Eleonora Paissoni

Demonstration of anaerobic membrane bioreactors for resource recovery in wastewater treatment applications

School of Water, Energy and Environment

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Supervisor: Prof. Ana Soares
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This thesis is submitted in partial fulfilment of the requirements for the degree of PhD

Academic integrity declaration

I declare that:

- the thesis submitted has been written by me alone.
- the thesis submitted has not been previously submitted to this university or any other.
- that all content, including primary and/or secondary data, is true to the best of my knowledge.
- that all quotations and references have been duly acknowledged according to the requirements of academic research.

I understand that to knowingly submit work in violation of the above statement will be considered by examiners as academic misconduct.

Abstract

Pilot-scale studies on anaerobic membrane bioreactors (AnMBRs) for municipal wastewater treatment at low temperature (<20°C) showed promising results, however, their application at larger scale is still relatively unknown. This study investigated the scalability of an AnMBR, comprising an upflow anaerobic sludge blanket (UASB) reactor and an external ultrafiltration membrane tank, operating AnMBRs both at pilot- and demonstration-scale and identifying how sludge physical and microbiological properties, membrane design and variations in influent temperature, chemical oxygen demand (COD) and sulphate (SO_4) influence the successful scale-up of the technology. At pilot-scale, the source and adaptation of the inoculum and the orientation and arrangement of the membrane fibres did not affect the performance of the reactors. However, the use of horizontal hollow fibres led to lower gas sparging energy consumption compared to a vertical module. The membrane improved removal efficiencies (from 49-57% to 88-92% COD removal), solids hydrolysed (from 0.82-0.86 g/(L·d) to 1.57-1.87 g/(L·d)) and methane production (from 2.3-2.7 L/d to 5.3-5.7 L/d). Methanogenesis percentages were linearly correlated to hydrolysis, which in turn was affected by temperature and inversely correlated to the Sauter mean diameter of the sludge particles. Higher substrate affinities were found at the operational temperature of the reactors (15-20°C), while hydrolytic enzyme activities in UASB reactors and AnMBRs were higher at 37°C. Methane was mainly dissolved in the effluent (70-90%), implying the need for a recovery process to improve the net energy balance. At demonstration-scale, low COD:SO_4 ratio caused competition between sulphate-reducing bacteria and methanogens, leading to a decrease in methane yield. This study proved that AnMBRs are a suitable technology to treat municipal wastewater, however site-specific control strategies to manage fouling and sulphate and appropriate post-treatments are necessary to ensure the successful application of the process at full-scale in temperate climates and the recovery of useful resources from wastewater.

Keywords:

demonstration-scale; dissolved methane; fouling; hollow-fibre; horizontally orientated membrane module; hydrolysis; hydrolytic enzyme activity; inoculum; low temperature; methane; municipal wastewater; pilot-scale; scalability; substrate affinity; sulphate-reducing bacteria; UASB; upflow anaerobic sludge blanket reactor.

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List of Abbreviations

AnMBR	Anaerobic membrane bioreactor
BOD	Biochemical oxygen demand
$CaCO_3$	Calcium carbonate
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
EU	European Union
GHG	Greenhouse gas
HDPE	High density polyethylene
HF	Hollow-fibre
HRT	Hydraulic retention time
LDPE	Low-density polyethylene
MBR	Membrane bioreactor
N	Nitrogen
N_2O	Nitrous oxide
NH_4-N	Ammonium
NTU	Nephelometric turbidity units
O_2	Oxygen
ORP	Oxidation-reduction potential
P	Phosphorus
PCR	Polymerase Chain Reaction
PFAS	Per- and polyfluoroalkyl substances
PO_4-P	Phosphate
PSD	Particle size distribution
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
sCOD	Soluble chemical oxygen demand
SDG	Sustainable development goal
SEA	Specific enzymatic activity
SGD_m	Specific gas demand per membrane area
SO_4	Sulphate
SRB	Sulphate-reducing bacteria

SRT	Sludge retention time
TMP	Transmembrane pressure
TS	Total solids
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket
UF	Ultrafiltration
UK	United Kingdom
VS	Volatile solids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

1 Introduction

1.1 Background

Energy demand of wastewater treatment plants (WWTPs) is estimated to account for 1-3% of the total electricity consumption of a country and is expected to grow by more than 20% in the next 15 years due to population growth, ageing infrastructure and the need to meet stricter effluent quality standards (Bashar et al., 2021; Capodaglio & Olsson, 2020). The energy consumption of a conventional WWTP ranges between 0.3-2.1 kWh/m^3 of treated wastewater, approximately 55-70% of which is used for aeration (Panepinto et al., 2016), and contributes to greenhouse gas (GHG) emissions (0.41-1.08 kg$_{CO2e}$/m^3), in particular with the release of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Cecconet et al., 2022). However, many countries have set a Net Zero carbon emissions target by 2050, which for the water sector in the United Kingdom is set to 2030, therefore, the reduction of net energy requirements and operational GHG emissions in wastewater treatment plants becomes essential to achieve this goal.

Anaerobic reactors can be a valid alternative to aerobic systems in treating municipal wastewater since they consume less energy, due to the absence of aeration, produce less sludge and generate methane-rich biogas. Among high-rate anaerobic reactors, which allow the decoupling of sludge retention time and hydraulic retention time, the upflow anaerobic sludge blanket (UASB) reactor, developed in the Netherlands in the 1970's, is considered a consolidated technology implemented at full-scale. It was originally used to treat highly soluble industrial wastewater, but it is now widely applied at full-scale for municipal wastewater treatment in tropical climates, e.g. Latin America, India, Middle East (Chernicharo et al., 2015). In the UASB reactor the wastewater is fed as uniformly as possible from the bottom of the reactor and flows upwards through a bed of sludge with good sedimentation properties formed by incoming suspended solids and biologically active biomass. Filtration through the sludge bed converts the organic matter found in wastewater into methane and carbon dioxide. Natural mixing from influent flow and gas production enhances contact between wastewater and biomass. In the upper part a three-phase separator collects and discharges the biogas produced, reduces liquid turbulence in the settling zone, allowing sludge particles, which have risen attached to gas bubbles, to settle, preventing them to be washed out and separating them from the effluent (Stazi & Tomei, 2018).

Hydrolysis of particulate matter, which is the greatest organic fraction in municipal wastewater, is considered the rate-limiting step in the anaerobic treatment of low-strength streams. Hydrolysis kinetics are particularly affected by temperature, as temperature influences other environmental parameters, like pH and viscosity. At low temperatures (<20°C), diffusion rates decrease, negatively affecting substrate utilisation kinetics, and gas solubility increases (Daud et al., 2018). Non-hydrolysed solids can accumulate in the reactor, reducing removal efficiencies and methane production and leading to biomass washout (Ribera-Pi et al., 2020). Therefore, the ability of the system to retain solids and increase sludge retention time (SRT) to improve the contact between substrate and biomass, hence hydrolytic rates, becomes key for the successful application of high-rate anaerobic reactors in temperate climates.

The combination of a UASB reactor with a submerged membrane process, in an anaerobic membrane bioreactor (AnMBR), is a promising solution to promote hydrolysis at low temperatures. AnMBRs, due to the presence of an ultrafiltration membrane, often with a hollow-fibre (HF) configuration, can provide complete retention of solids and biomass, increasing SRT and sustaining hydrolytic activity, and produce a high-quality effluent free of solids and pathogens (Stazi & Tomei, 2018). Furthermore, since the anaerobic treatment do not degrade ammonium and phosphate, the AnMBR effluent can be of interest for fertigation or nutrient recovery and subsequently water reuse (Robles et al., 2021). Recent large-scale studies, summarised in Table 1-1, have shown the potential application of AnMBR for municipal wastewater treatment at low temperatures, however few challenges still need to be addressed to successfully scale up the technology.

