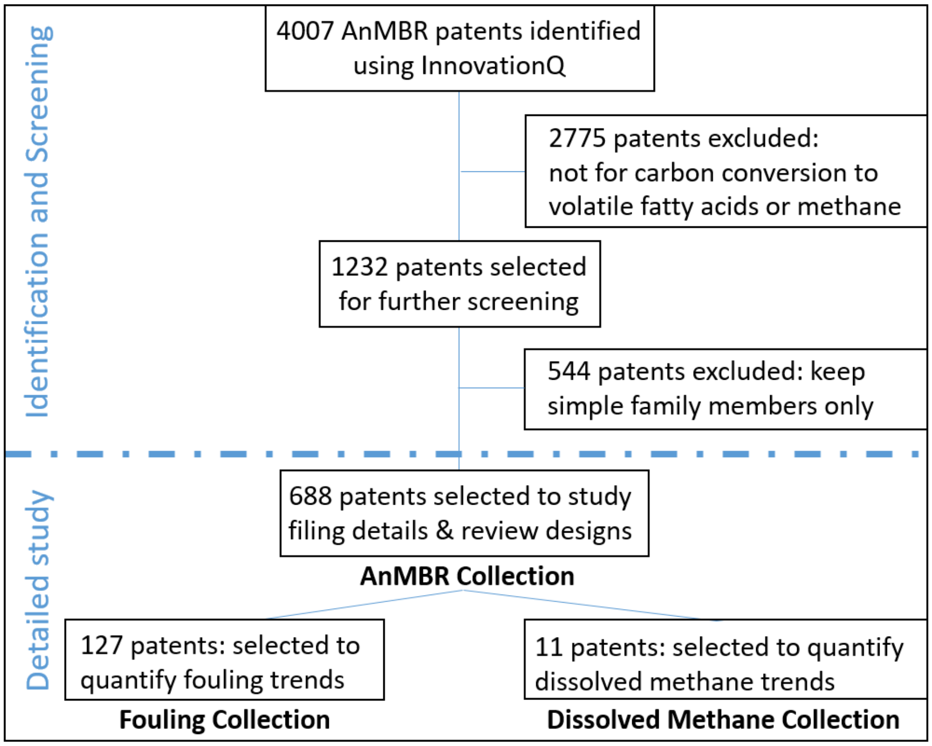

Figure 2.1: Flow chart of patent selection process.

InnovationQ outputs the following patent filing details: earliest priority date (the earliest filing date of patent documents within one simple family), licensing organizations (also referred to as "applicants/assignees"), first assignee/applicant (the assignee/applicant listed first during the application process), country of origin (the country where the first listed assignee/applicant is located), global markets (represented ftby "authorities"), and technology classes (international patent classification code (IPC) or cooperative patent classification (CPC) codes). These filing characteristics of all collected AnMBR patents were studied to obtain a broad overview of AnMBR innovation activity. We used IPC codes to determine the field within which AnMBR patents are filed as IPC codes were more consistently reported than CPC codes. InnovationQ did not output a country of origin for 464 of the 688 AnMBR patent documents. The missing countries of origin for the patent documents listing a company or organization as first assignee/applicant (432 patent documents) were filled in by researching the country of origin of each of these companies/organizations. The country of origin of less than 5% of the patents in the AnMBR patent collection (32 patent documents) was not further researched since these documents listed an individual as first assignee/applicant. The average amount of time an authority requires to publish a patent was calculated to estimate the amount of patent documents already filed but not yet published. The amount of time an authority requires to publish a patent was calculated by the difference between the publication date and the earliest priority date for patents solely filed to that authority. Since patents are often filed to other authorities only after they are granted by their first authority, the difference between the publication date and the earliest priority date was artificially augmented for patents with multiple authorities. The same numerical analyses were performed for two sub-collections comprising patents relating to (i) fouling challenges and (ii) dissolved methane mitigation. These two sub-collections were formed by selecting all patents mentioning specific keywords in their title, abstract, description, or claims (i.e., "fouling", 127 patents; and "dissolved methane" or "dissolubility methane", 11 patents, respectively). These keywords were selected based on an initial thorough read-through of 100 patents, which indicated that none of the patent documents targeted fouling or dissolved methane mitigation without mentioning at least one of these keywords. The low number of AnMBR patents targeting dissolved methane mitigation can at least partially be explained by the fact that dissolved methane is not only a concern for AnMBRs but for anaerobic treatment systems in general. Note that patents aiming to remove dissolved methane from anaerobic effluents other than those generated by AnMBRs were beyond the scope of this study.

Aside from the filing details listed on the front page of patent documents, the field's technological direction can be evaluated by examining the description and claims section of patent document (Johnstone et al. 2010, Míguez et al. 2018). We categorized the different designs within the AnMBR patent collection into four categories:(i) approaches developed to pre-treat AnMBR influents, (ii) patents aiming to improve AnMBR bioreactors, (iii) methods concentrated on membranes used in AnMBRs, and (iv) patents focused on post treatment of AnMBR effluents.

2.4 Results and Discussion

As explained above, a keyword search was performed using the InnovationQ patent software to identify 4,007 patent documents focused on AnMBR designs. After removing duplicates and only including those patents targeting anaerobic production of VFAs or methane, 688 AnMBR patents remained, which are further referred to as the 'AnMBR patent collection'. The technologies presented by the majority of these AnMBR patents are classified for the treatment of water, wastewater, sewage, or sludge (C02F, 80% see All technologies within the AnMBR patent collection were invented in one of 27 countries and the corresponding patents were filed to one (or more) of 47 authorities. Most patents in the AnMBR collection were invented (Figure 2.5) and filed (Figure 2.6) in China. Most AnMBR patent documents only have one reported authority (547 documents, or 80%), of which 72% are invented and 77% are filed in China. However, the United States Patent and Trademark Office and World Intellectual Property

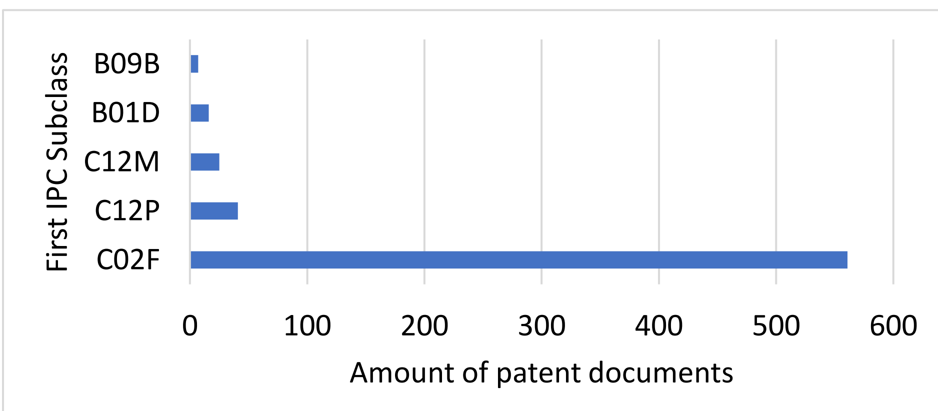

Figure 2.2: Amount of patent documents per first IPC subclass: treatment of water, wastewater, sewage, or sludge (C02F); fermentation or enzyme-using processes to synthesize a desired chemical compound or composition or to separate optical isomers from a racemic mixture (C12P); apparatus for enzymology or microbiology including installations for fermenting manure (C12M); separation processes (B01D); disposal of solid waste (B09B).

Organization almost consistently surpass the China National Intellectual Property Administration (CNIPA) as authorities for AnMBR patents filed to multiple authorities (data not shown). The countries of origin and amount of patent applications per authority are less skewed towards China for the patents that mention fouling (Figure 2.7). When comparing the overall AnMBR collection to the fouling collection, the respective fractions of patents invented in China were 58% and 28%, the respective fractions of patents applied to in China were 70% and 45%, the respective fractions of patents invented in Korea were 7% and 12%, and the respective fractions of patents filed in the United States were 18% and 32%. Similarly, a more evenly distributed regional activity (both regarding countries of origin and authorities) is observed for the dissolved methane patents (Figure 2.7). The Chinese dominance regarding country of origin and authority has been observed in other patent studies focusing on membrane technologies (Zhai et al. 2014) and wastewater treatment (Yuan et al. 2009, González-Cabrera et al. 2014) and is consistent with China treating the largest capacity of water with MBRs (Xiao et al. 2019). So, even though China dominates regarding installing and inventing MBRs and AnMBRs, our analysis indicates that China does not lead when considering inventiveness regarding fouling and dissolved methane mitigation. A comprehensive review on patent-based research demonstrated a strong relationship between the amount of patents generated by firms and their R&D expenditures, suggesting that patent data are indicators for inventive input and output and can be used as proxies for R&D data (Griliches 1998). The mismatch between inventiveness regarding sustainability challenges and implementation of full-scale MBRs highlights opportunities for increased R&D to achieve fouling and dissolved methane mitigation for Chinese firms.

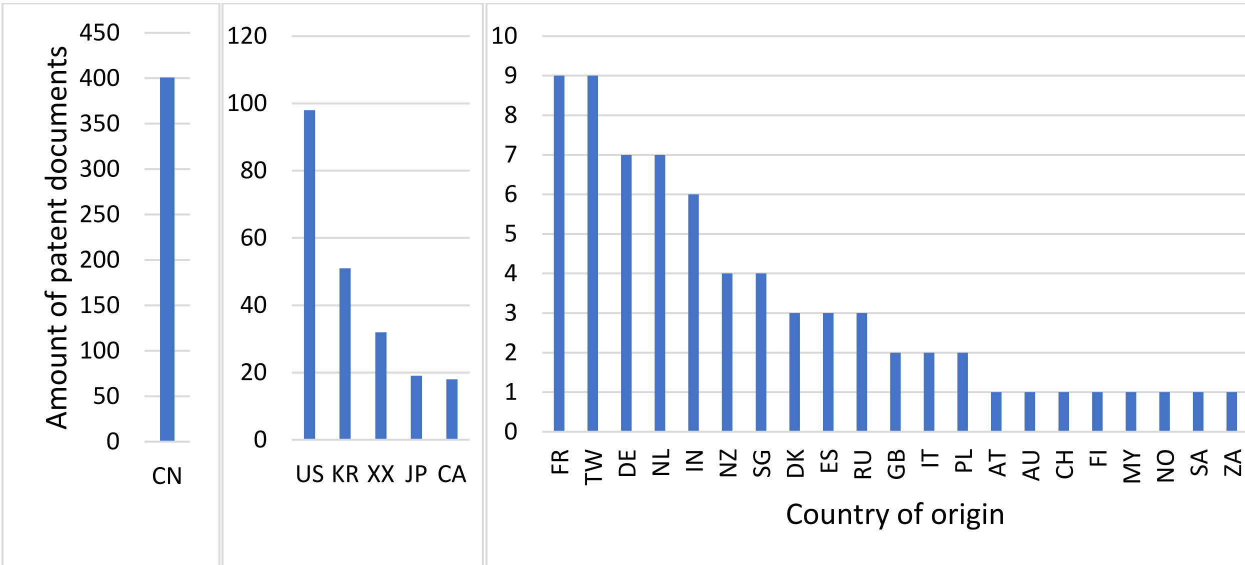

Figure 2.5: Amount of AnMBR patent documents per country of origin, represented by the following country codes: China (CN), United States (US), Republic of Korea (KR), Unknown (XX), Japan (JP), Canada (CA), France (FR), Taiwan (TW), Germany (DE), Netherlands (NL), India (IN), New Zealand (NZ), Singapore (SG), Denmark (DK), Spain (ES), Russia (RU), United Kingdom of Great Britain and Northern Ireland (GB), Italy (IT), Poland (PL), Austria (AT), Australia (AU), Switzerland (CH), Finland (FI), Malaysia (MY), Norway (NO), Saudi Arabia (SA), South Africa (ZA).

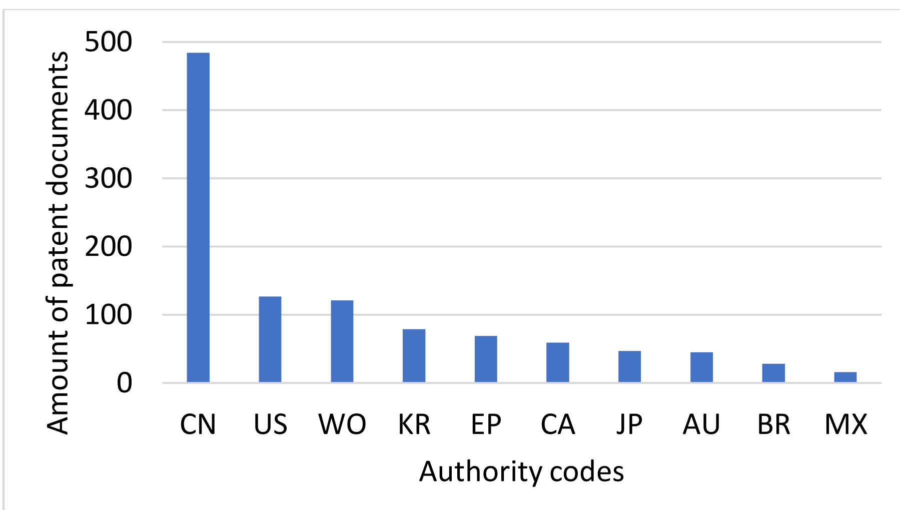

Figure 2.6: Amount of AnMBR patents filed (if application) or issued (if granted) per authority for the ten authorities with the largest amount of patents filed or issued, with CN = China, US = United States, WO = World Intellectual Property Organization, KR = Republic of Korea, EP = European Patent Office, CA = Canada, JP = Japan, AU = Australia, BR = Brazil, MX = Mexico.

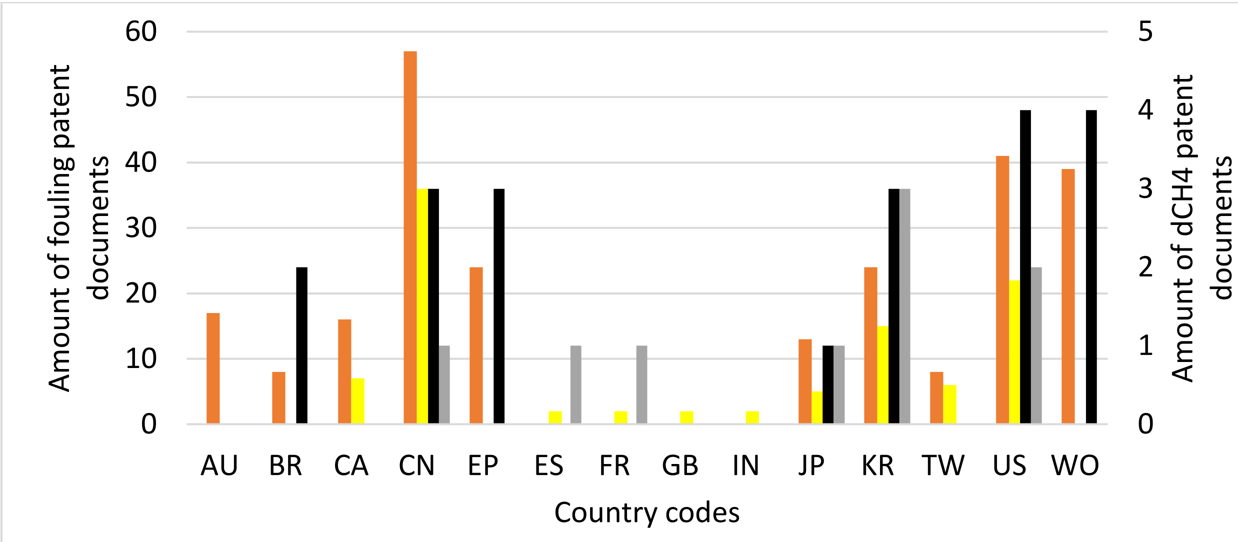

Figure 2.7: Amount of fouling patents per authority (orange) and per country of origin (yellow) and amount of dissolved methane patents per authority (black) and per country of origin (grey).

2.4.1.1 Analysis by first assignee or applicant

.2). In line with this observation, municipal wastewater treatment accounts for 75% of the total amount of wastewater treated by MBRs in China (Xiao et al., 2019). Through an additional keyword search, we learned that 127 patents (18.5%) targeted fouling and 11 patens focused on dissolved methane mitigation (1.6%). Below we discuss historical, regional, and institutional trends (Section 2.4.1) and specific AnMBR design categories (Section 2.4.2).

2.4.2 AnMBR patent collection trends

2.4.2.1 Historical development of AnMBR patents

The first AnMBR patent application was filed in 1979 and only a few additional patents were published from 1980 to 1995 (Figure 2.3). The number of AnMBR patents filed slowly increased from 1995 to 2005 and this increasing trend accelerated from 2005 until 2018. The China National Intellectual Property Administration required an average of about ten months to process patents within the AnMBR collection. All other authorities required an average of 29 months. The European patent office required slightly over 18 months, while the United States Patent and Trademark Office required 41 months on average. Therefore, the drop observed in the number of patent applications reported in 2018 can be explained by the fact that the patents included in this study were collected in May 2019 and the considerable time required to publish patent applications. Even the number of published patent applications for 2017 and 2016 are expected to increase slightly in the years to come. Other patent studies targeting related topics, such as nanofiltration membrane technologies (Zhai et al. 2014), MBRs (Xiao et al. 2019), water

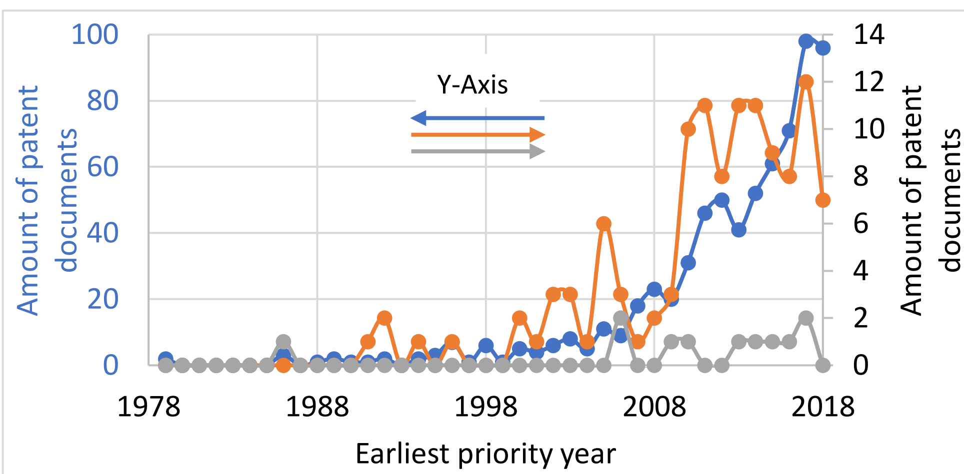

Figure 2.3: Amount of patent documents per earliest priority year per patent collection: AnMBR collection (blue), fouling collection (orange), dissolved methane collection (grey). Data collected in May 2019.

and wastewater treatment (Yuan et al. 2009, González-Cabrera et al. 2014), and MBR-based water or wastewater technologies (Woo 2018), have both shown similar and diverging temporal behaviors. As 80% of the AnMBR patents involve water or wastewater treatment, patent studies in these related fields are relevant for the current analysis. Three of these related studies displayed similar historical trends to the one observed for the current study (Yuan et al. 2009, Zhai et al. 2014, Xiao et al. 2019). In contrast, two studies reported a consistent steady number of patent publications per year (González-Cabrera et al. 2014, Woo 2018). It is difficult to discern why this different trend was observed as they either do not visually illustrate the temporal (González-Cabrera et al. 2014) or mention how their patents were found (i.e., specific keywords or patent classification codes) (Woo 2018).

The temporal characteristics for dissolved methane and fouling related patent applications differ from those of the overall AnMBR collection (Figure 2.3). The first fouling patent was published over ten years after the first AnMBR patent (1991 versus 1979). Furthermore, the number of fouling patent publications slowly increased from 1991 to 2009, spiked in 2010, and then stayed relatively constant until 2019. Even though it is difficult to validate if this observed trend corresponds to the real behavior of fouling AnMBR patents over time rather than it being an artefact of the database or search method used, at least one study reports a similar stepwise trend for academic papers focusing on fouling (Meng et al. 2017). Due to the limited number of dissolved methane related patents (only 11 patents), it is not meaningful to ascertain trends, but it is noteworthy that 10 of these 11 patents were published after 2005. By presenting the data shown in Figure 2.3 in a different manner, Figure 2.4 shows that the contribution of fouling patents to the overall AnMBR patent pool was substantial during several years between 1991 and 2006, but has been declining since then. Furthermore, Figure 2.4 highlights that the contribution of dissolved methane patents to the overall AnMBR patent collection is very low. These results suggest the AnMBR field holds substantial capacity for innovation to find solutions for the environmental costs associated with fouling and methane loss.

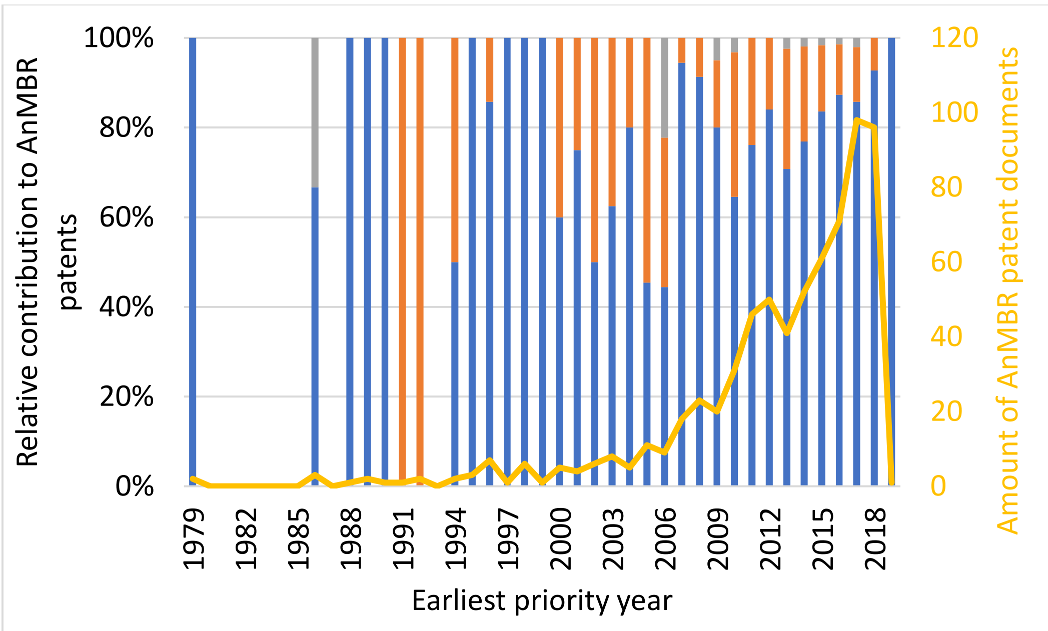

Figure 2.4: Relative contribution of fouling (orange), dissolved methane (grey), or other patents (blue) to total AnMBR patents (left y-axis) together with the total amount of AnMBR patent documents per earliest priority year (yellow, right y-axis).

2.4.2.2 Analysis by authorities and countries of origin

All technologies within the AnMBR patent collection were invented in one of 27 countries and the corresponding patents were filed to one (or more) of 47 authorities. Most patents in the AnMBR collection were invented (Figure 2.5) and filed (Figure 2.6) in China. Most AnMBR patent documents only have one reported authority (547 documents, or 80%), of which 72% are invented and 77% are filed in China. However, the United States Patent and Trademark Office and World Intellectual Property Organization almost consistently surpass the China National Intellectual Property Administration (CNIPA) as authorities for AnMBR patents filed to multiple authorities (data not shown). The countries of origin and amount of patent applications per authority are less skewed towards China for the patents that mention fouling (Figure 2.7). When comparing the overall AnMBR collection to the fouling collection, the respective fractions of patents invented in China were 58% and 28%, the respective fractions of patents applied to in China were 70% and 45%, the respective fractions of patents invented in Korea were 7% and 12%, and the respective fractions of patents filed in the United States were 18% and 32%. Similarly, a more evenly distributed regional activity (both regarding countries of origin and authorities) is observed for the dissolved methane patents (Figure 2.7). The Chinese dominance regarding country of origin and authority has been observed in other patent studies focusing on membrane technologies (Zhai et al. 2014) and wastewater treatment (Yuan et al. 2009, González-Cabrera et al. 2014) and is consistent with China treating the largest capacity of water with MBRs (Xiao et al. 2019). So, even though China dominates regarding installing and inventing MBRs and AnMBRs, our analysis indicates that China does not lead when considering inventiveness regarding fouling and dissolved methane mitigation. A comprehensive review on patent-based research demonstrated a strong relationship between the amount of patents generated by firms and their R&D expenditures, suggesting that patent data are indicators for inventive input and output and can be used as proxies for R&D data (Griliches 1998). The mismatch between inventiveness regarding sustainability challenges and implementation of full-scale MBRs highlights opportunities for increased R&D to achieve fouling and dissolved methane mitigation for Chinese firms.

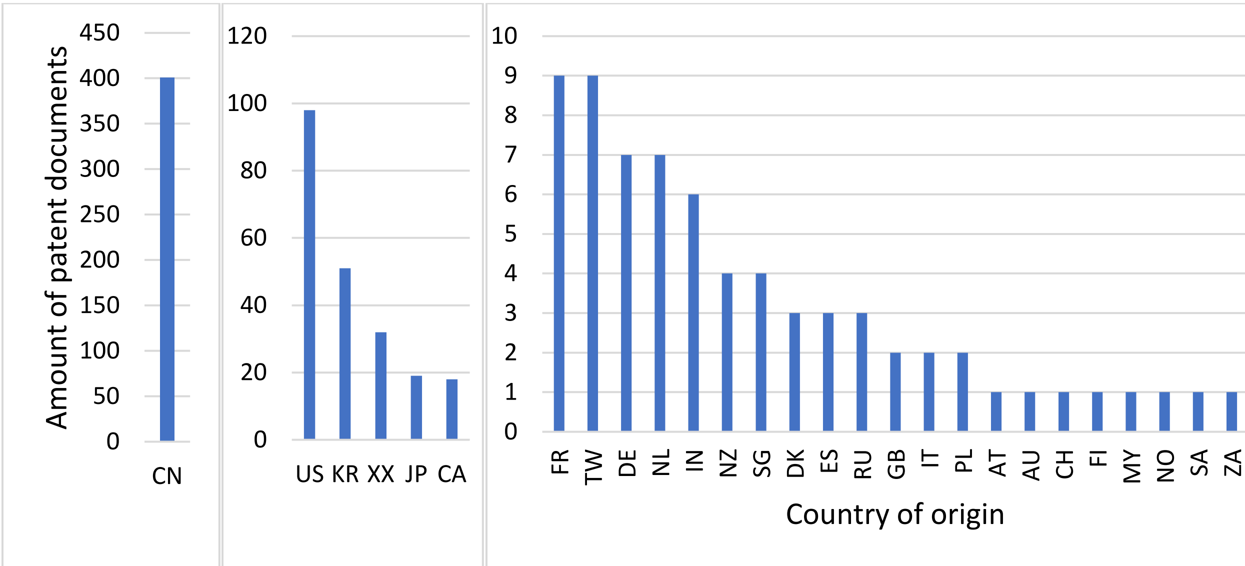

Figure 2.5: Amount of AnMBR patent documents per country of origin, represented by the following country codes: China (CN), United States (US), Republic of Korea (KR), Unknown (XX), Japan (JP), Canada (CA), France (FR), Taiwan (TW), Germany (DE), Netherlands (NL), India (IN), New Zealand (NZ), Singapore (SG), Denmark (DK), Spain (ES), Russia (RU), United Kingdom of Great Britain and Northern Ireland (GB), Italy (IT), Poland (PL), Austria (AT), Australia (AU), Switzerland (CH), Finland (FI), Malaysia (MY), Norway (NO), Saudi Arabia (SA), South Africa (ZA).

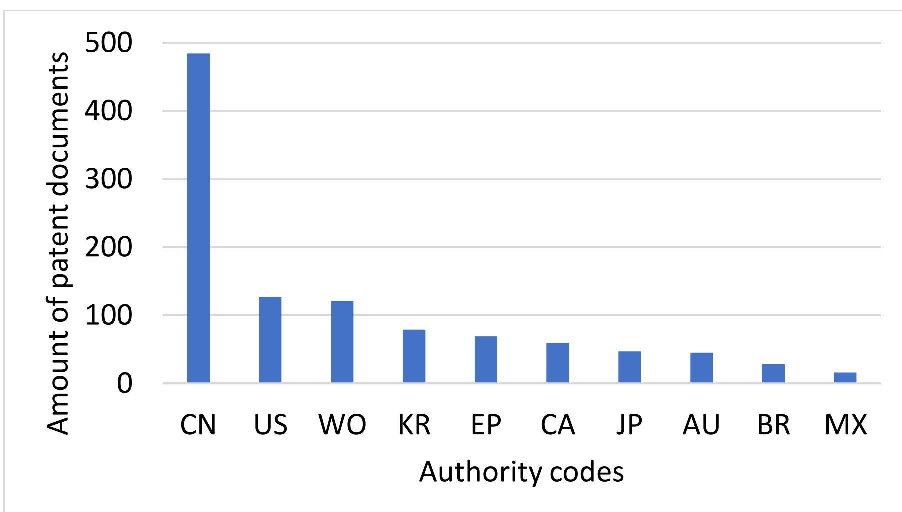

Figure 2.6: Amount of AnMBR patents filed (if application) or issued (if granted) per authority for the ten authorities with the largest amount of patents filed or issued, with CN = China, US = United States, WO = World Intellectual Property Organization, KR = Republic of Korea, EP = European Patent Office, CA = Canada, JP = Japan, AU = Australia, BR = Brazil, MX = Mexico.

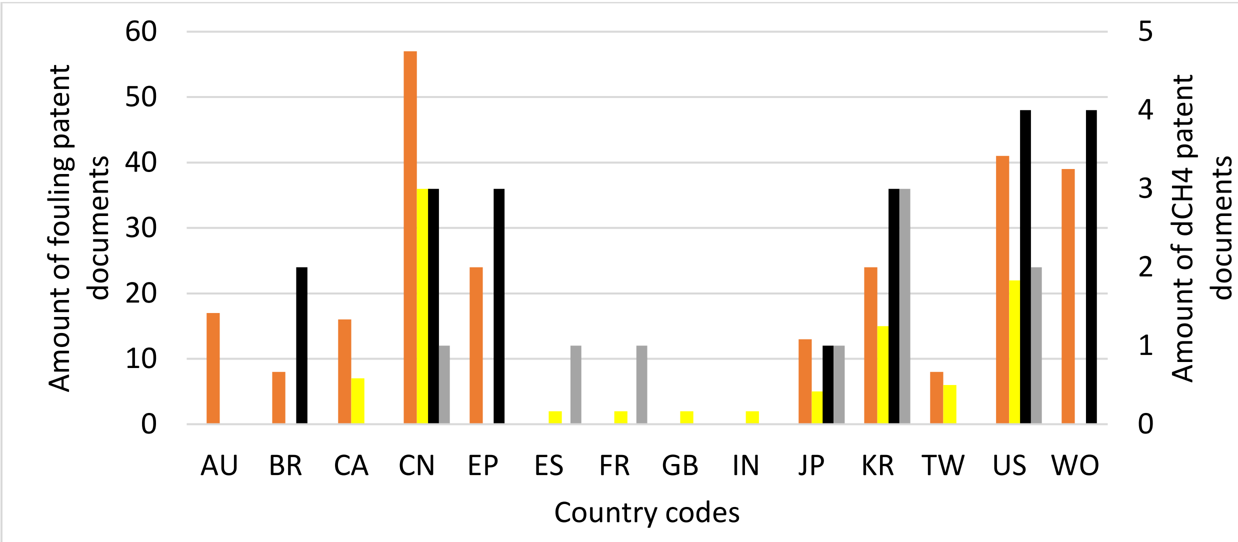

Figure 2.7: Amount of fouling patents per authority (orange) and per country of origin (yellow) and amount of dissolved methane patents per authority (black) and per country of origin (grey).

2.4.2.3 Analysis by first assignee or applicant

Figure 2.8 shows the amount of patent documents applied by or granted to the top ten first assignees/applicants for all AnMBR patents, and for the fouling and dissolved methane sub-collections. These top first assignees/applicants collectively produced 16%, 29%, and 100% of all AnMBR, fouling, and dissolved methane patent documents, respectively. Research institutes and universities were especially active in filing dissolved methane patent documents; they produced 78% of dissolved methane patents (Figure 2.9). González-Cabrera et al. (2014) commented on the considerable wastewater patent filing activity of universities and hypothesized that universities might be incentivized to publish patents as funding agencies might increasingly take the number of patent applications into account when appropriating research funds.

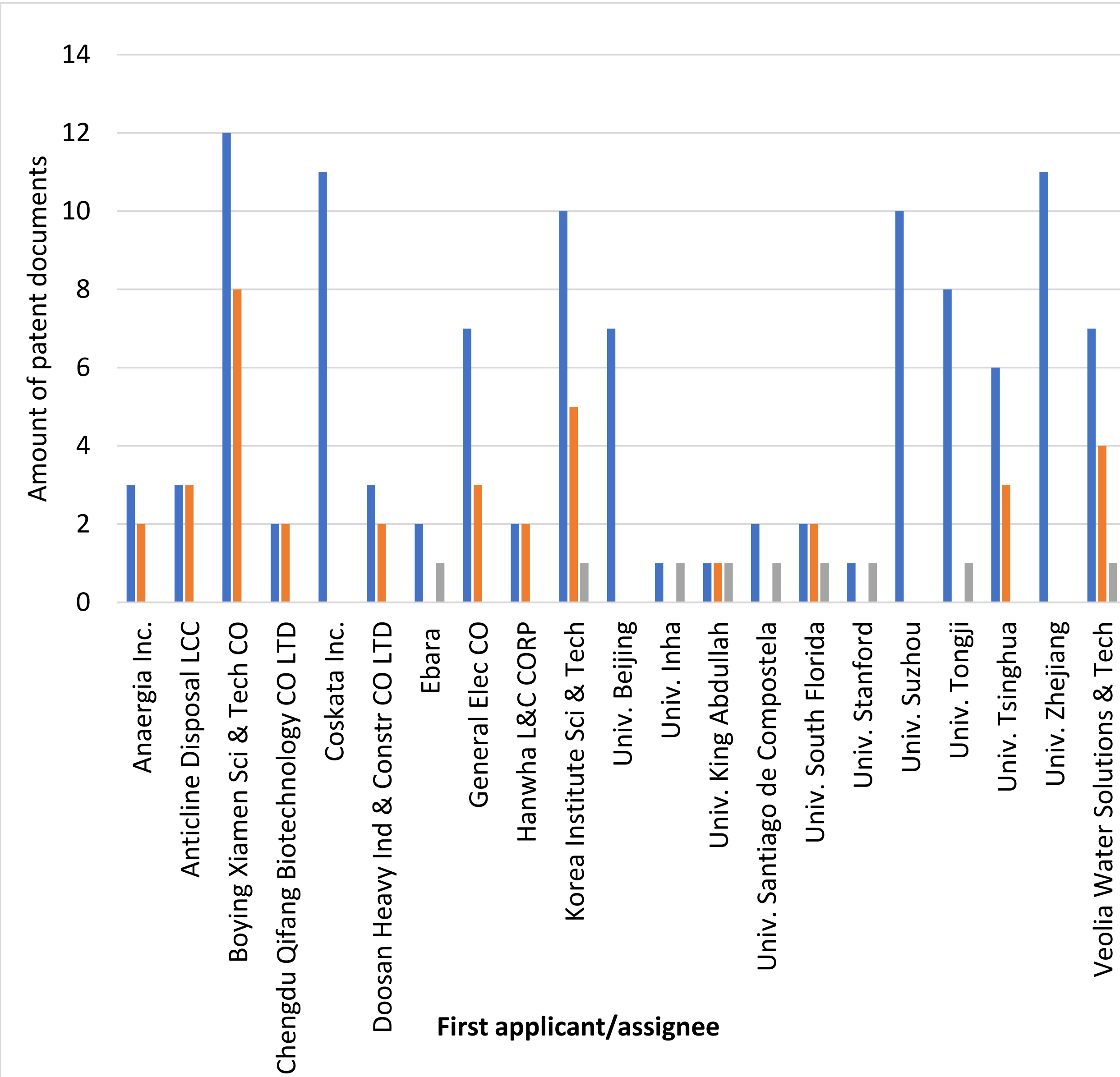

Figure 2.8: Amount of patent documents applied by or granted to the top ten assignees/applications for all AnMBR patents (blue) as well as for the fouling (orange) and dissolved methane (grey) sub-collections. Since some assignees or applicants only showed up for certain collections, the figure includes more than 10.

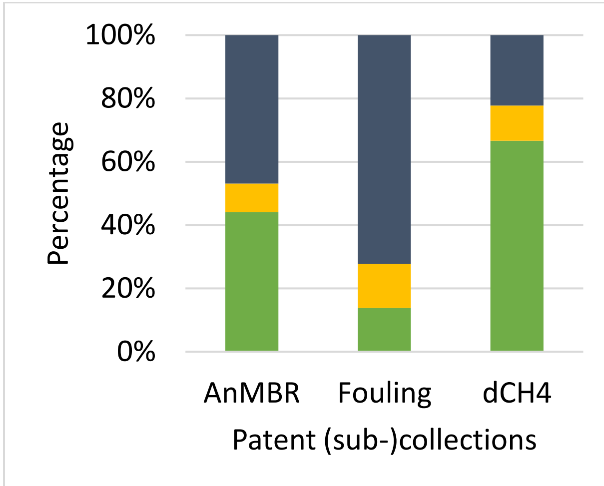

Figure 2.9: Relative contribution of universities (green), research institutes (yellow), and companies (grey) towards the top 10 first applicants/assignees of the AnMBR collection and the fouling and dissolved methane (dCH4) sub-collections.

2.4.3 Overview of AnMBR designs

The earliest patents in the AnMBR collection comprise anaerobic processes that include a membrane to purify the produced biogas (Messing 1979, Koichi 1986b), rather than a membrane to separate solids and liquids. The first inventions that include membranes to retain solids and obtain higher effluent quality were filed in the late 1990s ((Kazyuki et al. 1994, Chmiel 1997, Zhou et al. 1998), except for Hiroshi et al. (1991) who report an earliest priority date of 1991, but whose patent was only published in 2001.