Table 1-1 Large-scale AnMBR studies from literature treating municipal wastewater in temperate climates. COD: chemical oxygen demand. (*): $L_{CH4}/g_{COD,in}$.

Volume (m^3)	*HRT (h)*	*Temperature (°C)*	*Influent COD (mg/L)*	*COD removal (%)*	*Methane yield ($L_{CH4}/g_{COD,rem}$)*	*Reference*
0.31	7	18±2	892±271	87±1	0.18-0.23	(Gouveia et al., 2015)
0.5	8-10	10-28	1729±914	85-94	0.09-0.14	(Peña et al., 2019)
1.42	11±3	12.7-31.5	610±260	88±7	0.14±0.06*	(Lim et al., 2019)
2.1	24.4±0.4	27±1	510±87	88	0.108±0.018	(Seco et al., 2018)
5	12	25	403.1±28.1	90	0.25	(Kong et al., 2021)
40.8	25-41	10-27	755-1403	87.2±6.1	0.07-0.17*	(Robles et al., 2022)

The source and type of seed sludge to use in the anaerobic reactor at low temperature is currently still debated. Since at low temperature the activity of mesophilic microorganisms decreases, it can be argued that a psychrophilic inoculum could be more suited for this application (Petropoulos et al., 2017). Psychrophilic enzymes have developed higher flexibility of the active site and therefore lower stability of the protein structure to reduce the activation energy, which increases at low temperature, and thrive in cold environments (Collins & Margesin, 2019). However, at larger scale a psychrophilic inoculum may be difficult to source, since it is often located in hardly accessible areas, leading to higher costs, and the seasonal temperature variations may have a detrimental effect on psychrophilic enzymes due to their lower stability. Studies from literature (Gouveia et al., 2015; Hejnic et al., 2016; Ribera-Pi et al., 2020; Serrano León et al., 2018) have also employed a mesophilic inoculum, however it has not been clearly defined yet whether a period of adaptation before inoculation is needed or not. More research is also required on the sludge microbial community and the enzymes responsible for the hydrolysis of macromolecules to better understand how they adapt to the low-temperature operation and how they are affected by the presence of a membrane.

Regarding membrane operation, current research is mainly focusing on fouling control (Anjum et al., 2021; Hamedi et al., 2019; Robles et al., 2021; Wang et al., 2018b). Fouling is the deposition of colloids and particles inside or on the membrane surface. It is physically mitigated by relaxation, which is a stop in filtration, backwashing, namely reversing the flow, and/or gas sparging, which consists in the scouring of the membrane by gas bubbles and has been identified as the biggest contributor to operational costs. The energy consumption of gas sparging can be optimised reducing the time of its application. Several regimes have been studied: continuous, intermittent (e.g. 10 s on, 10 s off) and pseudo dead-end, in which filtration is performed without gas sparging and is followed by a period of relaxation or backwashing with gas sparging in sequential cycles. The pseudo dead-end has proven to be a promising option for AnMBRs (Wang et al., 2018a), however it still needs to be tested for different membrane geometries. In particular, the fibre orientation and arrangement in HF configurations can have an effect on the efficacy of gas sparging. This is because, while gas sparging is normally applied from the bottom of the tank with gas bubbles rising to the top, how the fibres are orientated and organised may vary the hydrodynamic environment around them and impact fouling control. Currently, the majority of products for membrane bioreactors are configured in a vertical orientation, however, a horizontal orientation may allow for reduced height requirements and therefore lower energy consumption of the gas blower. Nevertheless, the application of the horizontal HF configuration for large-scale AnMBRs has not been investigated yet.

Another issue that can impair the successful scalability of the AnMBR for municipal wastewater treatment is the highly variable characterisation of the influent wastewater. Robles et al. (2022) identified temperature and the ratio between influent COD and sulphate (SO_4) as crucial influent parameters to monitor to optimise the performance of the AnMBR. This is particularly important because these parameters can affect the effluent quality and therefore the possible post-treatments. Low temperature increases gas solubility, leading to an increase in the fraction of produced methane dissolved in the effluent, which, from the studies reported in Table 1-1, ranges between 20-50%. Without the recovery of this fraction, it would not be possible to achieve a neutral or positive energy balance and greenhouse gas emissions of the plant would increase due to the release of methane in the atmosphere from the effluent (Robles et al., 2022). Different technologies are available for the removal and recovery of dissolved methane (Crone et al., 2016), however their applicability has not been tested at full-scale yet. In anaerobic reactors sulphate-reducing bacteria (SRB) compete with methanogens for the available substrate; the ratio COD:SO_4 affects the competition and therefore the overall methane production. The reduction of sulphate leads to the production of dissolved sulphide, which can cause problems of corrosion and bad odours in the downstream processes. As for dissolved methane, the available removal technologies are currently applied at lower scale (Liu et al., 2018). Therefore, while AnMBRs could be a viable alternative to conventional aerobic systems, there is a current need for scaled up studies to investigate the key challenges described above to ensure the successful application of a large-scale AnMBR for municipal wastewater treatment in temperate climates, recovering clean water, energy and nutrients.

1.2 Aim and objectives

The overall aim of the thesis is to provide a robust scientific assessment of anaerobic membrane bioreactors (AnMBRs) for municipal wastewater treatment in temperate climates, by understanding the impact of adaptation of seed sludge, hydrolytic activity, influent wastewater variability and membrane design on the scalability of the process. To achieve that, a series of objectives were identified and investigated.

Objective 1: To understand the role of physical and microbiological properties of the seed sludge on the treatment of municipal wastewater in a pilot-scale upflow anaerobic sludge blanket (UASB) reactor at low temperatures (<20°C), in terms of hydrolytic efficiency, effluent quality and methane production.

Objective 2: To understand the impact of membrane ultrafiltration in pilot-scale UASB reactors on the activity of key hydrolytic enzymes and their substrate affinities at different temperatures.

Objective 3: To investigate the impact of influent wastewater fluctuations of chemical oxygen demand (COD), sulphate (SO_4) and oxidation-reduction potential (ORP) on effluent quality, methane production, competition between sulphate-reducing bacteria (SRB) and methanogens and scalability of a demonstration-scale AnMBRs for municipal wastewater treatment at low temperatures.

Objective 4: To understand the impact of fibre orientation (vertical or horizontal) and arrangement (coiled or not) in hollow-fibre membrane modules on fouling control, and therefore energy consumption, of AnMBRs treating municipal wastewater at different scales in temperate climates.

The objectives are graphically conceptualised in Figure 1-1.

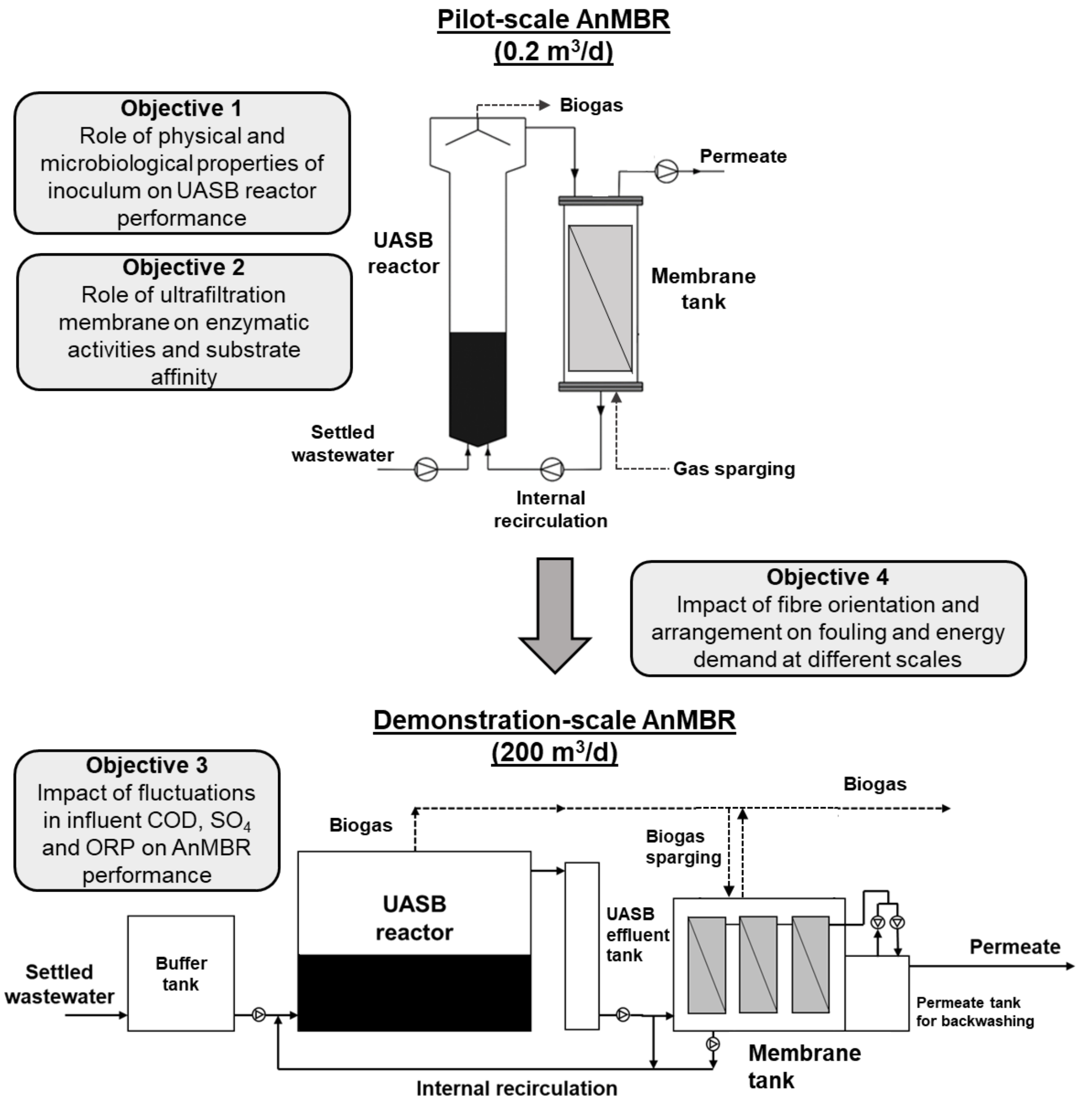

Figure 1-1 Schematic representation of the objectives of the thesis.

1.3 Thesis plan

This PhD thesis is presented as a series of chapters, formatted as journal papers. All chapters were prepared by the author, Eleonora Paissoni, and edited by the supervisor, Professor Ana Soares, and the associate supervisor, Professor Bruce Jefferson. The design of the pilot-scale experiments, collection and analysis of the samples and data analysis were performed by Eleonora Paissoni. Amer Qudah contributed to data collection for pilot-scale AnMBRs (Chapter 5). One of the two pilot-scale membrane tanks, built specifically for this work, was designed by Dr. Luca Alibardi and Eleonora Paissoni. The pilot-scale plants were operated by Eleonora Paissoni in the UKCRIC National Research Facility for Water and Wastewater Treatment at Cranfield University. The demonstration-scale plant was operated by Severn Trent Water and contractors at the Spernal wastewater treatment plant (WWTP). Analysis of the demonstration-scale plant samples were conducted by an external laboratory (ALS Environmental, Coventry, UK) and occasionally by Eleonora Paissoni. Data analysis for the demonstration-scale plant was performed by Eleonora Paissoni. Analysis of sludge bacterial community was performed by an external laboratory (Aqua Enviro, Wakefield, UK). This thesis has been carried out as part of the H2020 EU project NextGen (grant agreement No. 776541).

Chapter 2 addresses Objective 1 and investigated how the physical and microbiological properties of the seed sludge affected the operation of a pilot-scale UASB reactor, comparing results using inocula with different origin and adaptation. The performance was evaluated in terms of effluent quality, methane production, hydrolytic percentages and evolution of sludge characteristics, resolving the knowledge gap related to the choice of suitable inoculum for municipal wastewater treatment in temperate climates. (Paper in preparation: Paissoni, E., Jefferson, B. and Soares, A., Understanding the impact of the type of seed sludge on municipal anaerobic wastewater treatment in temperate climates).

Chapter 3 is related to Objective 2 and is consequential to Chapter 2. Once the impact of different types of seed sludge had been investigated, Chapter 3 focused on understanding better the hydrolysis stage, as it is the rate-limiting step in anaerobic treatment at low temperature, and how it was affected by the addition of a membrane. Hydrolytic enzyme activities and substrate affinity in UASB reactors and AnMBRs were studied at different temperatures, identifying optimal temperatures for enzyme activity and microbial growth in adapted sludge. (Paper in preparation: Paissoni, E., Jefferson, B. and Soares, A., Hydrolytic enzyme activity in high-rate anaerobic reactors treating municipal wastewater in temperate climates).

Since pilot-scale studies from previous Chapters proved the successful implementation of AnMBRs at pilot-scale for municipal wastewater treatment at low temperature, Chapter 4 addressed the operation of a large-scale AnMBR (fed with 200 m^3/d wastewater). Focusing particularly on the biological reactor scalability, the impact of variations in influent COD, SO_4 and ORP on the competition between SRB and methanogens, and therefore on the reactor performance, was investigated (Objective 3). (Paper in preparation: Paissoni, E., Palmer, M., Pitt, S., Jefferson, B., Vale, P. and Soares, A., Impact of influent characteristics on demonstration-scale anaerobic membrane bioreactor for municipal wastewater treatment in temperate climates).

Chapter 5 focused then on the scalability of the other component of the AnMBR, the membrane module. In an effort to reduce energy consumption of AnMBRs by managing membrane fouling, Chapter 5 investigated the role of fibre orientation and arrangement on fouling control and energy demand and how results compared at different scales (pilot- and demonstration-scale), addressing Objective 4. (Paper in preparation: Paissoni, E., Qudah, A., Jefferson, B. and Soares, A., Investigating the impact of horizontally orientated hollow fibres in pilot- and demonstration-scale anaerobic membrane bioreactors).

Chapter 6 includes an overall discussion of the work carried out in previous Chapters and the contribution to knowledge of the thesis and Chapter 7 presents the conclusions of the thesis and suggested areas for future research.