While some of the inventions among the 688 patents included in the AnMBR patent collection are still relevant for the operation of AnMBRs, others are not. The latter category includes a wide variety of processes, such as optimizing ethanol production through microbial gene modifications (Reeves et al. 2010) or monitoring oxygen utilization rates to control *aerobic* wastewater treatment (Goronszy 1996). Furthermore, a large fraction focuses on an extensive treatment train including anaerobic and anoxic processes as well as aerobic MBRs. Those treatment trains also constitute the dominant configuration of MBR applications in China (Xiao et al. 2014). Although not exclusively (e.g., Early et al. (2010)), these inventions often aim to treat a specific waste stream, such as sludge (Stephenson et al. 2013), distillery spent wash or molasses spent wash (Prasad et al. 2010), nutrient deficient streams (Masayo et al. 2009), or tobacco waste (Shiwen 2013). The novelty of these patents typically lies in the specific combination of treatment processes as opposed to the optimization of an anaerobic bioreactor or membrane setup, making these designs less relevant for AnMBR design innovation. Furthermore, fouling mechanisms in AnMBRs can differ from those in aerobic MBRs (Baek et al. 2006, Xiong et al. 2016), and effluent characteristics of AnMBRs are inherently different than those produced by a treatment train. Therefore, such treatment trains are not included in the sections below discussing designs reported in the AnMBR patents. Inventions targeting anaerobic fermentation processes to create value added products other than methane-rich biogas are excluded as well. For instance, multiple patents start out with syngas or waste gases from industrial processes such as oil refining to produce acetic acids, organic acids, or alcohols (Gaddy et al. 1994); ethanol, *n*-butanol, hexanol, or acetic acid (Shih-Perng et al. 2008); ethanol or acetic acid (Simpson et al. 2007); acetic acid (Gaddy 1998, Gaddy et al. 1998); or methanol (Datta et al. 2013). The following sections present approaches developed to pre-treat AnMBR influents (Section 2.4.2.1), patents aiming to improve AnMBR bioreactors (Sections 2.4.2.2), (iii) methods concentrated on membranes used in AnMBRs (Sections 2.4.2.3), and patents focused on post treatment of AnMBR effluents (Sections 2.4.2.4).

2.4.3.1 Innovations enhancing AnMBR pre-treatment

Rozich (2010) and Bi et al. (2016) present pre-treatment approaches that break down large organics in waste streams to be treated before they are sent to a bioreactor. These technologies improve the efficiency of the subsequent biological process and are thus relevant for AnMBR operation. The invention described by Yeh et al. (2017) focuses on a settling tank, optionally with a hydrocyclone, aimed to concentrate the waste stream to enhance AnMBR treatment of dilute wastewater streams.

2.4.3.2 Innovations specific to AnMBR bioreactors

Much consideration has been given to multi-stage reactor setups for improving anaerobic digestion. AnMBR patents with multi-stage reactor setups can be divided into two groups: those aimed at improving the anaerobic degradation of organic compounds by physically separating hydrolysis/acidification from methanogenesis, and those targeted at achieving nutrient removal in addition to organics removal through the inclusion of aerobic and anoxic tanks. As these latter inventions are not considered true AnMBRs, they were excluded from this critical review. Several two-stage AnMBR patents were published after the first academic study on a two-stage anaerobic digestion system in 1974 (Ghosh et al. 1974). Some two-stage patents focus on optimizing the first hydrolysis/acid stage by controlling its pH and redox potential (Uwe 1999) or by adding a specific mix of enzymes (Morgoun 2002), while other patents add a neutralization tank to optimize the subsequent methanogenesis stage (Ren-Yang et al. 2003, Chiu-Yue et al. 2015).

A second type of novel reactor configuration involves the promotion of biofilm growth to increase biomass concentration and ensure continued treatment throughout system perturbations. UASBs and EGSBs were originally designed to retain slow growing anaerobic microorganisms and avoid washout by selecting for granular biomass, which can also be thought of as layered biofilms (Calderón *et al.*, 2013). While one invention discusses the addition of a membrane to a UASB to help granule formation during startup (Hansen et al. 2006), most other inventions aiming to promote biofilms do so by including carrier material (Bae et al. 2010a, Josse et al. 2012, Austin et al. 2014, Gosselin et al. 2014a, Gosselin et al. 2014b, Uller 2014, Fuwei 2015, Huajun et al. 2018). To accelerate anaerobic biofilm growth on the surface of carrier material, one invention proposes to first allow faster growing aerobic biofilms to adhere to the carrier material before operating the system anaerobically (Huajun et al. 2018). Alternatively, some patents discuss specific materials to maximize biomass attachment such as carbon allotrope-silica composite materials (Gosselin et al. 2014a, Gosselin et al. 2014b), cross-linked polymeric material such as calcium alginate (Yeyuan et al. 2016), or a polymeric gel matrix comprising chitosan and lignosulphonate (Tartakovsky et al. 1996). While some inventions comprise typical moving bed systems (Josse et al. 2012), Uller (2014) aims to avoid channeling through the carrier material by enforcing a specific flow pattern. Three-phase separators (Fuwei 2015) or other solids separation devices such as cyclones (Austin et al. 2014) are used to retain carrier material in the bioreactor. As opposed to confining the carrier material, Jae-Ho et al. (2010) and Haeng et al. (2015) allow the carrier material to interact with their membranes to continuously scrape the fouling layer while still acting as support media for microorganisms and adsorb substances that could contribute to membrane fouling.

In line with this last invention, a third group of patents aim to operate or configure reactors specifically to address membrane fouling and increase membrane flux. Biopolymers, including polysaccharides and proteins secreted by biomass, are known to contribute to membrane fouling and reactor foaming. To counter the development of a thick fouling layer and reactor foaming, therefore, specific cationic but also amphoteric and zwitterionic polymers are proposed to be added to react with the biopolymers present (Seong-Hoon et al. 2005, Yoon et al. 2005, Collins et al. 2006). Abdelkader et al. (2014) proposed to add an organic sequestering agent consisting of organic phosphates to minimize membrane scaling (i.e., precipitation of inorganic compounds (e.g., calcium carbonate) onto the membrane surface). Aside from revising reactor operation to minimize fouling, many inventions focus on optimizing the reactor configuration to decrease the amount of solids or organics going into an external membrane unit. For example, Ewing (2010) and Grelot et al. (2012) proposed anaerobic reactor configurations that achieve solids stratification. Sludge coming from the reactor zone with the lowest solids concentration is sent to the membrane unit, while the highest solids concentration sludge is sent to an external solids separation unit such as a hydrocyclone. Early EGSBs and UASBs can be thought of as reactors with solids stratification, and indeed, Ramanath et al. (2014) and Yan et al. (2018) discuss the placement of an EGSB or UASB to avoid subsequent membrane fouling. To ensure maximal solids removal from a stream to be sent to the membrane unit, certain patents include coagulation and flocculation (Grelot et al. 2012, Chul et al. 2017). Separate solids treatment processes, including cyclones and screw presses, are also independently used to separate solids from the liquid stream so that the liquid effluent can be sent to the membrane unit (Nikhujs et al. 2012, Young 2014). Lastly, Lan et al. (2018) include consecutive baffles of decreasing size to limit the amount of solids entering the membrane unit while still compactly achieving different reaction zones.

A final group of patents that address reactor performance is focused on enhanced methanogenesis by optimizing the microbial and nutrient composition (Ashby et al. 2010), adding biocatalysts (Engineering et al. 1998), introducing monitoring and control ranging from simple real time control of pH and sludge levels (Yuansong et al. 2017) to combining online extended Kalman filters with dynamic anaerobic digestion models (Kumar et al. 2011), and installing specific stirring devices (Yang et al. 2018).

2.4.3.3 Innovations related to membranes used in AnMBRs

Most membrane innovations revolve around enhancing membrane life or increasing flux by developing new membrane materials or inventing new fouling mitigation techniques. Membranes used in AnMBRs are typically made of polymeric materials such as polyurethane or polyethylene terephthalate and operate in the ultra or microfiltration range. Such polymeric materials have low durability and tensile strength, which makes them susceptible to damage during cleaning or operation at high transmembrane pressures. Therefore, as opposed to installing polymeric membranes, patents have been awarded for the use of more durable ceramic membranes (Young 2014, Chidambaran et al. 2016, Gao et al. 2018). Furthermore, membranes can be coated with an antibacterial agent such as quaternary ammonium salts to minimize the formation of a fouling layer (Zhiwei et al. 2018) or be reinforced with metal powder (from transition metals such as oxides and alloys) to make the membrane more hydrophilic and thus increase flux (Dae et al. 2011). Another approach to increase flux while operating at lower transmembrane pressures is to utilize a support medium with larger pores such as in dynamic membranes (Schindler et al. 2006, Yang et al. 2008, Hua et al. 2011, Zhiwei et al. 2014, Baoshan et al. 2017, Binghua et al. 2018). These dynamic membranes have pore sizes ranging from 0.5-500 micrometers and can consist of polymers (Yang et al. 2008), nylon or stainless steel (Zhiwei et al. 2014, Baoshan et al. 2017, Binghua et al. 2018), and tyrylene or silk (Hua et al. 2011). Due to the relatively large pore sizes, the fouling layer that develops on these support structures provides most of the solid/liquid separation. To maintain a desired fouling layer thickness, Zhiwei et al. (2014) suggest to sparge the membrane surface while Hua et al. (2011) propose to employ physical cleaning with a brush or sponge.

Early fouling mitigation techniques found in the AnMBR patent collection include backwashing membranes with chemicals such as chlorine (Chmiel 1997) or introducing hydraulic shear on the membrane surface through sparging with biogas (Dirk et al. 2003). Some patents comprise relatively simple variations of these. For example, Hyup et al. (2015) include a baffle between membrane and bioreactor so that the membrane can be cleaned in situ without compromising the operation of the bioreactor. Novel sparging strategies as patented for AnMBRs include sparging with carbon dioxide obtained from the biogas coupled with the use of sensor feedback to acidify/basify the sludge as needed (Oh et al. 2017) or modifying the bubble generation method (Zimmerman et al. 2006). Another membrane configuration involves allowing the formed biogas to accumulate and pressurize the membrane tank up to 10 kPa so that a permeate flux can be achieved without needing permeate pumps (Hong et al. 2010). More complex membrane setups achieve hydraulic shear on the membrane surface by rotating a disc near the membrane surface (Guen et al. 2012a, Guen et al. 2012b, Guen et al. 2014), shaking the membrane itself back and forth (Chul et al. 2017, Oh et al. 2017, Yili et al. 2017, Chundi 2018, Xiaolan et al. 2018, Yili et al. 2018), rotating the membrane (Yang et al. 2010, Guangbin et al. 2014, Meilan et al. 2015), or rotating the reactor (Liang et al. 2015). In addition to mitigating fouling, these moving systems increase mixing inside the bioreactor, which further enhances anaerobic degradation and achieves gas-liquid equilibrium. Entirely different AnMBR setups include membrane distillation techniques, which achieve higher effluent purity (Fane et al. 2005, Guen et al. 2014), and electrochemical MBRs in which a cathodic membrane catalyzes anaerobic hydrogen production and hereby dislodges membrane fouling layers (Amy et al. 2014, Xia et al. 2018, Xie et al. 2018). Xia et al. (2018), furthermore, describe achieving additional phosphorus removal through electrocoagulation.

2.4.3.4 Innovations regarding post treatment of AnMBR effluents

AnMBR effluents can be treated to further purify (Heffernan et al. 2016) or upgrade these streams (Iversen 2011), generate additional energy (Shiwen 2013), and remove nutrients (Bae et al. 2012, Hwan et al. 2016, Chul et al. 2017, Oh et al. 2017, Bin et al. 2018, Yili et al. 2018) or dissolved methane (Koichi 1986a, Nemser et al. 1996, Lubbe et al. 2010, Garrido et al. 2013). The majority of post treatment nutrient removal processes for AnMBR effluents revolve around short-cut nitrification and anammox (Bae et al. 2012, Hwan et al. 2016, Chul et al. 2017, Oh et al. 2017, Bin et al. 2018, Yili et al. 2018), which require less carbon and oxygen than conventional nitrogen removal. These reactors are typically operated continuously (Bae et al. 2012, Hwan et al. 2016, Chul et al. 2017, Bin et al. 2018, Yili et al. 2018); however, some are operated as sequencing batch reactors (Oh et al. 2017). The earliest patent discussing dissolved methane removal from anaerobic effluents dates from 1987 and suggests to heat anaerobic effluents to strip dissolved gasses into the gas phase (Koichi 1986a). This latter patent, however, does not contain a membrane and can therefore not be considered an AnMBR. Patents that discuss removing dissolved methane from AnMBR effluents do so using methane gas recovery units including aeration, gas stripping, or gas vacuum extraction devices (Bae et al. 2010b, Lubbe et al. 2010); specific degasifying membranes (Nemser et al. 1996, Behmann et al. 2000) (although this latter patent does not mention the specific removal of dissolved methane); or biological methane oxidation (Scheller et al. 2015).

2.5 Discussion and conclusions

The present work offers a broad overview on AnMBR innovation through a comprehensive analysis of AnMBR patent documents. The number of AnMBR patent applications has grown substantially since 2009. Relatively few patents mentioned ‘fouling’ or ‘dissolved methane’, which are the main challenges from a sustainability point of view, averaging 18.4% and 1.4% for the period 2010-2018, respectively. Since other studies found that China is the primary country installing MBRs at this time, it is not surprising our analysis showed most of the AnMBR patents to have Chinese applicants or be filed to Chinese authorities. Given the importance of addressing previously mentioned sustainability challenges, it was significant to observe that China loses a considerable part of its lead when specifically looking at patents addressing problems associated with fouling and dissolved methane. This activity mismatch between inventiveness regarding sustainability challenges and implementation of full-scale MBRs highlights an opportunity for increased R&D involving fouling and dissolved methane mitigation for assignees/applicants present or active in China. The current AnMBR patent field is dominated by a few assignees/applicants such as Boying Xiamen Science and Technology CO, Coskata Inc, Korea Institute for Science and Technology, and Veolia Water Solutions & Technology. Furthermore, research institutes and universities are as active as industrial companies when it comes to publishing AnMBR patents.

In addition to presenting historical, regional, or institutional trends regarding AnMBR patent documents, this work provides a broad overview of the technological advances presented within AnMBR patents. AnMBR patents can roughly be divided into four groups: those associated with pre-treatment, bioreactors, membranes, and post treatment. Most inventions on the reactor side aim to enhance anaerobic degradation as well as minimize fouling in the subsequent membrane unit, but none focused on avoiding or minimizing permeate dissolved methane. Furthermore, even though the previous analytical investigation showed that only a low percentage of patents focus on fouling, most innovations directly targeting membranes aim to mitigate fouling or enhance membrane flux. Finally, while a handful of post treatment processes focuses on dissolved methane removal, the majority focuses on removing nutrients.

This work showed that AnMBRs remain an emerging area of interest, which resulted in minimally one new patent application every four days in 2018 (not including patents filed but not yet published). While this review found a fair amount of strategies presented in patents to mitigate concerns associated with membrane fouling, only a handful of patents addressed permeate dissolved methane removal. To ensure responsible AnMBR use, it is paramount to mitigate environmental costs associated with fouling and dissolved methane. The low percentage of patents focusing on these topics suggests that the AnMBR field holds capacity for further innovation.

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Chapter 3. Improving Anaerobic Digestion via Direct Interspecies Electron Transfer Requires Development of Suitable Characterization Methods

3.1 Abstract

Recent anaerobic digestion studies commonly attribute performance improvements (e.g., increased methane production, enhanced process stability, reduced startup times) to direct interspecies electron transfer (DIET), even though only indirect evidence of DIET is available and DIET alone does not explain enhanced performance in many cases. This review evaluates methods believed to confirm the occurrence of DIET in anaerobic systems. 16S rRNA gene sequencing and meta-omics approaches are necessary to further DIET knowledge but are limited in their ability to confirm the occurrence of DIET. *In situ* use of cyclic voltammetry should be explored further, as well as microscopy and image analysis procedures to quantify stained cytochromes. Furthermore, linking interspecies distance, interspecies mixing, and cellular activity to a DIET-based electron transfer model is promising but needs further validation for anaerobic digestion systems. In short, a combination of methods is necessary to confirm the occurrence and expand our knowledge of DIET.

Van Steendam, C., Smets, I., Skerlos, S. and Raskin, L., 2019. Improving anaerobic digestion via direct interspecies electron transfer requires development of suitable characterization methods. *Current opinion in biotechnology*, *57*, pp.183-190.

3.2 Introduction

Shortly after the suggestion of the importance of direct interspecies electron transfer (DIET) in anaerobic microbial communities [1,2] and its discovery in a co-culture in 2010 [3], researchers started to evaluate strategies to promote DIET with the aim of enhancing methane production and promoting stability in anaerobic digestion (AD) systems. During AD, complex communities of microorganisms mediate a series of biochemical reactions (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) to convert organic compounds in waste streams to biogas (a renewable energy source). Before the discovery of DIET, acetogens and methanogens were thought to transfer reducing equivalents through chemical intermediates such as hydrogen (H2) and formate (recently referred to as mediated interspecies electron transfer, MIET [4]). It is now clear that some microorganisms, when in direct contact with each other, can exchange electrons using electron transport proteins such as c-type cytochromes located in their outer cell surface [5,6]. By producing electrically conductive pili [7,8] or through the use of abiotic conductive materials, some microorganisms also perform extracellular electron transfer (EET) over distances on the centimeter scale [7].

Recent AD studies intended to exploit the enhanced electron exchange associated with DIET for the treatment of organic waste streams and have reported improved startup times, performance stability, and methane production rates when promoting DIET [9-11]. While many studies attribute improvements in methane production to DIET, often only indirect evidence of DIET is available [12,13] and DIET alone does not explain enhanced AD performance in many cases [14]. Since it is challenging to unequivocally evaluate whether DIET takes place in AD systems [12,15], it is critical to develop and use appropriate monitoring methods to advance the field. Although several recent reviews have focused on the mechanisms behind DIET [4,8,12,16-

18], no clear method or combination of methods has been presented to directly evaluate whether DIET takes place in real AD systems.

This review presents and critically assesses methods believed to be able to confirm the occurrence of DIET. Methods are classified according to the DIET characteristics they aim to observe: species, genes, transcripts, or proteins; electrical properties; morphological characteristics and spatial distribution; and metabolic pathways. Relevant DIET principles are only discussed as they relate to the methods reviewed since in-depth discussions of different DIET mechanisms are available in several recent reviews [4,8,12,18]. Our focus on monitoring methods is motivated by the urgent need to carefully evaluate the potential of DIET enhancement strategies in real AD systems to help inform researchers and practitioners when to exploit DIET to boost AD.