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2 Understanding the impact of the type of seed sludge on municipal anaerobic wastewater treatment in temperate climates

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Abstract

The application of upflow anaerobic sludge blanket (UASB) reactors for anaerobic wastewater treatment in temperate climates is being thoroughly investigated. Nevertheless, the impact of the type of seed sludge and the understanding of the hydrolysis stage at temperatures <20°C is still unclear and it needs to be elucidated to successfully scale up the process in temperate climates. Two pilot-scale UASB reactors, inoculated with seed sludges from different sources, i.e., non-adapted granular sludge from industry (UASB-R1) and heterogenous sludge adapted to municipal wastewater (UASB-R2) were operated at 13°C and 19°C for more than a year. Data was collected to compare effluent quality, methane production and hydrolysis. The two reactors showed similar removal efficiencies at the same temperature, achieving chemical oxygen demand (COD) and total suspended solids (TSS) removals of around 50% and 60%, respectively. Average methane yields were 0.10 $L_{CH4}/g_{COD,rem}$ for both reactors at 13°C and, when the temperature increased to 19°C, the values increased (0.15 $L_{CH4}/g_{COD,rem}$ for UASB-R1 and 0.17 $L_{CH4}/g_{COD,rem}$ for UASB-R2, statistically similar). The methanogenesis percentage showed that the conversion of COD into methane was mainly affected by hydrolytic efficiencies, which in turn were influenced by temperature and correlated to the particle size distribution of the sludge. Furthermore, microbial diversity tests revealed that in time the two sludges developed a similar bacterial composition. This study demonstrates that hydrolysis is the limiting step and enhanced solids retention in UASB reactors is required to sustain it. The source and type of seed sludge did not have an impact

on the UASB operation, suggesting that cold-adaptation prior inoculation or use of a psychrophilic inoculum is unnecessary for municipal anaerobic wastewater treatment in temperate climates.

Keywords: anaerobic treatment, hydrolysis, inoculum, low temperature, municipal wastewater, UASB

2.1 Introduction

High-rate anaerobic reactors are a viable alternative to secondary aerobic systems for municipal wastewater treatment, since they are designed to decouple the hydraulic retention time (HRT) from the sludge retention time (SRT), allowing higher biomass retention inside the reactor and reduced treatment times, compared to conventional anaerobic processes (Chong et al., 2012). They are compact, robust and simple technologies, implying low capital and operational costs and lower sludge production than aerobic treatments (Chernicharo et al., 2015). Furthermore, high-rate anaerobic reactors do not require aeration and have the potential to be energy-neutral due to the degradation of organic matter into methane-rich biogas, instead of standard oxidation, promoted by forced aeration, into carbon dioxide.

Among high-rate anaerobic reactors, the upflow anaerobic sludge blanket (UASB) reactor is one of most widely used. In UASB reactors, slow-growing anaerobic microorganisms are retained in a sludge bed with good sedimentation properties that accumulates at the bottom of the reactor. These reactors were firstly developed and employed for high-strength and highly soluble industrial wastewater (Stazi & Tomei, 2018). Additionally, successful full-scale applications can also be found in tropical climates, e.g. India and Latin America, for the treatment of municipal wastewater, which are defined as low-strength streams with high particulate organic matter content (Ozgun et al., 2013). Full-scale UASB reactors recently installed in Brazil, treating wastewater with an average influent chemical oxygen demand (COD) of 195±54 mg/L, achieved removals between 58-79% with average of 66±7% (Chernicharo et al., 2015).

It is argued that, due to the nature of municipal wastewater and sludge, hydrolysis is the limiting step in the anaerobic degradation of organic matter and it is further impacted by temperature that leads to slower kinetics of substrate utilisation when temperature decreases (Daud et al., 2018). Poor hydrolysis can cause subsequent accumulation of suspended solids in the anaerobic reactor, which in turn triggers biomass washout, reduction of treatment performance and loss of biogas production potential. Therefore, the ability of the system to retain solids becomes key especially at low temperatures.

Several pilot-scale studies have shown the potential application of UASB and UASB-configured reactors in temperate climates (Gouveia et al., 2015; Saha et al., 2015; Xu et al., 2018; Wang et al., 2019; Ribera-Pi et al., 2020; Owusu-Agyeman et al., 2021; Trego et al., 2021; Cecconet et al., 2022). However, a few aspects of the treatment, especially related to the adaptation and type of seed sludge and how this relates to hydrolysis and management of solids inside the reactor, remain unclear. Therefore, there is a need to better understand these elements in order to achieve the successful application of the technology at larger scale in temperate climates.

In UASB reactors, suspended solids are removed via settling and filtration through the sludge bed, which acts as a filter media. During the anaerobic treatment, particulate organic matter is hydrolysed while adsorbed and entrapped within the sludge bed (Gomec, 2010). Therefore, different morphological and filtrating properties of the biomass may affect the efficiency in solids hydrolysis of the reactor. Currently, there is not a unanimous position on the type of inoculum to choose for anaerobic wastewater treatment at low temperature. Granular sludge possesses superior settling properties compared to flocculent sludge, making it a more suitable choice in case of UASB reactors, especially treating municipal wastewater with variable influent flow, as it is subject to less biomass washout. However, flocculent sludge is more ubiquitous and less expensive than granular sludge. Moreover, a few studies showed that, with appropriate solids management strategies, UASB-configured bioreactors inoculated with flocculent sludge perform equally to those inoculated with granular sludge (Ribera-Pi et al., 2020; Wang et al., 2019).

The source of the seed sludge and its adaptation constitutes an important element to consider before the implementation of a high-rate anaerobic reactor because it has an impact on the time schedule of the start-up, potential sourcing and transport issues and the overall cost. Few studies have successfully deployed a psychrophilic sludge for the anaerobic treatment of wastewater. Xing et al. (2010) used a waterfowl lake sediment as seed sludge to treat synthetic brewery wastewater at 15°C and reached 80% COD removal. Petropoulos et al. (2017) was able to treat UV-sterilized raw domestic wastewater in batch reactors, down to 4°C, with a mixture of sediments from Lake Geneva and soils from Svalbard, observing a shift in microbial diversity with a decrease in temperature. More recently, Aguilar-Muñoz et al. (2022) reported successful results related to the biotechnological application of Antarctic soils and sediments for improved methane production at 5°C, observing a 40% greater rate in the Antarctic inoculum, compared with a mesophilic industrial sludge.

However, the use of a psychrophilic inoculum for large-scale applications requires resources for sourcing and sampling a large volume of sediments, often located in hardly accessible areas, and a suitable design and management of the anaerobic reactor to assure the development of an abundant psychrophilic microbial community and its retention within the system. Moreover, since temperature in temperate climates is characterised by seasonal variations, the psychrophilic biomass performance should be evaluated at higher temperatures before asserting its suitability for this type of treatment (Smith et al., 2012).

Most trials adopted the strategy of adapting a mesophilic inoculum to work at sub-mesophilic temperatures (Akila & Chandra, 2007; Gouveia et al., 2015; Hejnic et al., 2016; Lew et al., 2004; Ribera-Pi et al., 2020; Saha et al., 2015; Serrano León et al., 2018; Singh & Viraraghavan, 2004). Akila et al. (2007) performed the adaptation of a mesophilic inoculum from 37°C to 15°C, decreasing the temperature in steps with sequential transfers, in 21 months. Other studies (Lew et al., 2004; Serrano León et al., 2018) acted on extending the HRT in order to increase the contact between the wastewater and the biomass and gradually acclimatise the microorganisms to lower temperatures. Another strategy would entail using previously acclimatised sludge (Hejnic et al., 2016; Ribera-Pi et al., 2020; Saha et al., 2015). Nevertheless, cold-adapted mesophilic inocula, despite the ability of growing at lower temperatures, did not display psychrophilic characteristics, but rather a psychrotolerant behaviour, defined by optimum growth temperature in the mesophilic range (O'Flaherty et al., 2006). Ultimately, to avoid the adaptation period altogether, non-adapted seed sludge can be employed for the anaerobic treatment of municipal wastewater (Gouveia et al., 2015; Singh & Viraraghavan, 2004).

Therefore, in this study, a pilot-scale UASB reactor, inoculated with fresh industrial mesophilic sludge, was operated to treat municipal wastewater at low temperature (<20°C) in the same conditions as a second UASB reactor, seeded with adapted sludge. This setup allowed to investigate the performance of the two UASB reactors and whether the different origin or history of the sludge had an impact on solids hydrolysis, effluent quality and methane production.

2.2 Materials and methods

2.2.1 Inoculum

Two pilot-scale UASB reactors were operated in parallel, fed with the same settled wastewater, but inoculated with two very different types of sludge. The first reactor, UASB-R1, was inoculated with 11 L of fresh non-adapted granular sludge (89% volatile solids (VS) to total solids (TS) and 55 g_{VS}/L, Figure 2-1a), sourced from a mesophilic industrial UASB reactor treating food processing wastewater (Waterleau, Wespelaar, Belgium). In contrast, the second reactor, UASB-R2, was inoculated with 11 L of adapted sludge (79% VS/TS, 33 g_{VS}/L, Figure 2-1b), originally derived from a municipal digester treating a mixture of primary and secondary sludge and an industrial UASB reactor treating pulp and paper processing wastewater. However, the sludge in UASB-R2 was already acclimatised to the municipal wastewater and low temperature since it had been previously used for pilot-scale trials at Cranfield University for more than 5 years (Ribera-Pi et al., 2020; Wang et al., 2018). Figure 2-1 shows qualitative morphological differences between the two seed sludges, as the granules in UASB-R2 were rounder and smoother than the ones from the fresh industrial granular sludge in UASB-R1. In addition, further characterisation was carried out analysing particle size distribution and microbial diversity of the two inocula before start-up.

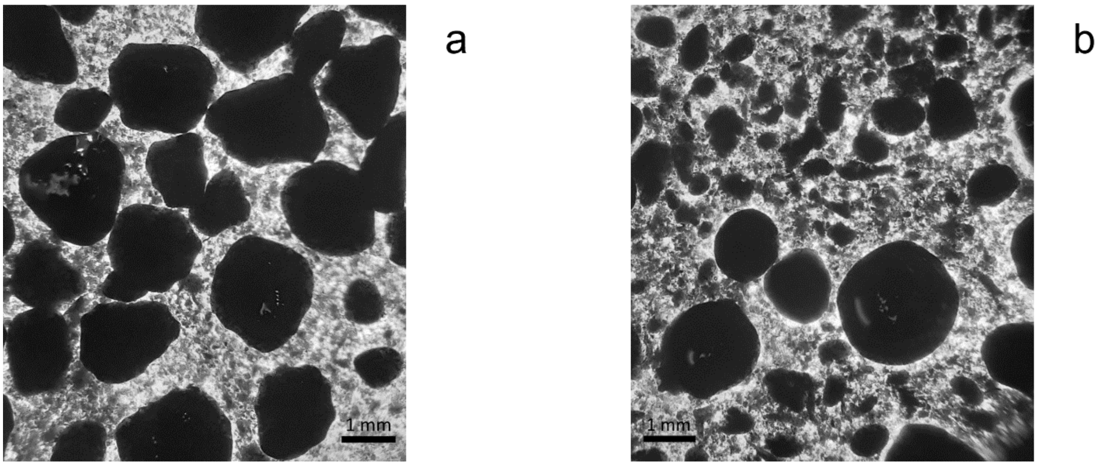

Figure 2-1 Pictures of seed sludge obtained with optical microscope. a: inoculum for UASB-R1 (fresh industrial granular sludge); b: inoculum for UASB-R2 (heterogeneous adapted sludge).

2.2.2 Description and operation of the pilot-scale plant

Two 70 L cylindrical UASB reactors (0.2 m diameter, 2.2 m height) were operated for over a year at ambient temperature in the UK (Appendix B, Figure B-1). Each reactor presented one sampling point at the bottom, five sampling points along the side, placed every 30 cm from the bottom, three lamella settlers just above the highest sampling point and a three-phase separator on top, where the effluent and recirculation pipes were positioned. Settled wastewater from Cranfield University wastewater treatment plant (WWTP) was pumped into the UASB reactors from the bottom through peristaltic pumps (520Du, Watson Marlow, Falmouth, UK), to achieve a hydraulic retention time (HRT) of 8 hours. The UASB effluent was recirculated from the top to the bottom using peristaltic pumps (620Du, Watson Marlow, Falmouth, UK) to sustain an upflow velocity of 0.8 m/h.

Sludge samples, collected from the bottom of the reactors, and influent and effluent wastewater samples were analysed all year round to assess the influence of the wastewater temperature, which varied from a minimum of 6°C in winter to a maximum of 23°C in the summer. Biogas production, from the top of the reactors, was monitored and its composition was analysed onsite. Liquid samples from the UASB reactors were collected for the analysis of dissolved methane. The performance of the treatment was evaluated in steady-state condition during two subsequent periods (days 101-327 and days 328-455), characterised by significantly different temperature datasets. Data of the pilot-scale plant operation prior day 101 was not included in this analysis due to lack of regular access to laboratory facilities, caused by national lockdowns during the coronavirus pandemic, which prevented a complete analysis of the methane production.

2.2.3 Analytical methods

Wastewater analyses were conducted in duplicates, multiple times a week. Total suspended solids (TSS), volatile suspended solids (VSS), biochemical oxygen demand (BOD) and alkalinity were measured according to Standard Methods (APHA, 2012). Total and soluble chemical oxygen demand (COD, sCOD), sulphate (SO_4), ammonium (NH_4-N) and phosphate (PO_4-P) were analysed with cell test kits (Merck KGaA, Darmstadt, Germany). Wastewater samples for cell test analysis, except COD, were filtered at 0.45 μm with retention membrane filters (Whatman, Cytiva, Little Chalfont, UK). Temperature and pH were measured on site right after sample collection with a portable Multi Meter (HQ40D, Hach Lange GmbH, Düsseldorf, Germany).

Biogas volume production from the UASB reactors was measured with gas meters (TG 0.5, Ritter, Bochum, Germany), one per each reactor, and the composition was determined with in-line CO_2 and CH_4 sensors (BCP-CO_2 and BCP-CH_4, BlueSens gas sensor GmbH, Herten, Germany). The connection to the sensors, from the outlet line of the gas meter, was switched between the two reactors every 2-3 days. During maintenance of the gas sensors, gas bags were used and biogas composition was measured via gas chromatography, as described below. Wastewater samples for dissolved methane measurements were collected twice a week in duplicates from the highest sampling point of the reactors in 22 ml glass vials with PTFE/silicone septum, avoiding any turbulence and leaving around 5 ml of headspace. Measurements were carried out at 20°C according to the method described by Cookney et al. (2016). The dissolved methane concentration was calculated according to the following mass balance as described in Equation (2-1) (Souza et al., 2011):

$$[\mathrm{CH_4}]_{\mathrm{diss}} = \frac{([\%\mathrm{CH_4}]_{\mathrm{gas}}/100) \cdot [\rho \cdot \mathrm{V_{gas}} + (\mathrm{P_T} - \mathrm{P_V}) \cdot \mathrm{K_H} \cdot \mathrm{V_{liq}}]}{\mathrm{V_{liq}}} \quad \textbf{(2-1)}$$

where, $[\mathrm{CH_4}]_{\mathrm{diss}}$ is the concentration of dissolved methane (mg/L), $[\%\mathrm{CH_4}]_{\mathrm{gas}}$ is the concentration of methane in vial's headspace (%), ρ is the density of methane (651 mg/L at 20°C and 0.988 atm), $\mathrm{V_{gas}}$ is the volume of gas in vial (mL), $\mathrm{P_T}$ is the atmospheric pressure (0.988 atm at Cranfield), $\mathrm{P_V}$ is the water vapour pressure (0.023 atm at 20°C), $\mathrm{K_H}$ is the Henry's constant for methane (24.9 mg L^{-1} atm^{-1}) at 20°C and $\mathrm{V_{liq}}$ is the volume of liquid in vial (mL). A water sample, once freshly saturated with 100% methane (SIP Analytical, Sandwich, UK), was collected following the same procedure and chromatographically analysed along with wastewater samples to determine the effect of sample collection and storage on the measurements. Methane yield was expressed as a ratio between methane produced in gas and liquid phases and the COD removed by methanogens, excluding the fraction of COD removed by the sulphate-reducing bacteria (SRB) (0.67 g of O_2 consumed per g SO_4 removed).