3.3 Detection of species, genes, transcripts, or proteins

Some studies solely rely on species identification through 16S rRNA gene sequencing to suggest DIET takes place in anaerobic digesters [19,20]. However, current DIET knowledge is too limited to suggest that the co-occurrence of certain populations in one system indicates DIET takes place. As opposed to microorganisms in co-cultures that have unequivocally been demonstrated to be involved in DIET through the use of gene deletion and substrate elimination experiments [3,5,21], most microorganisms linked to DIET (e.g., listed in [18,22]) are also able to perform MIET. Additionally, partial 16S rRNA gene sequencing often does not provide the resolution needed to identify populations below the taxonomic order or family levels and is thus limited in suggesting metabolic function. For instance, phylogenetically related species within the order *Desulfuromonadales* utilize different electron exchange pathways [23]. Similarly, archaeal community structure determined by 16S rRNA gene sequencing has been found to not always change upon DIET promotion [24]. Finally, it is unlikely that all microorganisms capable of DIET have been identified. Until 2018, *Geobacter* spp. were the only bacteria demonstrated to participate in DIET with methanogens. However, several anaerobic digesters utilizing conductive media to promote DIET primarily contained bacteria other than *Geobacter* spp. [19,20,25-27]. Furthermore, heterologous expression of pili genes from other bacteria into *Geobacter sulfurreducens* yielded conductive pili [28]. While some studies utilized preferential growth on conductive materials to suggest microorganisms likely participated in DIET (e.g., *Sporanaerobacter* [19] and *Syntrophomonas* [20]), the only bacterium outside of the *Geobacter* genus proven to participate in DIET through co-culture experiments is *Syntrophus aciditrophicus* [29]. The expectation that DIET will be found within other phylogenetic groups [28,29] is consistent with the recently discovered widespread occurrence of EET in a variety of environments (e.g., freshwater lakes [30], mammalian gut [31], marine sediments [32], and others [33,34]).

Detection of functional genes, transcripts, or proteins through (meta-)omic approaches can potentially identify whether DIET pathways are employed. A first strategy involves targeting DIET associated genes/transcripts/proteins (Table 3.1). Several studies identified genes essential for DIET in co-cultures by evaluating the ability of the co-cultures to still grow after gene deletion, including the deletion of *PilA* [3,5,6,11], Gmet_2896 [5], Gmet_1868 [6], and *OmcS* [3] genes. Shrestha et al. [5] observed upregulation of the *OmcT* gene in a DIET-performing co-culture relative to a MIET-performing co-culture. Other genes/transcripts/proteins in Table 3.1 have been linked to DIET by monitoring their up/down regulation or expression upon DIET promotion [13,35,36]. However, some of the genes/transcripts/proteins listed in Table 3.1 can be involved with other cell functions [37], precluding them as unique identifiers for DIET.

Additionally, some genes/transcripts/proteins are only associated with specific DIET pathways and the absence of such genes/transcripts/proteins does not imply an absence of DIET itself. For example, it has been hypothesized that genes associated with pili formation (e.g., *PilA* and *OmcS*) are downregulated when conductive materials are provided by allowing DIET over long(er) distances without the need for pili [13,36]. Furthermore, our knowledge regarding DIET mechanisms is still rapidly expanding [38-40]. An early study already noted minimal differences between both *PilA* gene expression and protein abundance in co-cultures interacting through DIET and MIET [5], and it has recently been demonstrated that pilus-free *Geobacter metallireducens* and *G. sulfurreducens* can still perform DIET [39], hypothetically using cytochromes alone. Conversely, the same pilus-free microorganisms were not able to grow syntrophically in another study but only grew in the presence of granular activated carbon (though not with magnetite) [40], suggesting that cytochromes are not always sufficient for DIET. Furthermore, not all cytochromes are essential to DIET, as the expressions of several outer surface cytochromes (e.g., *OmcZ*) were downregulated in a DIET-performing co-culture [5].

A second strategy involves detection of carbon dioxide (CO_2) reduction genes in *Methanothrix* spp. (formerly *Methanosaeta*). The full set of genes necessary for CO_2 reduction was found in several *Methanothrix* spp. even though *Methanothrix* spp. are acetoclastic methanogens and cannot utilize H_2 or formate as electron donors (typical electron donors for CO_2 reduction during hydrogenotrophic methanogenesis). Using a *G. metallireducens*-*Methanothrix harundinaceae* co-culture, Rotaru et al. [11] demonstrated that *M. harundinaceae* reduces CO_2 by accepting electrons through DIET. Thus, *Methanothrix* spp. are thought to only reduce CO_2 through DIET and the transcription of CO_2 reduction genes in *Methanothrix* spp. is used as direct proof that DIET is occurring [36,41]. Since *Methanothrix* is not consistently present as the dominant methanogen in AD systems [24,27], the applicability of this DIET-detection strategy is limited.

A third strategy targets the detection of MIET genes/transcripts/proteins to infer DIET is not taking place. Semenec et al. [21], for instance, noticed upregulation of HybA (uptake hydrogenase protein critical to interspecies H_2 transfer) and downregulation of c-type cytochrome proteins (OmcC, MacA, OmcS, PgcA) in subsequent co-culture transfers as microbial communities adapted to syntrophic growth. They concluded that DIET was particularly important during the initial transitory phase from pure culture growth to co-culture growth. Similarly, Shrestha et al. [5] observed reduced expression of genes associated with the use of H_2 and formate as electron donors in a DIET-performing co-culture relative to a MIET-performing co-culture. Nevertheless, microorganisms that exhibit expression of H_2 transfer genes/transcripts/proteins might still participate in DIET through the CO_2 reduction pathway. If *ex situ* substrate assays indicate a microbial community is not exhibiting H_2-based methanogenesis but meta-omics methods suggest H_2 transfer genes/transcripts/proteins are expressed, it is possible that methanogens are involved in DIET via the CO_2 reduction pathway [35].

Table 3.1 Genes (italicized), transcripts (in bold), and proteins (underlined) associated with DIET and MIET. Only Geobacter spp. and Syntrophus aciditrophicus have been demonstrated to participate in DIET, so all other electron donating microorganisms listed are putatively involved.

Associated with DIET				
Function	*Genes*/**Transcripts**/<u>Proteins</u>	Electron donating microorganisms	Electron accepting microorganisms	System for each reference
E-pili structural protein	*pilA* [3,5,6,36] **pilA** [5,11,13] <u>pilA</u> [5]	*Geobacter* spp. [36] *Geobacter metallireducens* [5,6,11] *Thauera* spp. [36] *Syntrophus* spp. [36] *Pseudomonas* spp. [36]	*G. sulfurreducens* [3,5]	[36]: Mixed AD culture [6]: *G. metallireducens* – *Methanosarcina barkeri* co-culture [3]: *G. metallireducens* - *G. sulfurreducen*s co-culture with and without gene-deletion (ΔomcS, ΔpilA, Δhyb) [11]: Mixed AD culture and *G. metallireducens* – *Methanothrix harundinacea* co-culture [41]: Mixed rice paddy soil culture [5]: *G. metallireducens* - *G. sulfurreducen*s and *P. carbinolicus*- *G. sulfurreducens* co-cultures with and without gene-deletion (ΔpilA, Δgmet 2896) [35]: Mixed AD culture [21]: G. *sulfurreducens* – *Pseudomonas aeruginosa* co-cultures with and without gene-deletion (Δphz, ΔomcZ, ΔomcS) [13]: Mixed AD culture
		Unassigned [13,41]		
E-pili accessory proteins	**pilB, pilC, pilM, pilQ** [13,41] **pilA-C, pilD, pile, pilN, pilO, pilR, pilS, pilT, pilV, pilW, pilY** [41] Unspecified *genes* and **transcripts** [41]	*Geobacter* spp. [41], Unassigned [13]		
			Desulfobacula, *Desulfobacterium*, *Deferribacter*, *Geoalkalibacter* [41]	
c-type cytochromes	*omcS* [3], **omcS** [5,41], <u>omcS</u> [3,21] <u>omcZ</u> [21] *gmet 2896* [5] *gmet 1868* [6] *omcT* [5] <u>omcC, macA, pgcA</u> [21] **Unspecified transcripts** [13]	*G. metallireducens* [5,6]	*G. sulfurreducens* [3,5,21,41]	
		Unassigned [13,36]		
CO_2 reduction pathway	*Pathway genes* [36] **Pathway transcripts** [11,13] **fmd, ftr, mch, mtd, mer, frh** [41]		*Methanothrix* spp. [11,36,41]	
		Unassigned [13]		
Acetate decarboxylation pathway	*Pathway genes* [36] **acs, cdh** [41] **Pathway transcripts** [13]		*Methanothrix* spp. [36,41]	
		Unassigned [13]		
Associated with MIET				
H_2 transfer	*hyb* [3] **hyb, hyp, hya, hox, mvh, hdr, and ehr** [5]		*G. sulfurreducens* [3,5]	
Formate transfer	Formate dehydrogenase *genes* [35] and **transcripts** [5]		*G. sulfurreducens* [5], *Methanosarcina spp.* [35]	

While meta-omics methods are necessary to identify DIET target genes, it is unclear if such genes will be found. The transcriptome of Methanosarcina barkeri was recently shown to differ during DIET versus MIET growth [42], which aligns with the expectation that cells produce specific cytochromes/proteins to allow electrons to traverse impermeable and electrically nonconductive cell envelopes [17]. However, universal DIET redox-active proteins may not exist. For instance, some microorganisms are known to use different sets of c-type cytochromes for EET [16], and various Geobacter spp. have been found to utilize different cytochromes [43].

3.4 Detection of electrical properties

Another set of methods aims to detect EET: cyclic voltammetry (CV) detects electron exchange between microorganisms and conductive materials, while conductivity measurements characterize the ability of biomass to conduct electrons. CV characterizes electron exchange between microorganisms and electrodes by determining the standard EET rate constant (k_{app}) [24,35,44] or the type of redox reactions occurring through electron transfer (with acetate oxidation and CO_2 reduction peaks at -0.35 V [45] and -0.32 V [46,47], respectively). The specific pathway through which microorganisms participate in DIET can differ from the one used in EET between cells and electrodes (e.g., in *G. sulfurreducens* different genes were found for cell-electrode and cell-mineral EET [48]). However, bacteria most commonly described to participate in DIET (i.e., *Geobacter* spp.) also perform EET with minerals and no microorganism capable of EET with electrodes but incapable of DIET has been identified to date. As such, an increased electron transfer rate upon DIET promotion signifies an increase in electro-active microorganisms likely capable of DIET. So far, CV has only been applied *ex situ*. Specifically, biomass from a system to be investigated for the occurrence of DIET is transferred into an electrochemical cell to allow biofilm formation on the electrode. A caveat is that such biofilm growth likely results in a shift in community structure, which thus may not be representative of the biomass in the system under investigation. In principle, CV could be applied *in situ* by connecting the electrode with conductive materials often present in systems in which DIET is being promoted. Since CV cannot assess the effect of non-conductive media (necessary as controls when studying the effect of conductive materials [14,49]), electron transfer rates need to be evaluated as a function of time and directly linked with DIET-associated performance characteristics such as CH_4 production rates or system resilience upon disturbances. Yin et al. [35], for instance, correlated the difference in CH_4 production rates between sequencing batch reactors with and without conductive materials to the difference in k_{app} evaluated *ex situ* for biomass collected from both reactors. While the close correlation between performance and k_{app} over time is no definite proof of DIET, it supports causality. Since continuously polarized electrodes are hypothesized to promote DIET in bioelectrochemical cells [50,51], the effect of brief electric fields generated by using CV *in situ* should be characterized before applying this method.

Another increasingly common method involves characterizing biomass conductivity (or conductance). While biofilms typically act as insulators, biofilms involved in DIET can be conductive [7,15]. Bulk biomass conductivity in anaerobic digesters amended with conductive attachment media such as carbon cloth and granular activated carbon is often higher than the bulk biomass conductivity in the corresponding control reactors (9.77 μS/cm versus 5.47 μS/cm [52], 498.8$\pm$ 75.2 μS/cm versus 6.3 $\pm$ 0.2 μS/cm [53], up to 27x increase [54]). The hypothesis that higher biomass conductivity is due to DIET was originally supported by a strong correlation between conductivity and pili protein abundance [7] or pilA expression [15]. However, biomass conductivity also was observed to increase upon the addition of non-conductive plastic materials [55], which would not promote DIET. Furthermore, biomass conductivity does not always increase upon DIET promotion [56]. Finally, biomass conductivity depends on the applied redox potential [7,57], which should therefore be recorded and reported, though this is not standard practice.Detection of cellular characteristics and spatial distribution

A third set of methods employs microscopy to visually assess characteristics and spatial distributions associated with DIET. As a first approach, components associated with DIET, such as pili [7], cytochromes [3,58-60], and redox mediators [61,62], can be visualized. While the visualization of pili was valuable when initially confirming the existence of DIET, recent discoveries show that the presence of pili is neither necessary [13,36,39] nor sufficient [5] for DIET. Alternatively, visualizing cytochromes in and around cells can provide insight in EET pathways [3,58-60]. It is no longer useful to solely stain specific c-type cytochromes (e.g., OmcS using antibodies [3]) as none have been conclusively linked with DIET. Instead, all cytochromes can be visualized using a 3,30-diaminobenzidine stain, which reacts with redox active transition metal ions bound to heme groups [59]. This technique was used early on to associate the presence of heme groups with an EPS-bound mineral (UO_2-EPS) with which *Shewanella oneidensis* was extracellularly exchanging electrons [60]. More recent studies have used this technique to visualize heme-groups in cellular membranes [59], intracellular membrane invaginations [59], extracellular space [59], and filamentous structures bridging cells [58]. Since cytochromes can perform functions other than DIET and are often redundantly present [63], it is not sufficient to qualitatively evaluate the presence of cytochromes to confirm DIET. Rather, a robust quantitative image analysis procedure needs to be developed to confirm that (membrane-bound or extracellular) cytochrome concentrations increase upon DIET promotion. Furthermore, future research should include testing such cytochrome visualization methods in AD consortia since such applications have not been reported thus far. Scanning electrochemical microscopy is another visualization strategy, which not only focuses on cytochromes but also on other electrochemical compounds, such as peptides, protein complexes, DNA, and enzymes by recording the electrical current response on conductive surfaces [61,62]. For example, Ren et al. [62] measured higher current peaks in cathode biofilms of a graphite-amended electrochemical cell than in those of non-graphite amended controls, suggesting higher concentrations of redox mediators or conductive small molecules, components essential to the occurrence of DIET. Given the complexity of current scanning electrochemical microscopy setups, this technology is currently only used *ex situ*.

A second microscopy strategy involves studying cellular spatial arrangements. For instance, transmission electron microscopy (TEM) [58] and fluorescence *in situ* hybridization (FISH) [64,65] are used to visualize juxtapositioning of syntrophic partners. While TEM relies on cell morphology for identification, FISH can identify syntrophic partners using oligonucleotide probes designed to target unique sequences in rRNA molecules. A related strategy involves using microscopy (e.g., scanning electron microscopy [9,10,66,67] or FISH [68]) to observe how closely cells are positioned to each other or to attachment media. The hypothesis here is that the observation of isolated cell growth on conductive attachment media indicates cell-media-cell DIET. Furthermore, scanning electron microscopy combined with energy dispersive X-ray analysis can ascertain if conductive nanoparticles (e.g., magnetite) are incorporated into cell aggregates, indicating the possibility of DIET via such conductive particles [13,69,70]. In addition to providing general spatial observations of microbial cells, microscopy can characterize distances between specific groups of microorganisms using techniques such as FISH [59,71]. McGlynn et al. [59] combined FISH with nanoscale secondary ion mass spectrometry and stable isotope probing and observed single cell metabolic activity to be independent of species intermixing or distance between syntrophic partners. The authors theorize that such a distance-independent trend of syntrophic partners is more likely to correspond with DIET than diffusion-based syntrophy since the global electric potential for each consortium is highest upon spatial segregation of the participating cells, as confirmed by cellular activity models based on DIET. Since this study did not include a non-DIET control, further research is necessary to confirm that the same activity/distance trend is not observed for other syntrophic pathways. For instance, Felchner-Zwirello et al. [71] observed a random distribution of their syntrophic partners in an anaerobic digester in which DIET was not promoted (nor investigated). However, the (uncharacterized) cellular activity could still have been dependent on interspecies distance. Interestingly, Felchner-Zwirello et al. [71] observed the average interspecies distance to decrease with time, which, according to the polar charge separation theory, could correspond with a decreased occurrence of DIET. Such a time-dependent trend has been confirmed by proteomics in a syntrophic co-culture of *G. sulfurreducens* and *Pseudomonas aeruginosa* [21].

3.5 Detection of cellular characteristics and spatial distribution

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3.6 Metabolism evaluation

A final set of methods evaluates methanogenic metabolisms through substrate assays [72], carbon isotope labeling [13,73], and specific inhibition experiments [64,74]. Substrate assays aid the identification of active methanogenic pathways by performing *ex situ* batch experiments and monitoring acetate or H_2 consumption and methane production. Similar to metatranscriptomics based techniques [41], substrate assays denoting CO_2 reduction as the main methanogenic pathway can be used to infer DIET when *Methanothrix* is the primary active methanogen [72]. More broadly applicable information can be obtained from substrate assays when combined with carbon isotope labeling (i.e., monitoring the biogas isotope ratio of CH_4 and CO_2). When isotope labeling demonstrates increased CO_2 reduction while substrate assays do not show intensified H_2 consumption, the enhanced CO_2 reduction pathway is likely a result of DIET [13]. In the absence of such substrate assays, the use of isotope labeling can confirm CO_2 reduction [73], which supports but does not prove DIET. A final set of methods determines how AD performance is affected when specific metabolisms are inhibited (e.g., acetoclastic [64], hydrogenotrophic [74], or overall methanogenesis [75]). For example, when AD is not affected by increasing H_2 partial pressures [74,76], it is likely to be occurring through DIET.

Table 3.2 summarizes DIET characterization methods as discussed in this paper, organized according to their ability to detect DIET.

Table 3.2: DIET characterization methods organized according to their ability to detect DIET. The superscripts indicate the DIET characteristic they aim to detect.1: species, genes, transcripts, and proteins, 2: electrical properties, 3: morphology and spatial distribution, 4: metabolic pathways. SECM = scanning electrochemical microscopy, FISH-nanoSIMS = fluorescence in situ hybridization nanoscale secondary ion mass spectrometry, TEM = transmission electron microscopy.

Methods requiring complementary tests	Methods that could detect DIET provided future development	Methods that provide inconclusive proof of DIET occurrence
Substrate assays[4]	FISH-nanoSIMS combined with stable isotope labeling[3]	16S rRNA gene sequencing[1]
Carbon isotopes[4]	Cytochrome staining[3]	Conductivity measurements[2]
Inhibition tests[4]	Cyclic voltammetry[2]	Pili staining[3]
Meta-omics approaches[1]	SECM[3]	Standalone FISH or TEM[3]

3.7 Conclusions and Future Work

Over the last decade, many AD studies have aimed to enhance methane production and promote performance stability through the stimulation of DIET. It is now apparent that performance improvements reported in these studies are not solely due to DIET. A complex set of methods is necessary to evaluate the potential of DIET enhancement strategies in real AD systems and not only suggest, but confirm, the occurrence of DIET (see Table 3.2). For instance, microbial community characterization through 16S rRNA gene sequencing cannot independently confirm DIET, but remains attractive to further our knowledge regarding DIET-performing microorganisms. Furthermore, the detection of genes/transcripts/proteins associated with MIET (e.g., H_2 and formate transfer) versus DIET (e.g., utilization of the CO_2 reduction pathway for

Methanothrix spp.) using meta-omics approaches is helpful, but is best accompanied by other methods. For example, *ex situ* batch experiments can confirm that upregulation of H_2 transfer genes does not coincide with a concomitant increase in H_2 consumption. Another promising method entails the *in situ* use of CV to directly link performance characteristics with electron transfer rates over time. When performing CV, however, the possible effect of electric fields on DIET needs to be studied. Microscopy can provide conclusive proof of DIET in a few situations. Monitoring the abundance of cytochromes across TEM images is one such method, though it requires quantitative image analysis. The 3,30-diaminobenzidine stain, furthermore, is known to bind to cytochromes but has not yet been validated for AD systems. Additionally, scanning electrochemical microscopy can characterize biomass electric current responses, however, presently only *ex situ*. The combination of FISH with nanoscale secondary ion mass spectrometry and stable isotope probing, on the other hand, can determine if the correlation between interspecies distance, interspecies mixing, and cellular activity corresponds to a DIET model versus a diffusion based MIET model. While this strategy has provided promising results, further research is necessary to confirm the previously identified distance-independent trend over time in DIET versus non-DIET promoted systems.

3.8 Acknowledgements

This work was supported by the U.S. National Science Foundation (Project CBET 1604069). CVS was supported by a University of Michigan (UM) Predoctoral Fellowship from the Rackham Graduate School and an Integrated Training in Microbial Systems (ITiMS) Fellowship. The ITiMS program “Instructional Program Unifying Population and Laboratory Based Sciences” at UM was funded by the Burroughs Wellcome Fund. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funding sources.

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Chapter 4. Novel Anaerobic Membrane Bioreactor Designs for Low Temperature Domestic Wastewater Treatment

4.1 Abstract

Two novel anaerobic membrane bioreactor (AnMBR) configurations were designed and evaluated for domestic wastewater treatment in temperate climates: the biofilm-enhanced AnMBR (BfE-AnMBR) and the MagnaTree reactor. Both reactors push influent and mixed liquor through meshes to allow development of a biofilm, referred to as 'flow-through' biofilm. The BfE-AnMBR uses two conductive meshes to divide the bioreactor into three compartments and regularly reverses the flow through these compartments to avoid clogging of the meshes while allowing for substrate staging and partial biomass migration between the different compartments. We hypothesize that substrate staging will result in an accumulation of volatile fatty acids in the first (hydraulic) compartment. The bioreactor is connected to an external membrane filtration unit containing a continuously rotating ceramic disc. The BfE-AnMBR was operated for 268 days during which volatile fatty acids did not accumulate in the compartment with the highest loading rate. This lack of localized reactor souring might have been caused by the relatively low organic loading rate due to the combination of low strength substrate (domestic wastewater) and flux limitations with the rotating disc membrane system. When operating the BfE-AnMBR at 15°C and an organic loading rate of 1 g COD/L/day, the BfE-AnMBR only achieved a COD removal of 45%. Furthermore, an energy evaluation of full-scale MBR systems suggested that utilizing rotating discs as a fouling mitigation technique might only entail an incremental improvement compared to existing MBR systems. The MagnaTree reactor contains

a submerged branched structure consisting of two concentric cylinders: one of which serves to continuously recirculate influent and mixed liquor while the other one produces a continuous permeate stream. The branches of the tree have openings and are wrapped with conductive meshes on which a flow-through biofilm developed, which serves as the barrier for solids-liquid separation. This reactor is currently operated at 21°C and achieves a COD removal of 86%. Once stable operation is achieved, the MagnaTree will be tested at lower temperatures to determine its performance limits at psychrophilic temperatures.

4.2 Introduction

Anaerobic membrane bioreactors (AnMBRs) are increasingly researched for domestic wastewater treatment due to their potential for energy recovery in the form of biogas and water reuse for irrigation or even drinking water production (with additional treatment) (Smith et al. 2014, Pretel et al. 2015). Although promising, challenges exist that impede widespread AnMBR implementation. Membranes are costly and require considerable energy for permeation as well as mitigate the fouling layer that unavoidably develops upon the membrane surface. For domestic wastewater, treatment at ambient temperatures is necessary as the low concentration of organic carbon in domestic wastewater produces insufficient biogas to heat the wastewater. In cold to temperate climates, the temperature of domestic wastewater is generally below 20°C and can drop to below 5°C during the coldest days of the year. However, low temperature anaerobic treatment is often accompanied by a reduction in degradation rates and microbial activity (Matsushige et al. 1990, Van de Last et al. 1992, Schalk et al. 2019, Schmidt et al. 2019). Furthermore, membrane fouling is intensified at low temperatures (Gao et al. 2014a, Smith et al. 2015b), which requires high pressures to maintain wastewater flow and increases operational costs. Finally, operation of AnMBRs at low temperatures leads to high methane losses through the effluent (Lettinga et al. 2001, Smith et al. 2012, Smith et al. 2014, Smith et al. 2015a), resulting in less energy captured as biogas and substantial greenhouse gas emissions. A life cycle analysis confirmed that these downsides prohibit implementation of current AnMBR designs operated at 15 and 25°C (Smith et al. 2014).

Despite initial concerns regarding impaired microbial activity, recent research has shown that acceptable pollutant removal can be achieved in AnMBRs operated at ambient temperatures in temperate climates (Lei et al. 2018, Petropoulos et al. 2019) and even at temperatures as low as 6°C by exploiting biofilms (Smith et al. 2015a). The aim of this study is to integrate microbial findings from previous AnMBR studies with novel design strategies and design, construct, and evaluate two novel AnMBR reactors (the Biofilm Enhanced-AnMBR (BfE-AnMBR) and the MagnaTree reactor). Ultimately, we aim to identify design characteristics that will enable AnMBRs to achieve reduced global warming impacts, increased energy recovery, and high-quality effluent for water reuse when treating domestic wastewater in cold to temperate climates.

4.3 Design rationale

4.3.1 BfE-AnMBR design

The BfE-AnMBR was designed to address a variety of concerns observed when using AnMBRs to treat domestic wastewater at temperatures below 25°C (Lettinga et al. 2001, Gao et al. 2014a, Smith et al. 2015a), including: (*i*) reduced carbon removal and permeate methane oversaturation, (*ii*) low hydrolytic and/or methanogenic activity, (*iii*) suboptimal syntrophic interactions between bacteria and methanogens, and (*iv*) high energy consumption for membrane fouling. The respective design strategies corresponding to the above limitations are discussed in detail below and schematically represented in Figure 4.1. Briefly, the BfE-AnMBR consists of a three compartment bioreactor followed by an external membrane filtration unit. The compartments are separated by coarse meshes (pore size >40μm) on which biofilms develop while minimizing transmembrane pressures between compartments. The flow through the three-compartment reactor is periodically reversed by which the respective end compartments are fed alternatingly. The external membrane filtration unit contains one rotating ceramic disc membrane (0.2 μm pore size, 0.12 m^2 surface area).

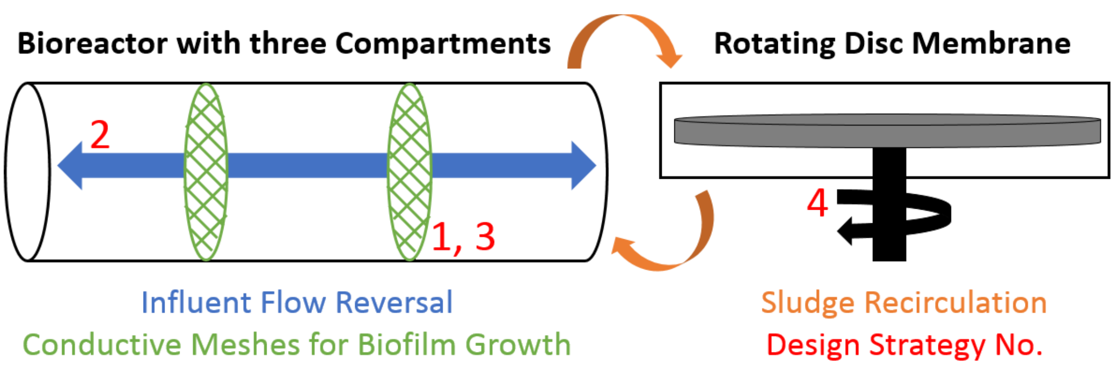

Figure 4.1: Conceptual design of the novel BfE-AnMBR with its specific design strategies enumerated in red.

1. Biofilm methane generation in bioreactor instead of on membrane surface. While previous research shows that increased relative activity of membrane-biofilms (as opposed to suspended biomass) can compensate for decreased carbon removal observed in psychrophilic AnMBRs (Smith et al. 2015a), relying on membrane-biofilms causes oversaturation of dissolved methane via direct dissolution into the effluent (Giménez et al. 2012, Smith et al. 2012, Ozgun et al. 2013, Smith et al. 2014, Smith et al. 2015a). The degree of methane saturation is defined as the ratio of the measured dissolved methane concentration over the dissolved methane concentration predicted using Henry's Law. The degree of methane saturation equals one at equilibrium, but has often been observed to be larger than one in conventional AnMBRs due the microbially active membrane biofilm and the concomitant direct dissolution of methane into the effluent. (Giménez et al. 2012, Smith et al. 2012, Ozgun et al. 2013, Smith et al. 2014, Smith et al. 2015a). The BfE-AnMBR design relies on biofilm development within the bioreactor to achieve carbon removal at low temperatures without relying on biofilm development on the membrane for treatment. To mimic reduced mass transfer limitations as experienced within membrane-biofilms, the wastewater in the novel BfE-AnMBR flows through several coarse meshes with biofilms. This flow-through biofilm design was expected to provide superior treatment compared to biofilms developed on attachment media.

2. Increasing hydrolysis and methanogenesis rates through substrate staging. To boost the hydrolysis and methanogenesis rates at low temperatures, the coarse meshes in the

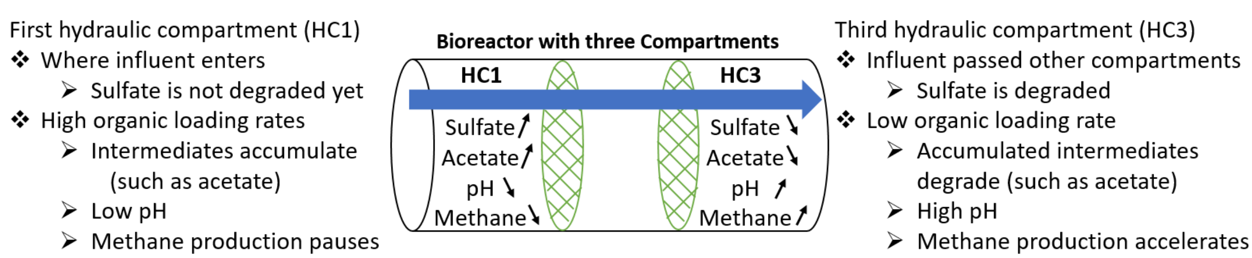

Figure 4.2: Schematic representation of expected effect of substrate staging.

BfE-AnMBR divide the bioreactor into compartments and allow for partial biomass migration and substrate staging. The flow in the BfE-AnMBR is regularly reversed while the biomass is allowed to partially migrate between compartments. Each compartment thus develops a unique microbial community, but there is sufficient biomass migration so that all populations of the anaerobic foodweb are represented in each compartment, although in different proportions (Angenent et al. 2001, Angenent et al. 2002a, Angenent et al. 2002b). As opposed to staged systems (Van Lier et al. 2001), the pH in the BfE-AnMBR compartments is not allowed to drop below 6.2. This pH level is low enough to enhance hydrolysis and acidogenesis without inhibiting methanogenesis. This approach avoids complete reactor souring, hereby eliminating the need for a neutralization tank or buffer addition, often required with staged systems. The alternating first compartment (further referred to as the hydraulic first compartment, HC1) is exposed to a high loading rate, promoting hydrolytic and acidogenic activity. When the flow is reversed, syntrophic and methanogenic activities in the last compartment (the hydraulic third compartment, HC3), which previously was the first compartment, ensures removal of accumulated intermediates and methane production. By reversing the influent flow through the system, the outer compartments are alternately exposed to high/low loading rates, which promotes alternating increased hydrolytic/methanogenic activity. The expected effect of such substrate staging on specific performance characteristics such as sulfate, acetate, and methane concentrations as well as pH is illustrated in Figure 4.2. Finally, reversing the flow through the meshes dislodges biomass and controls the thickness of the biofilm developing on the meshes. Chapter 2 demonstrated that none of the currently patented AnMBR designs utilize substrate staging with regular flow reversals as proposed in the BfE-AnMBR design.

3. Promotion of methanogenic activity through direct interspecies electron transfer (DIET). The discovery of DIET as a means to transport reducing equivalents between syntrophic bacteria and methanogens (Rotaru et al. 2014) is of specific interest for systems designed to exploit biofilms, as DIET enables microorganisms to directly exchange electrons via conductive surfaces (Liu et al. 2012, Chen et al. 2014). Such direct electron transfer is hypothesized to minimize chemical energy losses, which leaves more energy for microbial growth and methane production. To promote the occurrence of DIET in our BfE-AnMBR, the coarse meshes used to separate compartments are made of conductive material. Chapter 2 also demonstrated that no current AnMBR patent discusses promoting DIET.

4. Low energy fouling control. Lower temperatures lead to higher rates of membrane fouling, which is generally recognized as one of the greatest challenges in MBR operation as it results in a rapid increase in transmembrane pressures and operating costs. The BfE-AnMBR does not rely on sparging for fouling mitigation, as this method has often been denoted to be energy intensive (Smith et al. 2014, Krzeminski et al. 2017). Instead, we hypothesized that using a rotating ceramic disc to impose hydraulic shear on the surface of the rotating membrane would eliminate cake formation in an energy friendly manner (Poudel 2016). In support of this hypothesis, Bilad et al. (2012) showed that a vibrating membrane system ensured higher fluxes and lower fouling rates when compared to conventional aerobic MBRs. Applying intermittent vibration further reduced the energy use. Increased flux and lower fouling rates have also been reported for rotating ceramic disk systems. Frappart et al. (2006) illustrated this for a system treating dairy process water with nanofiltration. They reported that a system with rotating disks yielded better performance than a vibrating system due to a higher attainable membrane shear rate with the rotating disks.

4.3.2 Magna Tree design

The BfE-AnMBR design proved to be complex, required intense operator supervision, and did not behave as expected. Furthermore, our case study regarding the energy consumption of full-scale systems showed that utilizing rotating discs as a fouling mitigation technique provided only incremental improvement compared to other existing systems. To continue our search for design characteristics ideal for low temperature AnMBR treatment of domestic wastewater, we decided to simplify the system.

Nishant Jalgaonkar and Timothy Fairley, graduate students in Mechanical Engineering and Environmental Engineering at the University of Michigan, respectively, designed the MagnaTree reactor (Skerlos et al. 2019). Similar to the BfE-AnMBR, the MagnaTree reactor includes conductive meshes to support flow-through biofilm growth inside the bioreactor. The MagnaTree reactor consists of one compartment in which a branched structure is submerged. The structure consists of two concentric cylinders, each connected with their own respective set of branches wrapped with conductive meshes. Influent and mixed liquor are continuously recirculated through one set of branches, while the other set produces a continuous permeate stream. Thus, the MagnaTree reactor does not contain a final micro or ultrafiltration membrane. The lack of such a membrane causes washout of some suspended biomass during reactor startup, while the coarse meshes encourage attached biomass growth. Over time, the biofilm on the meshes becomes thick enough to provide solids/liquid separation and produce a relatively clear effluent. Figure 4.3a shows a schematic representation of the MagnaTree while Figure 4.3b and c

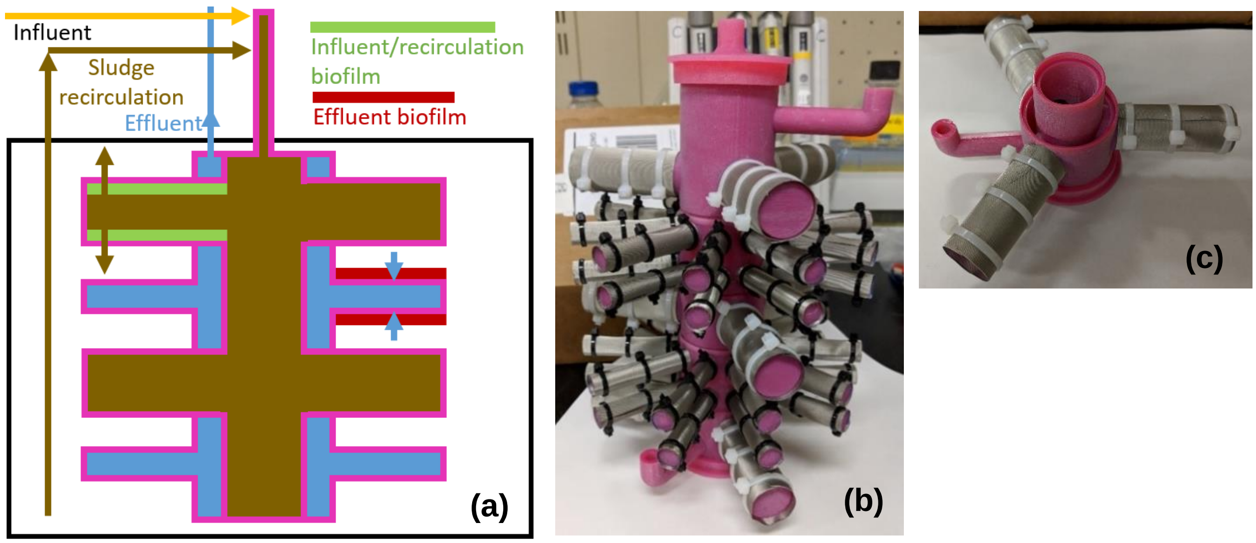

Figure 4.3: (a) Schematic representation of MagnaTree reactor design. Influent (yellow line) is sent into the middle concentric cylinder together with recirculated sludge (brown line) and is pushed out of the larger branches of the tree, hereby forming an influent/recirculation biofilm (green line) on the inside of meshes on the larger branches. Effluent (blue line) is pulled into the outer concentric cylinder through the smaller branches, hereby forming an effluent biofilm on the outside of the smaller branches. The effluent leaves the reactor from the outer concentric cylinder. (b) Pictures of the MagnaTree structure before being submerged into the reactor. The MagnaTree design maximizes mesh surface area using a tree-like structure consisting (b) of two concentric cylinders (c), each connected with their own set of hollow branches wrapped with stainless steel meshes.

depict the actual MagnaTree structure before being submerged into the reactor. Influent and recirculated sludge are pushed into the middle concentric cylinder and out of the larger branches of the tree, so that an influent/recirculation biofilm is formed on the inside of those meshes. Effluent is pulled into the outer concentric cylinder through the smaller branches, hereby forming an effluent biofilm on the outside of the smaller branches. The effluent leaves the reactor from the outer concentric cylinder.

The MagnaTree structure itself is 3D-printed out of water and gas impermeable material, and each cylindrical branch contains two slits through which water is pushed or pulled. The specific surface area of the meshes wrapped around these branches can therefore be distinguished into two separate categories: the parts of the meshes through which influent/mixed liquor or permeate is pushed or pulled (164 and 88.56 cm^2, respectively) and the parts of which the inside is blocked by a support structure (88,400 cm^2). While the surface area through which mixed liquor is pushed is smaller in the MagnaTree than in the BfE-AnMBR (253 versus 1044 cm^2, respectively), the total mesh surface area upon which biofilm can grow is substantially higher in the MagnaTree reactor (reaching 88,400 cm^2).