Percentages of hydrolysis and methanogenesis were calculated according to Equations (2-2) and (2-3) (Elmitwalli et al., 2002):

$$\mathrm{Hydrolysis\ (\%)} = 100 \cdot \frac{\mathrm{CH_4\ as\ COD} + \mathrm{sCOD_{out}} - \mathrm{sCOD_{in}}}{\mathrm{COD_{in}} - \mathrm{sCOD_{in}}} \quad \textbf{(2-2)}$$

$$\mathrm{Methanogenesis\ (\%)} = 100 \cdot \frac{\mathrm{CH_4\ as\ COD}}{\mathrm{COD_{in}}} \quad \textbf{(2-3)}$$

where, the subscripts "in" and "out" indicate the influent and effluent wastewater, respectively, and 0.25 L_{CH4}/g_{COD} was used for the conversion between methane and COD. While the formulas are widely used in literature to express percentages of hydrolysis and methanogenesis in anaerobic reactors, some limitations need to be addressed. In particular, the hydrolysis percentage, using soluble COD, assumes as a first approximation that solubility equates hydrolysis, without taking into consideration the fraction of COD or soluble COD that is in fact hydrolysable. However, to compare results with the ones from other studies, the formulas have been applied as described in Equations 2-2 and 2-3.

Sludge blanket height was measured every 2-3 days. Removal of the top layer of the sludge blanket of both UASB reactors was required roughly every 3 months to prevent sludge washout and displacement of the lamella settlers. Sludge samples were collected and analysed periodically during the study to observe variations in the particle size distribution and total (TS) and volatile solids (VS) content. Total and volatile solids were measured according to Standard Methods (APHA, 2012). Particle size distribution of sludge samples was assessed via a laser particle size analysis system (Mastersizer 3000, Malvern Instruments Ltd, Malvern, UK).

The bacterial diversity of the sludge was analysed to determine the main biological differences at the beginning and during the study. Sludge samples were kept in the cold room and shipped to an external laboratory for analysis. DNA extraction was performed with Qiagen DNeasy Powersoil Pro Kit, according to kit's instructions. PCR analysis and sequencing were carried out with the Nanopore 16S Barcoding Kit (SQK-RAB204) and the Nanopore MinION Flowcell (R9.4.1), respectively.

Statistical analysis was performed to evaluate first outliers, then normality through Shapiro-Wilk tests. Welch's t-test was applied for normal distributions, while Mann–Whitney U test was used in the other cases. The analysis was based on a 95% confidence limit.

2.3 Results and discussion

2.3.1 Effluent quality and removal efficiencies

The average influent temperatures of the two periods, selected for further analysis, were 13°C and 19°C (Table 2-1), which are typical of winter and summer in temperate climates and similar to previous pilot-scale and laboratory studies (Gouveia et al., 2015; Hejnic et al., 2016; Petropoulos et al., 2019; Wang et al., 2019). The average total COD was 214-221 mg/L and TSS was around 120-124 mg/L, both typical of settled municipal wastewater and not statistically different between the two periods (Table 2-1). Soluble COD was around 45 mg/L during the entire operational period, implying a very significant fraction of total COD was particulate. The influent ratio COD:BOD ranged between 2.5-2.8, higher than the typical values of 1.7-2.4 for municipal wastewater (Von Sperling & Chernicharo, 2005). The influent sulphate concentration was around 68 mg/L at 13°C and 56 mg/L at 19°C and showed a significant difference between the values at the two different temperatures (Table 2-1).

Effluent COD in UASB-R1 was around 98 mg/L at 13°C and 91 mg/L 19°C, while in UASB-R2 the concentrations were marginally higher, namely 110 mg/L at 13°C and 101 mg/L at 19°C. The TSS concentration in the UASB-R1 was lower at 19°C (31 mg/L) than at 13°C (39 mg/L), while in UASB-R2 effluent was 40 mg/L at 19°C and 44 mg/L at 13°C (Table 2-1). Conversely, soluble COD concentration was slightly lower in UASB-R2 than in UASB-R1 (Table 2-1), however at the same temperature the differences between the two reactors were not significant ($p>0.05$).

Table 2-1 Average influent and effluent (UASB-R1 and UASB-R2) wastewater characterisation over two different operational periods (days 101-327 and 328-455).

	Influent		*Effluent UASB-R1*		*Effluent UASB-R2*	
	days 101-327	*days 328-455*	*days 101-327*	*days 328-455*	*days 101-327*	*days 328-455*
Temperature (°C)	13.1±1.7	19.0±1.1	11.9±1.6	18.3±1.5	12.0±1.7	18.4±1.6
pH	8.12±0.34	7.75±0.20	7.62±0.23	7.36±0.21	7.78±0.31	7.55±0.12
COD (mg/L)	214±58	221±52	98±28	91±18	110±32	101±22
sCOD (mg/L)	45±8	45±9	39±7	33±7	38±8	31±6
BOD (mg/L)	87±24	80±15	48±11	36±4	53±8	43±9
SO_4 (mg/L)	68±5	56±6	43±15	38±11	49±13	42±10
TSS (mg/L)	120±33	124±26	39±9	31±9	44±9	40±7
VSS (mg/L)	103±29	107±21	36±10	29±8	40±9	37±8
Alkalinity (mg_{CaCO3}/L)	300±58	295±61	278±23	270±19	276±16	256±15
NH_4-N (mg/L)	20.0±4.1	22.8±4.3	24.1±4.5	28.3±3.5	24.7±3.5	27.1±3.6
PO_4-P (mg/L)	3.5±0.7	4.3±0.6	4.0±0.7	4.9±0.7	3.9±0.6	4.7±0.7

The removal of pollutants in UASB-R1 and UASB-R2 at 13°C and 19°C are reported in Figure 2-2. Both reactors showed COD and TSS removals between 50-60%, all year round, consistent with previous UASB studies at similar temperature (Bandara et al., 2012; Rizvi et al., 2015; Wang et al., 2019). However, there was a significant increase ($p<0.05$) in sCOD removal from 13°C to 19°C, from 13% to 26%, respectively, in UASB-R1, and from 18% to 29%, respectively, in UASB-R2 (Figure 2-2). Furthermore, there was a slight, although not statistically different, decrease in sulphate removal, from 38% to 32% in

UASB-R1 and from 28% to 26% in UASB-R2, at 13°C and 19°C, respectively (Figure 2-2). Statistical differences between the two reactors were detected for COD and SO_4 removals at 13°C (54% and 38% for UASB-R1, respectively, and 49% and 28% for UASB-R2, respectively) and TSS removal at 19°C (Figure 2-2). At 19°C, UASB-R1 removed 75% of TSS, while UASB-R2 achieved a TSS removal of 65%. For all parameters in Figure 2-2, except soluble COD, UASB-R1, inoculated with non-adapted sludge, displayed higher removal efficiencies than UASB-R2, seeded with acclimatised sludge, suggesting that the mesophilic industrial sludge adapted quickly to the wastewater and temperature and there was no need of an acclimation period (effluent quality of the two UASB reactors for days 1-100 can be found in Appendix B, Table B-1). Overall, Figure 2-2 clearly shows that the two reactors performed similarly, regardless of temperature, since differences in removals were mainly not statistically relevant.

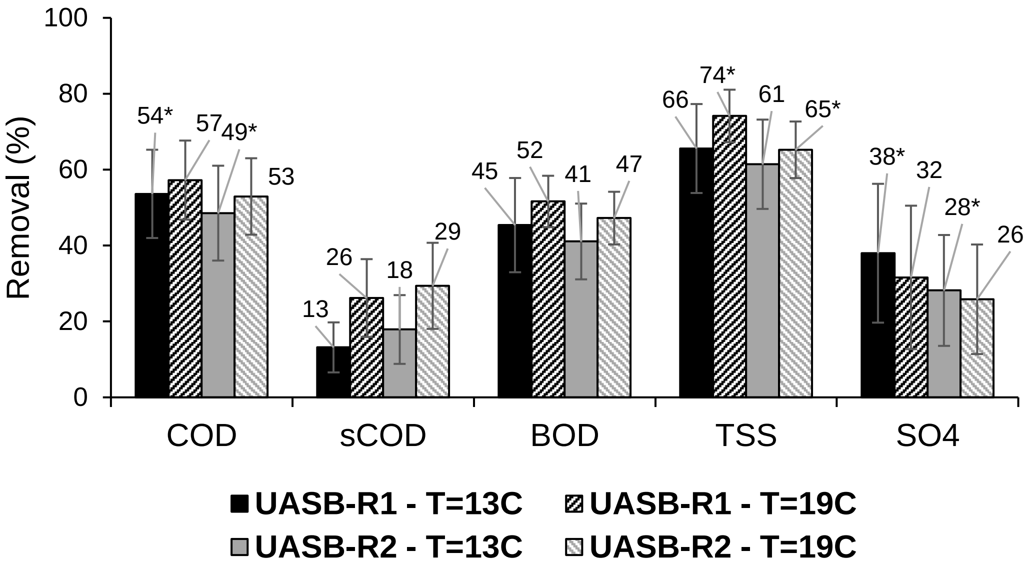

Figure 2-2 Average removals of COD, soluble COD, BOD, TSS and SO_4 in UASB-R1 and UASB-R2 over two different periods at the average temperature of 13°C (days 101-327) and 19°C (days 328-455). The star (*) indicates the parameters for which the two UASB reactors showed significantly different removals at the same temperature.

2.3.2 Methane production

Methane was observed both in liquid and gas phases in the UASB reactors. At 13°C, the production of methane in biogas was 0.19 L/d for UASB-R1 and 0.32 L/d for UASB-R2 (Table 2-2). At 19°C, both reactors displayed a significant rise in the methane gas production (0.76 L/d for UASB-R1 and 0.74 L/d for UASB-R2, Table 2-2) and the average volume of methane was not statistically different between the two reactors ($p>0.05$). Methane produced in the liquid phase, reaching 1.64 L/d for UASB-R1 and 1.34 L/d for UASB-R2 at 13°C and 2.93 L/d for UASB-R1 and 2.14 L/d for UASB-R2 at 19°C (Table 2-2), was similar between the two reactors at the same temperature ($p>0.05$). The dissolved methane represented the highest fraction of the total methane produced, attaining over 80% in winter and 70% in summer in both reactors. At 13°C, the ratio of dissolved methane over total methane reached 89% in UASB-R1 and 80% in UASB-R2, while, at 19°C, it reduced to 80% in UASB-R1 and 74% in UASB-R2 (Table 2-2). Similarly, other studies on anaerobic treatment of municipal wastewater observed that a great fraction of the methane produced is dissolved and consequently lost in the effluent. Souza et al. (2011) measured between 36-41% dissolved methane (in relation to the total) in pilot-scale and demonstration-scale UASB reactors operated at 25°C. Giménez et al. (2012) observed that in a semi-industrial anaerobic membrane bioreactor the dissolved methane accounted for 46% of the total methane production at 21°C. As expected, a higher fraction of 85% was found in a pilot-scale UASB reactor working at 10°C (Xu et al., 2018). The presence of dissolved methane in the anaerobic effluent poses different challenges, especially at lower temperatures since the solubility increases. Firstly, its removal is mandatory if the effluent is to be discharged in a closed conduit to avoid the creation of a potentially explosive atmosphere (Cookney et al., 2016). Moreover, the loss of a substantial part of the total methane produced may jeopardise the net energy efficiency of the anaerobic system (Smith et al., 2015). Finally, in an effort to mitigate climate change and achieve the Net Zero carbon emissions target from the water sector, the dissolved methane fraction needs to be removed to avoid unwanted greenhouse gas emissions from the effluent, which constitutes the biggest contributor to the global warming impact of an anaerobic treatment (Smith et al., 2014).

The total methane yields were approximately 0.10 $L_{CH4}/g_{COD,rem}$ at 13°C and 0.16 $L_{CH4}/g_{COD,rem}$ at 19°C and these values were similar to previous studies. Ribera-Pi et al. (2020) achieved 0.13 $L_{CH4}/g_{COD,rem}$ at around 10°C, operating a 70 L flocculent UASB reactor with HRT of 8 h, while Serrano León et al. (2018) obtained 0.16 $L_{CH4}/g_{COD,rem}$ in a 11 L UASB reactor operated with HRT of 6 hours at 19°C. Despite differences in total methane produced in the two reactors and COD and sulphate removals, the methane yields were not statistically different ($p>0.05$) both at 13°C and at 19°C (Table 2-2).

Table 2-2 Methane production in gas and liquid phases and methane yields (for gas, dissolved and total methane) in UASB-R1 and UASB-R2 over two different periods, at the average temperature of 13°C (days 101-327) and 19°C (days 328-455). The star (*) indicates significantly different methane production between the two UASB reactors at the same temperature.

	13°C		***19°C***	
	UASB-R1	***UASB-R2***	***UASB-R1***	***UASB-R2***
Gas CH_4 (L/d)	0.19±0.11*	0.32±0.25*	0.76±0.43	0.74±0.29
Gas CH_4 yield ($L_{CH4}/g_{COD,rem}$)	0.010±0.004	0.017±0.009	0.030±0.011	0.039±0.018
Dissolved CH_4 (L/d)	1.64±0.77	1.34±0.64	2.93±1.01	2.14±0.52
Dissolved CH_4 yield ($L_{CH4}/g_{COD,rem}$)	0.092±0.046	0.081±0.038	0.125±0.033	0.138±0.053
Total CH_4 yield ($L_{CH4}/g_{COD,rem}$)	0.10±0.05	0.10±0.04	0.15±0.04	0.17±0.05
Dissolved CH_4/Total CH_4 (%)	89±5	80±13	80±7	74±9

Overall, the methane content in the biogas was lower than in similar studies. This was, in average, between 24-35% for UASB-R1 and 35-45% for UASB-R2. Gouveia et al. (2015) reported a methane content of 80-83% in a pilot-scale UASB reactor treating municipal wastewater at 18°C, while Hejnic et al. (2016) observed up to 63% in a laboratory UASB reactor operated at 15°C. However, a similar biogas composition (45.7% methane) was measured by Giménez et al. (2012) in a large-scale anaerobic membrane bioreactor at 21°C. The content of carbon dioxide in the biogas was always below 5% during this study.