4.4 Materials and Methods

4.4.1 BfE- AnMBR construction

A schematic representation of the laboratory-scale BfE-AnMBR is depicted in Figure 4.4, while Figure 4.5 shows the BfE-AnMBR as set up in the laboratory. Two 304 stainless steel meshes with a combined submerged surface area of 1,044 cm^2 and a pore size of 180 μm separate the bioreactor into three compartments. Each compartment has a working volume of 4.07 L, determined by the minimum volume required to permit glass blowing of compartments with sufficient sampling (gas and sludge), sensor, and mixing ports. Two impeller setups were consecutively installed to avoid (gas) leaks: self-assembled impellers using ball-bearings around the rotating impeller shafts and agitator setups acquired through Chemglass. Even though each compartment has a water jacket, these water jackets proved to be incapable of controlling reactor temperature at high flow rates. Separately cooling the influent was insufficient for temperature control. Therefore, the BfE-AnMBR was moved to a temperature controlled room. Influent flow reversal was achieved using three-way solenoid valves, which were switched on and off based on a set time-cycle. Influent was always sent to the first hydraulic compartment (HC1), while sludge exiting the ceramic disc tank was either recirculated to the hydraulic third (HC3) compartment during the first run or, for specific time intervals, to HC1 during the second run in the temperature controlled room. A transmembrane pressure across the meshes is required to ensure influent and sludge to flow from HC1 to HC3, which was achieved by two-way solenoid valves on the separate headspaces of HC1, the hydraulic second compartment (HC2), and HC3. Automatic coding ensured that HC1 and HC2 headspaces were closed, while the level in HC3 was allowed to fluctuate. The influent pump was programmed to turn on when on-line liquid level readings indicated the sludge volume in HC3 to be lower than its working volume. Inline probes were used to continuously monitor and record the pressure in each compartment, the temperature and pH in both outer compartments, and the redox potential in the middle compartment. One pump was used to feed the bioreactor, while a second pump recirculated sludge between bioreactor and membrane filtration unit. The membrane filtration unit was designed to contain one rotating ceramic disc with a nominal pore size of 0.2 µm and a membrane surface area of 0.12 m^2 (Grundfos, Langå, Denmark) and had a working volume of 5.7 L. While the gearbox and motor required to rotate the disc were acquired from Grundfos, the ceramic disc housing was designed to meet the physical characteristics of the disc. A 3-D drawing of components required for this membrane filtration unit was created in DS SolidWorks and is depicted in Figure 4.6. The Department of Mechanical Engineering at the University of Michigan manufactured the pieces for the stainless steel coupler and shaft as well as the plexiglass housing. Vac-Met Inc (Warren, Michigan, USA) then connected the coupler pieces by furnace brazing them. The leak-tight simmerings, designed to hold the rotating shaft in the static housing, were provided by Freudenberg Sealing Technologies, while the hubs that hold the disc in place were 3D-printed by Materialise (stereolithography with the material Perform). The constructed membrane filtration unit can be seen in Figure 4.5

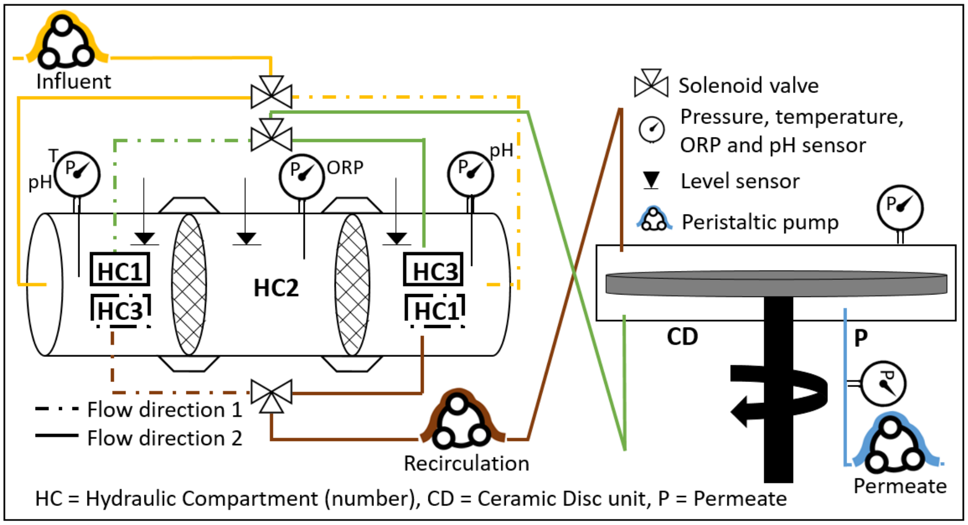

Figure 4.4: Schematic representation of the BfE-AnMBR. The influent (yellow line) is alternatingly fed to the left or right compartment. When the influent enters on the left (represented by a solid line), the left compartment is called the hydraulic first compartment (HC1) while the right compartment becomes the hydraulic third compartment (HC3). When the influent enters on the right (represented by an interrupted line), the right compartment becomes HC1 and the left compartment becomes HC3. Sludge is continuously recirculated from HC3 (brown line) to the ceramic disc unit (CD) and back (green line). Permeate (P, blue line) is collected from the CD unit. The schematic representation additionally depicts all online sensors (level, pressure (P), temperature (T), oxidation reduction potential (ORP), and pH).

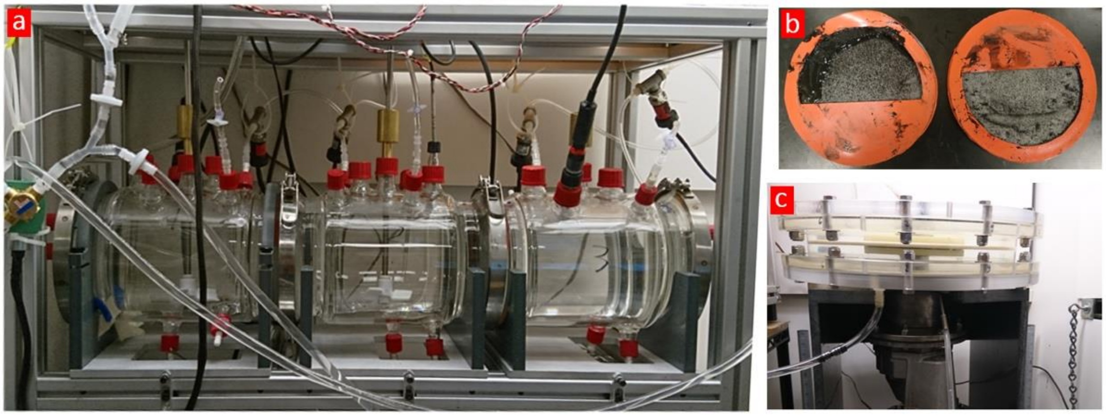

Figure 4.5: The BfE-AnMBR treats wastewater by sending it through three compartments in series (a) separated by meshes that serve for biofilm attachment (b) and filtering it using a rotating disk membrane (c). Picture (b) shows the mesh-biofilms.

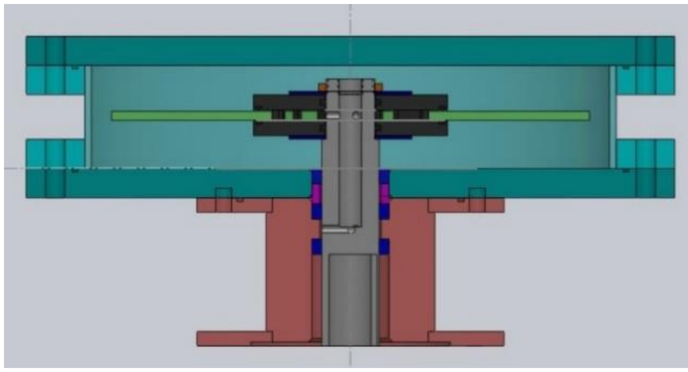

Figure 4.6: Schematic of membrane filtration unit as drawn in DS SolidWorks. Turquoise = plexiglas housing, brown = nut, dark blue = washers, dark grey = disc hubs, green = ceramic disc, light gray = shaft, blue = simmerings, purple= spacer, and red = coupler.

4.4.2 BfE-AnMBR and MagnaTree operation

The BfE-AnMBR was seeded on January 5th, 2018 with sludge previously used in our lab for the anaerobic treatment of domestic wastewater treatment at ambient temperatures (unpublished data) and operated for 196 days at temperatures between 15 and 25°C. This operational period is referred to as the first run. The BfE-AnMBR was shut down in July, 2018 to be moved to a temperature controlled room to enable adequate temperature control at high influent flow rates. The system was restarted on August 6th, 2018 for a second run and finally shut down the 26th of November, 2018. The system was fed with synthetic domestic wastewater according to the recipe reported in a previous low temperature AnMBR study (Smith et al. 2012)). During operation, the hydraulic retention time (HRT), solids retention time, and reactor temperature were varied between 8-40 h, 50-110 days, and 15-25°C, respectively. The sludge recirculation flow rate was varied between 70-400 ml/min (2-54 times higher than the influent flow rate) to ensure sufficient upflow velocity to keep the biomass suspended in each compartment. The duration between influent flow reversals was typically 8 h, although for short-term experiments flow was reversed up to once per hour or not at all. Given that holding a transmembrane pressure across the meshes was crucial to force the mixed liquor to flow from HC1 to HC2 and to HC3, each compartment needed to be gas tight. Since the two mixing assemblies used did not enable the reactor to reliably hold pressure, the compartments were mixed by introducing influent/recirculation sludge into HC1 or HC3 from the bottom (depending on the feed cycle) starting on day 134. A separate recirculation line was set up in the middle compartment to achieve similar mixing conditions for all compartments. For the first run, meshes with a 180-μm pore size were used, whereas for the second run we used meshes with a pore size of 43 μm to which single weave activated carbon cloth (www.buyactivatedcharcoal.com) was attached. This smaller pore size caused pressure buildups across the compartments. Therefore, the 180- μm meshes were reintroduced when restarting the BfE-AnMBR for the second run.

The MagnaTree reactor was started May 9th 2019 and seeded with biomass previously used to anaerobically treat synthetic domestic wastewater at 15°C (Smith et al. 2012). The system was run at 15°C for 27 days, after which the temperature was increased to 21°C to facilitate faster startup. During startup, the reactor was run at a relatively constant HRT of 26 h, with temporary fluctuations when the reactor was opened up for reactor modifications or when the permeate flow rate needed to be increased to mitigate clogging in the permeate line.

4.4.3 Monitoring Performance by Chemical assays

The BfE-AnMBR was sampled by collecting gaseous samples from the headspace of each compartment and membrane unit and liquid samples of the influent, each compartment, membrane unit, and permeate. For the MagnaTree reactor, gaseous samples from the headspace and liquid samples of the influent, the recirculation line, and permeate were collected. The sampling frequency for both reactors depended on the stage of the experiment, but generally two to three samples were collected per week. The reactor performance was assessed by determining total and volatile suspended solids (TSS and VSS), chemical oxygen demand (COD, total and soluble), volatile fatty acids (VFAs), sulfate, and gaseous and dissolved methane concentrations.

Procedures for COD, TSS, and VSS analyses were developed according to Standard Methods for the Examination of Water and Wastewater (2005). Each sample was analyzed in duplicate for COD and VSS/TSS, and in triplicate for gaseous and dissolved methane, VFA, and sulfate concentrations. VFA, sulfate, and soluble COD samples were filtered through a 0.45 µm filter (EZflow, 25-mm diameter membrane filters). COD samples were diluted as needed, acidified with 20 µL of 5 N sulfuric acid per 3 ml of sample, stored at 4°C, and analyzed once per week using low range Lovibond COD tube tests (<150 mg O_2/L), a Hach digester, and a Hach Colorimeter. VFA and sulfate samples were diluted as needed, basified with 20 µL of 1 N sodium hydroxide per 1.8 ml of sample, stored at 4°C, and analyzed once per week using a Dionex IonPac AS11-HC-4µm column in an ion chromatography system (Dionex Integrion HPIC) connected with a temperature controlled autosampler (4°C, Agilent 1100 series). Formate, acetate, propionate, butyrate, valerate, and sulfate anions were eluted through the column using the Dionex EGC 500 KOH RFIC and a HPIC Eluent generator cartridge. Biogas methane samples were collected in a 1-L Tedlar bag. On a daily basis, biogas content was analyzed using a gas chromatograph (Gow-Mac, Bethlehem, PA) coupled with a thermal conductivity detector (TCD). The volume of biogas produced was quantified by repeatedly expelling the collected biogas from the 1-L Tedlar bag into a gas-tight 100-ml syringe until depleted. Dissolved methane samples were taken as described by Smith et al. (2012). Briefly, 15 ml of sample was taken with a gas-tight syringe and supplemented with 15 ml of N_2 gas. The syringe was shaken by hand to achieve methane gas-liquid equilibrium in the syringe. Using liquid displacement, 10 ml of the syringe headspace was added to a capped 20 ml bottle filled completely with Millipore water. Triplicate samples were stored at room temperature and the vial headspaces were analyzed once per week using a gas chromatograph (Hewlett Packard HP 6890 Series) with a flame ionization detector. Dissolved methane concentration in the original sample in the syringe was calculated using Henry's Law for gas-liquid equilibrium for both the sample vial as well as the syringe (see Equation 4.1), assuming that the syringe and the sample vial achieved gas-liquid equilibrium and were stored at a temperature of 25°C. Based on these assumptions, Henry's molar constant was taken to be 31.4 L_{liquid}/L_{gas} (Benjamin et al. 2013). Reactor dissolved methane saturation was calculated by dividing the expected reactor dissolved methane concentration based on a gas-liquid equilibrium between the reactor headspace and reactor samples by the measured dissolved methane concentration (Equation 4.2). As the permeate does not have a headspace, the headspace of the reactor was used to calculate permeate dissolved methane oversaturation as well. Equation 4.3 (Henry's law, based on partial pressure and molar concentration) and Equation 4.4 (Van 't Hoff relationship) were used to calculated the expected amount of reactor and permeate dissolved methane while taking reactor temperature into account.

$$H_{mm} = \frac{\frac{n_{gas,i}}{V_{gas}}}{\frac{n_{liquid,i}}{V_{liquid}}} \qquad \text{Equation 4.1}$$

$$CH_4 saturation = \frac{expected\ CH_4 concentration\ based\ on\ Henry's\ law}{measured\ CH_4 concentration} \qquad \text{Equation 4.2}$$

$$H_{px} = \frac{P_i}{\frac{n_{liquid,i}}{n_{liquid,total}}} \qquad \text{Equation 4.3}$$

$$\log H_{px} = \frac{-A'}{T} + B' \qquad \text{Equation 4.4}$$

With H_{mm} = Henry's constant based on molar concentrations [L_{liquid}/L_{gas}], $n_{gas,i}$ = amount of moles of species i in gas phase [mol], V_{gas} = volume of gas phase [L], $n_{liquid,i}$ = amount of moles of species i in liquid phase [mol], V_{liquid}= volume of liquid phase [L], H_{px} = Henry's constant based on partial pressure and molar fraction [atm], P_i = partial pressure of species i in gas phase, $n_{liquid,total}$ = total amount of moles in solution for which the total molar concentration was assumed to be 55.4 mol/L, $A'$and B' = empirical constants for Van 't Hoff equation, taken to be 675.74 and 6.88, respectively (Hung et al. 2007).

4.5 Results and Discussion

4.5.1 BfE-AnMBR reactor performance

While the BfE-AnMBR achieved substrate staging, the expected accumulation of VFAs, as illustrated in Figure 4.2, was not observed in HC1. Figure 4.7 shows the sulfate and acetate concentrations in influent and first and third hydraulic compartments during the first 45 days of the first BfE-AnMBR run. The sulfate concentration in HC1 was, with the exception of one data point during startup, consistently higher than or close to that in the third compartment. Given that the hydraulic first and third reactor alternated between being the left and right compartment due to regular flow reversals, these data show that HC1 indeed had a higher loading rate and thus that substrate staging was achieved. The higher loading rate in HC1 was expected to result in the accumulation of VFAs, intermediates in the anaerobic degradation pathway (such as acetate, see Figure 4.2). This accumulation was not observed as illustrated in Figure 4.7b, which shows that acetate was not consistently higher in HC1.

We hypothesized that the organic loading rate (OLR) to the BfE-AnMBR might not have been high enough to induce reactor souring. Therefore, we increased the OLR by decreasing the HRT. For the first run, the HRT fluctuated heavily during the initial 43 days due to operational issues (ranging between 19-30 h) and was kept constant around 20 h from days 43 to 88 (see Figure 4.8). The HRT was decreased to 8 h on day 88. Frequent chemical cleans became necessary at this flow rate as membrane fouling decreased the flux, resulting in an increased HRT. From day 130 until the remainder of the run, the BfE-AnMBR was operated at an HRT of 14 h to avoid frequent chemical cleans.

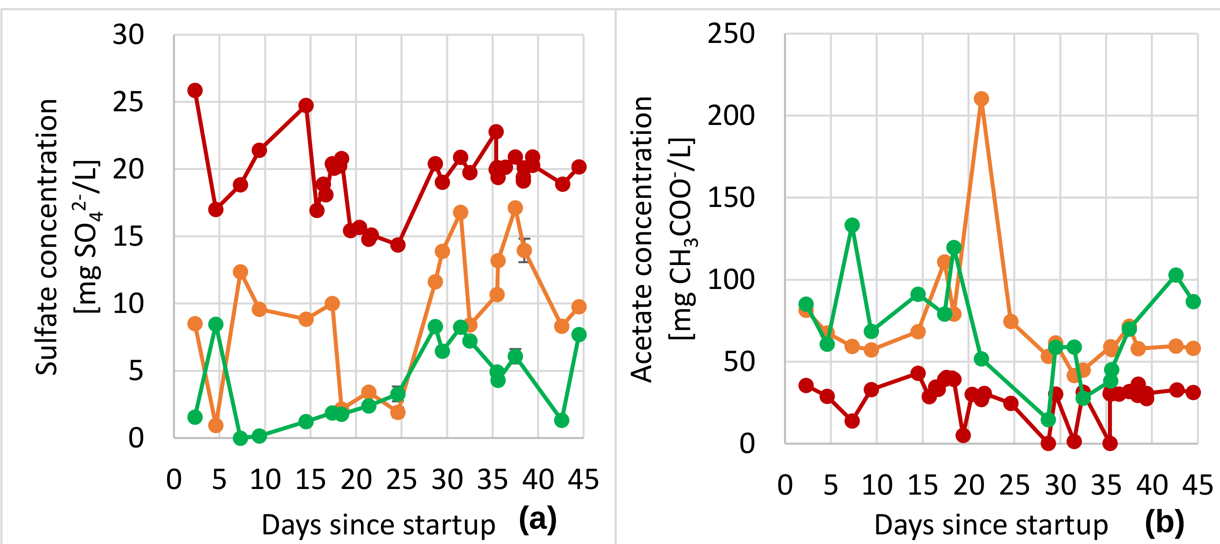

Figure 4.7: Sulfate (a) and acetate (b) concentrations in the influent (red) and first (orange) and third (green) hydraulic compartments during first 45 days of the first BfE-AnMBR run.

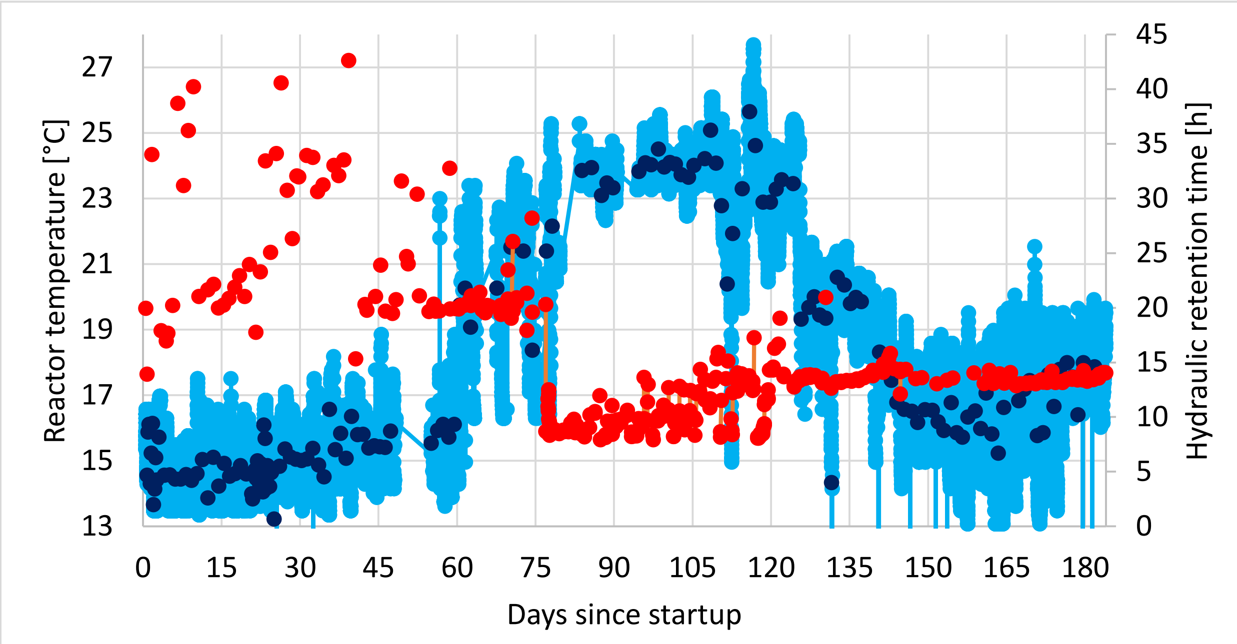

Figure 4.8: Reactor temperature (light blue: in-line probe readings, dark blue: 24-h average, left y-axis) and hydraulic retention time (HRT, red, right y-axis) during the startup of the first reactor run.

Concomitant with the HRT decrease to 20 h and then 8 h, the reactor temperature increased from 15°C to 21°C and then to 24°C (Figure 4.8). While we aimed to test the BfE-AnMBR at 15°C, the water jackets were no longer able to control the temperature of the reactor at the flow rates required to achieve HRTs of 20 h and 8 h. The reactor temperature decreased to 18°C upon separately cooling the influent, but the target temperature of 15°C could not be reached. Therefore, the BfE-AnMBR was moved to a temperature controlled room to achieve the desired 15°C while running at an HRT of 14 h.

After moving the reactor to the temperature controlled room, the acetate concentration continued to be similar in HC3 and HC1 (Figure 4.9), suggesting that the increase in OLR was insufficient to induce souring in HC1. The maximum OLR used was 1.65 g COD/L/d (or 0.3 g COD/g VSS/d). This OLR was higher than the one used in some studies using AnMBRs for treatment of domestic wastewater (e.g., between 0.44-0.66 g COD/L/day (Smith et al. 2012) or

1.29 g COD/L/d (Gao et al. 2014b)), but lower than in others (between 2-2.5 g COD/L/d (Gouveia et al. 2015)). Given that the COD concentration of our synthetic wastewater was consistent with a medium strength domestic wastewater (Tchobanoglous et al. 2004), we decided against increasing the OLR by increasing the strength of the wastewater. Furthermore, the maximum OLR for HC1 (i.e., 7.28 g COD/L/d or 1.32 g COD/g VSS/d) was higher than reported by most domestic wastewater studies. However, the OLRs achieved with medium strength synthetic domestic wastewater in our AnMBR study were lower than the one reported for a study that successfully employed substrate staging (and thus achieved localized reactor souring) (Angenent et al. 2001). This study used an OLR of 30 gCOD/L/d using a high strength sucrose-based substrate (Angenent et al. 2001).

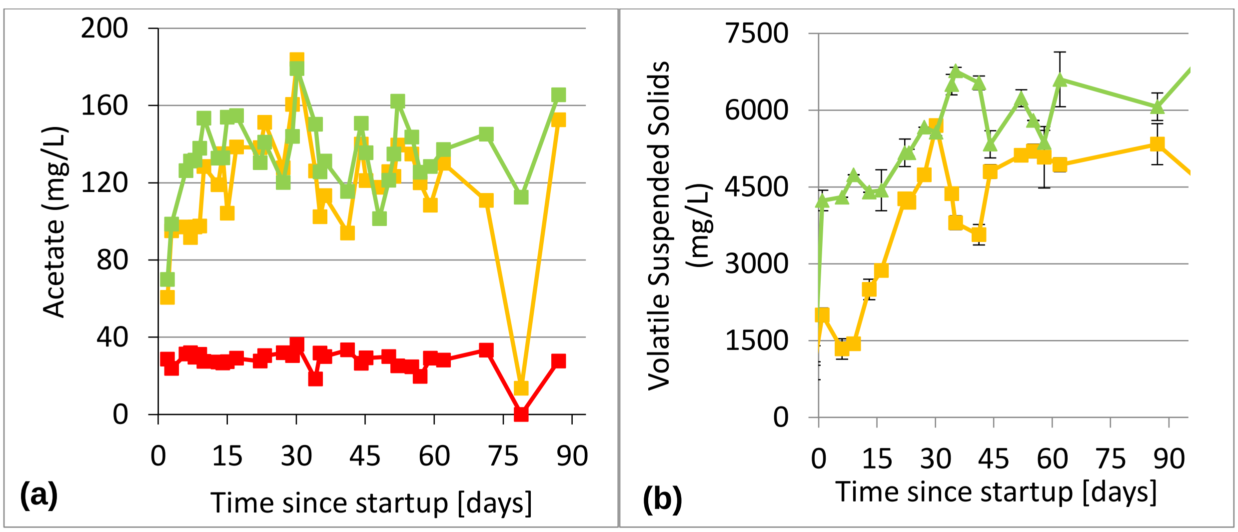

Figure 4.9: (a) Acetate concentrations in influent (red) and first (orange) and third (green) hydraulic compartments during second BfE-AnMBR run in temperature controlled room. (b) Volatile suspended solids concentrations, used as a proxy for biomass concentration, in the first (orange) and third (green) hydraulic compartments during the second BfE-AnMBR run in the temperature controlled room.

Besides the relatively low OLR, the lack of reactor souring in HC1 could also be due to the higher concentration of biomass generally measured in HC3 (in both runs). For the second run, we tried to keep more biomass in each individual compartment by using meshes with smaller pore size and attaching carbon cloth to the meshes. However, these changes resulted in transmembrane pressures beyond the expected pressure tolerance of the glass compartments. From day 1 to day 4, the system was only operated during the day so that the pressures could be closely monitored. The carbon cloth was removed from the meshes on day 4, after which we started to continuously operate the reactor flow reversals every 8 hours. However, the pressures still gradually built up to unsafe levels due to the smaller pore size of the meshes. The reactor was stopped a few times to dilute the biomass, and overall, the original biomass was diluted by a factor of eight. To further minimize the pressure buildups in the separate compartments, the operation of the reactor was changed so that the meshes would be 'backflushed' for one minute every ten minutes by temporarily changing the flow direction from HC3 to HC1. This backflushing appeared to work initially: only minor pressure buildups were observed during normal operation, and whatever pressure had built up disappeared completely when the flow was reversed. However, after a few hours of operation, the compartment pressure after backwash would immediately return to where it had left off. For instance, if the pressure in HC1 had built up to 118 kPa, it would drop to zero during backwash, but immediately go back up to 118 kPa after backwash. The system was therefore stopped on August 14th, upon which the meshes were replaced with the same meshes used in the first run and the reactor was reseeded with undiluted inoculum. Instead of trying to retain biomass in HC1 by installing meshes with a smaller pore size or attaching carbon cloth to the meshes, we decided to recirculate biomass from the ceramic disc tank back to HC1 for 10 minutes every hour on day 18. Figure 4.9 shows that all of these changes did cause the biomass concentration in HC1 to get closer to HC3, however, VFAs still did not accumulate in the first compartment. A final effort to achieve such an accumulation was started on day 51, after which sludge was continuously recirculated to HC1 and, instead of reversing the flow every 8 hours, the system was continuously run in one direction. This manner of operation again resulted in too high pressure buildups in the separate compartments. These pressures were monitored several times per day, and, when too high, the amount of time recirculated sludge was sent to HC1 was (temporarily) decreased. Unfortunately, this approach required close operator attention and VFAs still did not accumulate in HC1 (see Figure 4.9).

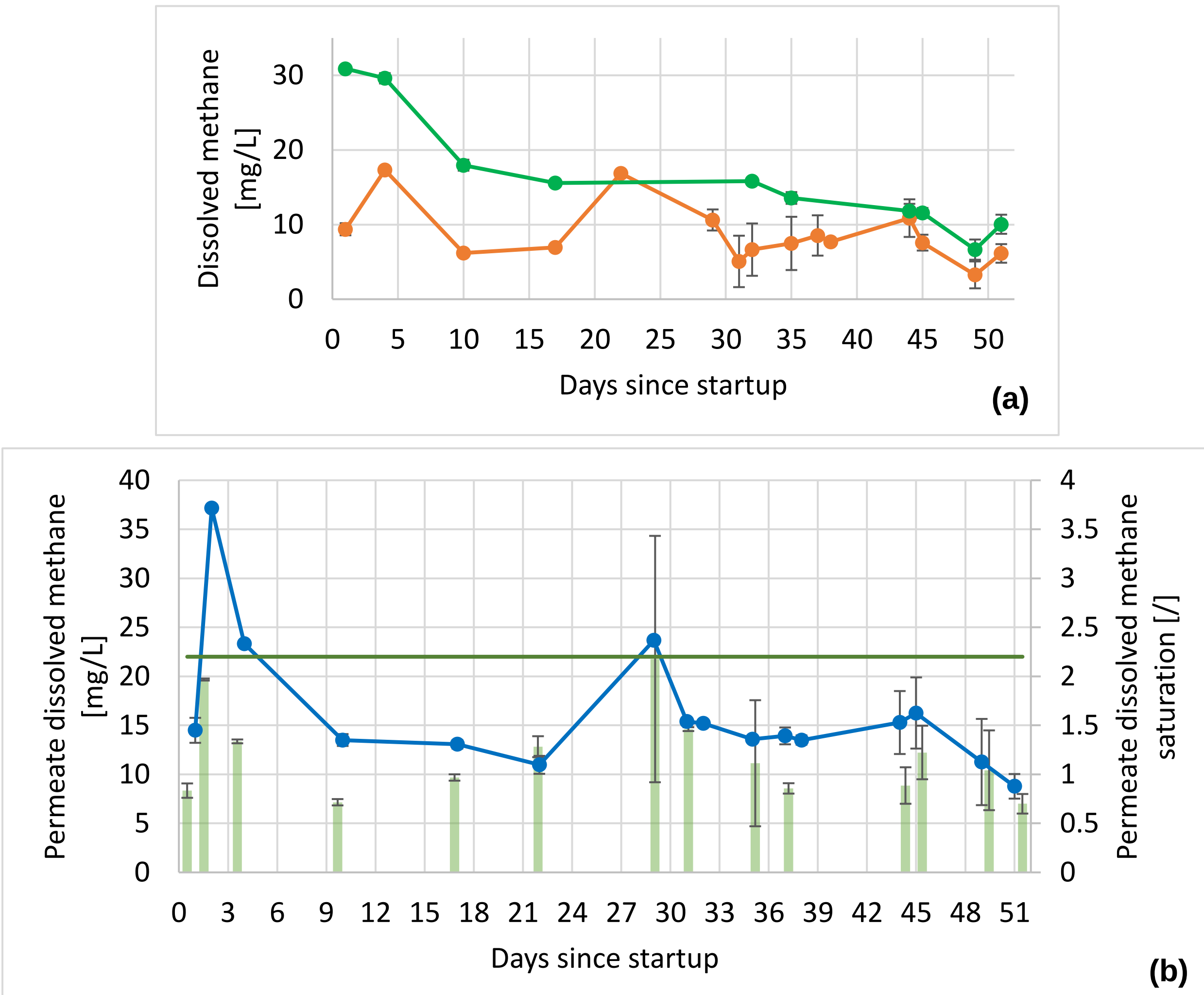

Figure 4.10: (a) Dissolved methane concentrations as measured in the hydraulic first (orange) and third (green) reactor of the first run of the BfE-AnMBR, (b) Permeate methane concentration (blue line) and saturation (green bars) as measured during the first run of the BfE-AnMBR as well as the saturation as reported by Smith et al. (2012) in a conventional AnMBR (green line).

The methane production across the BfE-AnMBR (Figure 4.10) behaved as expected (Figures 4.2). The methane concentration was consistently higher in HC3 (data shown for the first BfE-AnMBR run, Figure 4.10a). Furthermore, the measured permeate methane oversaturation was substantially lower for the BfE-AnMBR than for a conventional AnMBR previously run in our lab (Smith et al. 2012). Thus, even though the OLR to the BfE-AnMBR was too low to achieve an accumulation of acids in HC1, the novel setup succeeded at curbing methane oversaturation in the permeate.

4.5.2 Rotating discs energy consumption assessment

Given the objective to achieve low energy fouling control, it is imperative to assess the BfE-AnMBR's energy consumption associated with fouling mitigation. This assessment is difficult to accomplish with lab-scale data due to the poor scalability of the power required for pumping and disc rotation. Therefore, the energy consumed by full-scale membrane systems utilizing different methods of fouling mitigation was compared for plants operating at low (~450 m^3/d) and high flow rates (~45,000 m^3/d, see Table 4.1). The GE/Zenon ZeeWeed 500D utilizes a conventional sparging technique involving a continuous stream of small gas bubbles, while both the GE ZeeWeed 500 LEAP and Evoqua Mempulse provide sparging by intermittently generating larger bubbles to additionally shake the membrane and as such cause further fouling removal. The fourth membrane system studied represents the full-scale implementation of the membrane system installed in the BfE-AnMBR: rotating ceramic discs (Grundfos). The energy consumptions for three domestic wastewater treatment plants (Plants A, B, and D, see Table 4.1) were calculated based on industrial data obtained from the operators and the consulting engineers in charge of monitoring the systems (Carollo Eng.). These consulting engineers answered a detailed questionnaire and disclosed information such as the amount of time each dutyblower and air compressor was in operation. The 2008 housing crisis severely impacted the housing development in Plants A and B and limited their average daily flow to only 7 and 47% of their design flow rates, respectively. This mismatch between plant design and operation also affected the average membrane flux of these plants. Plant B, for instance, continuously keeps all membrane trains online and wet, but only permeates from one train at a time. Even though this plant was designed to operate at a flux of 13.1 L/m^2/h (LMH), current daily flows limit membrane flux to 3.7 or 5.3 LMH depending which specific train is permeating. Plant D does operate at its design flow rate, and achieves an average flux of 19.7 LMH. The energy consumption for the rotating disc systems was assumed to the best case scenario as disclosed by Grundfos for a dairy wastewater treatment facility, which equals 0.4 kWh/m^3 (Poudel 2016). Grundfos reported an average flux of 35 LMH. This study by no means represents an absolute comparison of different fouling mitigation techniques given the lack of replicates for each studied mitigation technique a well as the mismatch between original design and eventual operation for two of the studied plants, but rather serves as a first-order indicator of the energy consumption range within which these different fouling mitigation techniques are operated.

Figure 4.11 shows how for the studied water treatment plants operated at low flow rates, the novel sparging technique involving intermittently shaking the membrane with large gas bubbles (GE ZeeWeed 500 LEAP) required less energy per cubic meter of water treated than the conventional sparging technique (GE/Zenon ZeeWeed 500D). The best-case energy consumption as published by Grundfos for their rotating ceramic disc system is lower than for the novel

Table 4.1: Plants for which the energy associated with membrane operation was calculated.

	Plant	Fouling mitigation technique	Membrane setup	Flow rate
Low flow	Plant A	Novel sparging	GE/Zenon ZeeWeed LEAP	371 m^3/day
	Plant B	Conventional sparging	GE/Zenon ZeeWeed 500D	549 m^3/day
	Plant C	Disc rotation	Grundfos ceramic discs	425 m^3/day
High flow	Plant D	Novel sparging	Evoqua MemPulse	47,696 m^3/day

sparging technique operating at a low flow rate (0.4 kWh/m^3 versus 0.59 kWh/m^3). However, the normalized energy consumption of the rotating discs is higher than that of the novel sparging technique operating at a high flow rate (0.4 kWh/m^3 versus 0.17 kWh/m^3).

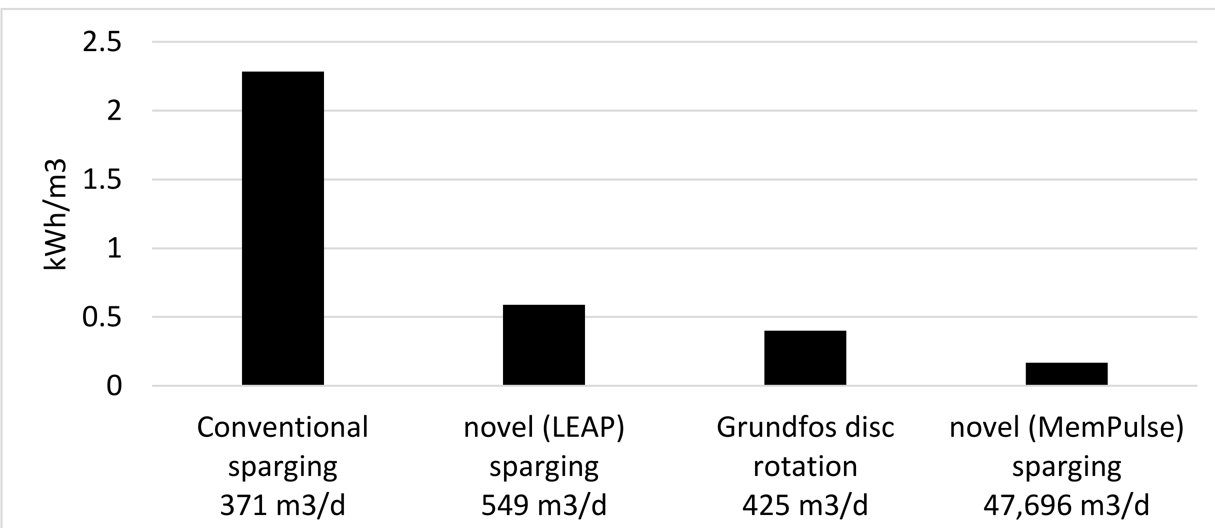

Figure 4.11: The amount of energy consumed per amount of wastewater treatment for four different wastewater treatment plants utilizing a variety of fouling mitigation techniques. These techniques include conventional sparging (GE/Zenon ZeeWeed 500D), novel sparging (GE ZeeWeed 500 LEAP), or disc rotation (Grundfos).

4.5.3 MagnaTree startup reactor performance

Operation of the BfE-AnMBR proved challenging due to the complexity of the design. Since we did not observe the expected performance enhancement, operation of the BfE-AnMBR was discontinued. Instead, we decided to evaluate the MagnaTree reactor at temperatures typical for domestic wastewater treatment in cold to temperate climates.

Figure 4.12 shows that the startup of the MagnaTree reactor was slow, with almost no biogas production and very little COD removal in the initial months. Biogas production increased slightly upon increasing the reactor temperature to 21°C on day 27, which was accompanied by a slow increase in headspace methane concentration and a decrease in effluent COD. Total influent COD readings fluctuated between 170 and 500 mg COD/L during the first 36 days but stabilized around 450 mg COD/L once the influent mixture was moved closer to the reactor to minimize the length of influent tubing. After performing additional pressure tests and observing leaks in the MagnaTree reactor headspace, the reactor was opened up on day 54 to retighten the reactor lid. Figure 4.12a shows how biogas production, as well as the methane percentage in the headspace, increased after tightening the reactor lid. These regular leaks influenced headspace methane concentrations and hereby dissolved methane saturation calculations (Equation 4.2). Figure 4.12b, for instance, shows several high peaks of permeate dissolved methane oversaturation during the first 54 days of operation. However, no oversaturation was observed once the headspace was made leak tight (Figure 4.12c). COD removal increased to 73% after 65 days of operation (see Figure 4.12c) and continued to increase to 87% after 105 days of operation (data not shown). Regarding COD removal, the performance of the MagnaTree reactor is getting close to that of other domestic wastewater AnMBR studies (91.9 ± 1.5% and 91.3 ± 2.1% (Chen et al. 2017), >94% (Seib et al. 2016a, Seib et al. 2016b), 90 ± 2% (Dong et al. 2015)). Once stable operation is achieved, the reactor temperature will be brought back down to 15°C to finish the low temperature experiments.

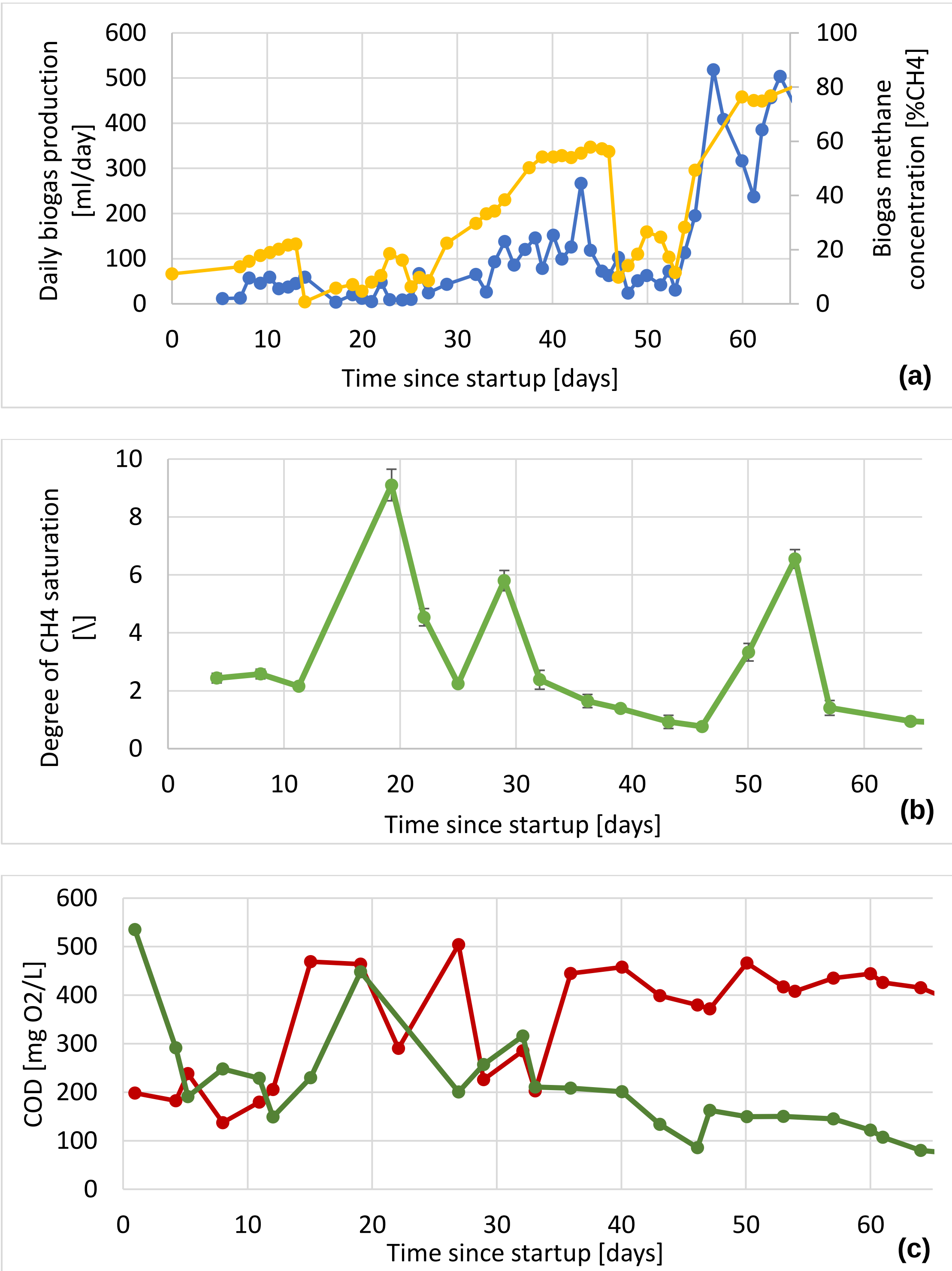

Figure 4.12: (a) Amount of biogas produced (blue) and percentage of methane in MagnaTree headspace (yellow), (b) degree of permeate methane oversaturation during startup of the MagnaTree reactor (green), (c) total influent (red) and effluent COD (green) during startup of the MagnaTree reactor.

4.6 Conclusions

Two novel AnMBR reactors were designed, built, and evaluated to identify design characteristics essential for low temperature domestic wastewater treatment. Our design approaches focused on achieving reduced global warming impacts, increased energy recovery, and production of high-quality effluent to allow water reuse. The BfE-AnMBR utilized three compartments separated by a stainless steel mesh to promote substrate staging and flow-through biofilm growth. This approach was used to achieve adequate treatment at high organic loading rates (and thus methane production), while avoiding dissolved methane oversaturation. Furthermore, the BfE-AnMBR used an external rotating disc membrane to minimize energy consumption for fouling mitigation. The BfE-AnMBR was run for a total of 268 days during which the acetate concentration in HC1 never surpassed the concentration in HC3 by more than 10 mg/L. The similar concentrations of acetate observed across the BfE-AnMBR illustrate that VFAs did not accumulate in the compartment with the highest loading rate and that substrate staging was not achieved. The low organic loading rate of 1.65 g COD/L reactor/d we achieved with our domestic wastewater (total COD of approximately 550 mg/L) and low flow rates due to excessive membrane fouling above 30.5 L/day was likely not high enough to cause localized reactor souring.

A comparison of the energy consumed by different fouling mitigation systems through a full-scale case study illustrated how a plant utilizing rotating discs (0.4 kW/m^3, Arla, Denmark) consumed less energy for fouling mitigation than two other plants utilizing conventional (0.59 kW/m^3, Plant A) or novel (2.28 kW/m^3, Plant B) sparging, but more than Plant D (0.17 kW/m^3, novel sparging). Due to the limitations of this initial case study including the lack of replicate fouling mitigation systems but also the suboptimal conditions two of the studied plants are operating at, this comparison merely illustrates that rotating discs can be promising but might not consistently entail energy consumption reductions. Future work is necessary to establish a more detailed comparison between these different fouling mitigation techniques. The BfE-AnMBR was discontinued and replaced by the MagnaTree reactor. The MagnaTree reactor utilizes a branched structure wrapped with conductive meshes to solely rely on flow-through biofilm growth for wastewater treatment. Additionally, influent and sludge is continuously recirculated through these meshes to further enhance such biofilm treatment. This reactor achieved a COD removal of 86% and no permeate dissolved methane oversaturation when operated at 21°C. Once stable operation is achieved, the MagnaTree will be tested at decreased temperatures to determine its performance limits at temperatures below 20°C.

4.7 Literature Cited

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Chapter 5. Conclusions and Future Perspectives

5.1 Overview

This dissertation research evaluated anaerobic membrane bioreactor (AnMBR) design characteristics in their ability to increase organics removal, eliminate permeate dissolved methane oversaturation, and minimize energy consumption associated with membrane fouling mitigation when treating domestic wastewater in cold to temperate climates. In doing so, this research advances sustainable domestic wastewater treatment with a focus on net positive energy production and greenhouse gas emission reductions.

A patent review study (Chapter 2) revealed that only a small fraction of AnMBR designs focused on addressing membrane fouling and no more than 11 out of 688 AnMBR patents addressed dissolved methane mitigation. At first glance, the low number of patents targeting these environmental sustainability concerns might suggest the field has exhausted all possible solutions. In contrast, we believe the lack of innovation regarding these topics indicates the need for continued research and novel technology development. The results of the patent review study furthermore suggest that our envisioned design characteristics were distinct from those presented in existing patents, confirming the novelty of our research approach.

A second literature review study revealed substantial complexity associated with confirming the occurrence of direct interspecies electron transfer (DIET) in anaerobic digestion systems (Chapter 3). Future work aimed at evaluating the effectiveness of DIET promotion in our novel AnMBRs will require methodological advances to study DIET. Finally, we evaluated novel AnMBR design characteristics by operating the Biofilm-Enhanced AnMBR (BfE-AnMBR) and MagnaTree reactor (Chapter 4).

5.2 Main findings and significance for the wastewater treatment field

The BfE-AnMBR consists of a three-compartment bioreactor followed by an external rotating disc membrane filtration unit. One focus of this research was to facilitate substrate staging within the three-compartment bioreactor of the BfE-AnMBR to ensure satisfactory organics removal at a high organic loading rate and, hence, a small physical footprint. In line with a previously developed anaerobic migrating blanket reactor (Angenent et al. 2001), we hypothesized that by regularly reversing the flow through the three-compartment BfE-AnMBR, hydrolysis and acidogenesis would be alternatingly promoted in the outer compartments without reaching a pH low enough to inhibit methanogenesis. Such an approach allows all populations represented in a typical anaerobic microbial community to be present throughout the system, providing the potential for greater operational stability. The approach further eliminates the need for an equalization tank or for buffer addition, often required with staged systems. Unfortunately, the BfE-AnMBR never achieved conditions ideal for hydrolysis and acidogenesis, i.e., the pH did not decrease in the compartment with high substrate levels. We hypothesize that this lack of localized reactor souring was caused by the low organic loading rate we were able to achieve (1.65 g COD/L/d compared to 30 g COD/L/d by Angenent et al. (2001)) due to relatively low influent organics concentration (inherent to domestic wastewater) and restricted influent flow rates (due to membrane flux limitations). While outside the scope of this dissertation, focused on domestic wastewater treatment, further work could evaluate if the previously observed benefits of substrate staging can be replicated in a modified AnMBR setup treating higher strength wastewater.

This dissertation research strengthens a previously developed hypothesis that biofilms can harness sufficient microbial activity to enable anaerobic treatment of domestic wastewater at temperatures around and below 20°C (Chapter 4). Even though other anaerobic treatment processes promote biofilm growth by adding packed, fluidized, or suspended attachment media, we hypothesized that pushing the wastewater through a biofilm would minimize any substrate mass transfer limitations and further enhance organics removal and methane production. The consistent buildup of transmembrane pressures across the meshes separating the compartments of the BfE-AnMBR confirmed such biofilm development. Nevertheless, COD removal remained limited to 45%. The underlying cause of this limited organics removal was difficult to ascertain due to the complexity of the BfE-AnMBR. Since not all operational characteristics of the BfE-AnMBR materialized as anticipated during the design phase (e.g., substrate staging), we decided to continue evaluating low temperature domestic wastewater treatment with the simpler MagnaTree reactor. This system comprises a tree-like structure with a hollow stem and branches with large openings wrapped with stainless steel meshes to support 'flow-through biofilm' development. Influent and mixed liquor are continuously recirculated through these flow-through biofilms before being extracted as permeate. Most suspended biomass was removed through the meshes during startup, and the only biomass that remained in the system over time was the biomass that accumulated as biofilm on the meshes. It is too soon to conclude whether the MagnaTree achieves adequate treatment at 15°C, since the system was initially operated at 21°C to accelerate startup. Yet the results are encouraging since, after 105 days of operation, COD removal reached 86% and the permeate dissolved methane was at equilibrium (i.e., methane oversaturation was eliminated). As other domestic wastewater AnMBR studies report COD removals of over 90% (Dong et al. 2015, Seib et al. 2016a, Seib et al. 2016b, Chen et al. 2017), additional performance improvements are necessary before the MagnaTree reactor can be considered for pilot- and full-scale applications. It is important to note that these systems either did not report dissolved methane concentrations or did not eliminate permeate dissolved methane oversaturation. Future work will reveal the minimum temperature at which the MagnaTree can be operated and still achieve adequate effluent quality.

5.3 Recommendations for future research

The results from this dissertation allowed us to identify a number of additional research questions and future research directions. First, the surface area available for biofilm growth in the MagnaTree reactor should be increased by expanding the mesh area since the flow-through biofilms contribute most to wastewater treatment. The packing density of the current MagnaTree reactor is 5.05 m^2/m^3, while other attached growth systems have a packing density of one or two orders of magnitude larger (Kermani et al. 2008, Londoño et al. 2019). The meshes/biofilms in the MagnaTree reactor can be classified into three groups: mesh/biofilm area through which influent and mixed liquor are continuously recirculated, mesh/biofilm area through which permeate is pulled, and biofilm attached to the impermeable parts of the stem and branches in the MagnaTree reactor.

The relative importance of each type of mesh/biofilm needs to be evaluated to better understand the MagnaTree reactor operation. For example, COD removal could be monitored in (i) a MagnaTree reactor with meshes through which the influent and mixed liquor are recirculated once (mimicking permeate branches), and (ii) a MagnaTree reactor with meshes through which the influent and mixed liquor are recirculated five times (mimicking influent/recirculation branches) before being extracted through a separate set of permeate branches, and (iii) a MagnaTree reactor with meshes through which the influent and mixed liquor are recirculated ten times (mimicking influent/recirculation branches) before being extracted through a separate set of permeate branches. Furthermore, microbial treatment performance can be evaluated by comparing the microbial community structure and activity of the different types of biofilms.

In the current MagnaTree design, the mesh surface area for permeation is larger than that through which influent and mixed liquor are recirculated (164 versus 88 cm^2, respectively). However, mixed liquor only passes through the permeate meshes once, and as such, the biofilm growing on the meshes through which influent and mixed liquor are continuously recirculated likely contributes more to organics removal and methane production. When increasing packing density, therefore, the ratio between these different types of mesh/biofilm areas needs to be revised, and a multitude of approached can be applied to accomplish this goal. A straightforward design iteration entails using a similar concentric cylinder setup but changing the proportion of impermeable versus permeate versus influent/mixed liquor surface areas. A more intricate upgrade involves submerging multiple MagnaTree-like structures in one reactor and modify the design of these MagnaTree-like structures to only contain one inner cylinder instead of two concentric cylinders. One set of MagnaTree-like structures could then solely serve to support ‘impermeable’ attached biofilm growth, another for influent and mixed liquor recirculation, and a final set for permeation.

Since the MagnaTree primarily relies on biofilms for treatment, it can be advantageous to further maximize biological activity within the biofilms by promoting DIET. It would be interesting to determine if DIET occurs to a greater extent at the interface between the MagnaTree biofilms and meshes compared to within biofilms not associated with meshes, and if DIET substantially contributes to overall treatment. Until -omics based approaches can be used to determine if DIET takes place in mixed microbial communities, other methods will need to be used towards this goal. Such methods could include in situ measurements of electron transfer rates using cyclic voltammetry or microscopically quantifying the abundance of cytochromes in biofilms versus in suspended biomass. Applying any of these methods to an anaerobic mixed-community bioreactor system will be challenging. A cost-benefit analysis should be performed to ensure that an investment in adding more conductive materials would sufficiently increase biogas production or decrease the treatment footprint to cover additional costs of mesh materials. Importantly, Semenec et al. (2018) suggested that the relative importance of DIET decreased with time, which could mean that DIET only contributes to treatment during the reactor startup phase. The previously made observation that the interspecies distance between propionate degraders and methanogens decreases when a community develops (Felchner-Zwirello et al. 2013) also supports a decrease in the occurrence of DIET, since the polar charge theory dictates DIET to become more active with increasing distance between syntrophic partners (McGlynn et al. 2015). Furthermore, the question arises if the relative importance of DIET, as opposed to mediated interspecies electron transfer, is substantial enough to be of consequence in full-scale domestic wastewater treatment. In addition to determining if DIET is occurring, therefore, it will be paramount to determine the extent to which DIET is contributing to wastewater treatment to inform the value of DIET promotion in full-scale domestic wastewater treatment.

Finally, the MagnaTree reactor needs to be operated with real domestic wastewater, as opposed to the synthetic wastewater used in this dissertation research. The synthetic wastewater primarily contains readily biodegradable compounds and only a low concentration of inert materials. When using real domestic wastewater, (inert) solids have the potential to accumulate inside the MagnaTree structure. If accumulation is substantial, it may be necessary to introduce the influent directly into the reactor in which case additional mixing would be required to keep suspended solids from settling. The current MagnaTree is designed to push influent and recirculated mixed liquor from the inside of the structure to the reactor through a mesh/biofilm, meaning that most of the active biofilm resides inside the MagnaTree structure. To ensure that volatile suspended solids maximize their contact with the biofilms, it might be advantageous to switch that direction of flow and push the sludge from the outside into the MagnaTree structure. Additionally, it is necessary to evaluate the performance of the MagnaTree upon system disturbances typical for domestic wastewater applications such as seasonal changes in wastewater temperature and diurnal and seasonal changes in influent composition. Furthermore, an in-depth effluent characterization is necessary when using the MagnaTree for mainstream domestic wastewater treatment to identify pollutants that require additional treatment. For instance, sulfate reducing microorganisms convert sulfate present in domestic wastewater to sulfide, which can accrue serious health effects when released into the environment (Hayes 1999). Finally, as anaerobic treatment does not remove nutrients, nitrogen and phosphorus removal may become necessary, unless the effluent is used for irrigation.

Overall, the results presented in this dissertation demonstrate that domestic wastewater treatment with low temperature AnMBRs is attainable. However, before they can be applied at the full-scale level to treat domestic wastewater in cold to temperate climates, additional research and technology innovation are needed. We suggest focusing attention towards increasing MagnaTree's mesh/biofilm packing density, evaluating the value of DIET promotion within MagnaTree, and testing MagnaTree's ability to treat real domestic wastewater to further enhance MagnaTree's critical design characteristics.

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