Further investigation led to the identification of correlations between biogas composition and organic concentration of the influent wastewater. More specifically, previous studies have shown that biogas from the anaerobic treatment of municipal wastewater presents high concentrations of nitrogen when the influent COD concentration is low (Cakir & Stenstrom, 2005). This is due to dissolved nitrogen being stripped out into the gas phase within the anaerobic reactor (Noyola et al., 2006). Since at constant pressure the gas-liquid equilibrium is dependent on temperature, temperature and, in particular, the temperature gradient between the influent and the reactor can affect the biogas composition and its nitrogen fraction (Giménez et al., 2012). However, at higher wastewater strengths, the production of methane and carbon dioxide is enough to reduce the nitrogen concentration in the gas. According to the biogas composition model by Cakir & Stenstrom (2005), developed for a temperature of 20°C, an increment of influent COD from 100 mg/L to 200 mg/L would decrease the nitrogen content in the biogas from around 55% to 38% and increase the methane from around 35% to over 50%.

Figure 2-3 shows the methane concentration in the biogas correlated to the influent COD concentration for UASB-R1 and UASB-R2 compared with literature data of UASB reactors treating municipal wastewater at low temperature (Singh & Viraraghavan, 2004; Souza et al., 2011; Syutsubo et al., 2011; Bandara et al., 2012; Gouveia et al., 2015; Hejnic et al., 2016; Serrano León et al., 2018; Cecconet et al., 2022) and simulation data (extracted from the Cakir & Stenstrom (2005) model). Data from UASB-R1 and UASB-R2 extend the dataset reported in Figure 2-3, as the influent concentration of COD from literature was observed to be higher than the one treated in this study. Although the data points do not completely overlap with the simulated data, the statistical tests, completed on the trends, suggest that the slopes were not statistically different ($p>0.05$). These observations hold for all data depicted in Figure 2-3 with influent COD concentrations between 60-600 mg/L, implying an influence of the strength of wastewater on the concentration of methane in the gas phase, as previously reported.

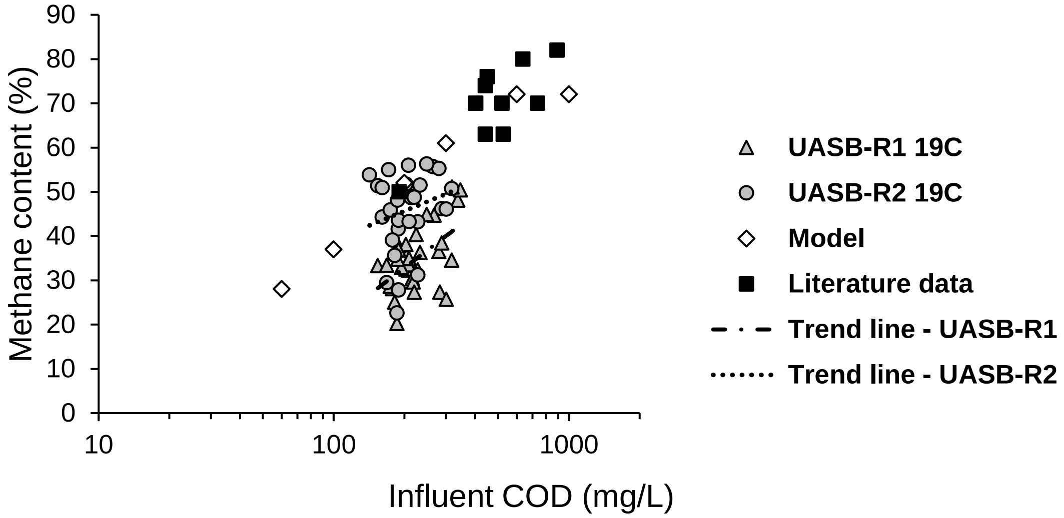

Figure 2-3 Relationship between methane content in the biogas of the two UASB reactors and influent COD concentrations during the study, compared with literature data and simulation data extracted from Cakir & Stenstrom (2005).

Taking into consideration the initial organic concentration in the anaerobic reactor is important in terms of the energy balance since the presence of nitrogen in biogas constitutes a challenge for the recovery and utilisation of the gas for energy purposes. Moreover, the continuous monitoring of methane content in the biogas with the in-line gas sensors, highlighted that the release of methane was not constant, and it happened through peaks followed by low values. This was due to the release of big gas bubbles (also visually observed), instead of continuous gas release. A possible explanation could be that as gas was produced, it fed a few growing bubbles that burst through once the buoyancy force had become sufficient to overcome the weight of the sludge bed above. In a UASB reactor the upflow movement of gas bubbles, the influent liquid flow and sometimes the effluent recirculation, constitute the self-mixing necessary for adequate contact between biomass and organic matter (Chernicharo, 2007). To avoid dead zones in the digestion compartment, especially during municipal wastewater treatment, which could lead to insufficient biogas production, the recirculation could happen exclusively within the digestion chamber. However, during this study, despite an upflow velocity of 0.8 m/h, we observed the formation of preferential pathways, probably due to the reactors' design, which caused the entrapment of gas bubbles and their subsequent uneven release. Furthermore, hydraulic short circuits could have an impact on mass transfer and substrate utilisation rates, which could affect hydrolysis of particulate matter.

2.3.3 Percentages of hydrolysis and methanogenesis

Calculations of hydrolysis and methanogenesis were carried out to understand the impact of adaptation of the seed sludge on the different stages of the anaerobic process. The percentages of hydrolysis for UASB-R1 and UASB-R2 were 20.8% and 17.8% at 13°C and 33.6% and 28.9% at 19°C, respectively. Therefore, hydrolysis was influenced by temperature ($p<0.05$), with temperatures of 19°C promoting an increase of around 12%. On the other side, statistical tests did not highlight any significant difference between the two different types of sludge on the solids hydrolysis inside the anaerobic reactor in each of the two periods. The higher hydrolytic percentages observed in UASB-R1 at 19°C (33.6%) could be related to greater TSS removal of 74% during this period (Figure 2-2). Methanogenesis for UASB-R1 and UASB-R2 was 18.8% and 17.3% at 13°C and 32.2% and 30.2% at 19°C, respectively. Similar to hydrolysis, an increase in temperature led to higher methanogenesis percentages, roughly an increment of 13%. As already observed in methane yields and hydrolysis, the two reactors achieved statistically similar methanogenesis percentages at the same temperature ($p>0.05$). While not many studies investigated the variation of hydrolysis and methanogenesis with temperature in UASB reactors, Halalsheh et al. (2005) reported for both parameters an increase of around 30% from 18 to 25°C.

Comparing the results of this study with literature data on UASB and UASB-configured reactors, it was observed that methanogenesis was linearly correlated to hydrolysis (Figure 2-4) (Mahmoud et al., 2004; Halalsheh et al., 2005; Al-Shayah & Mahmoud, 2008; Al-Jamal & Mahmoud, 2009; Mahmoud & Van Lier, 2011). In this study, the mean values of hydrolysis and methanogenesis for the same reactor at a certain temperature were not statistically different ($p>0.05$). As hydrolysis is rate-limiting, anything the impacts hydrolysis will impact methanogenesis. This is especially true for low-strength wastewater, where a significant proportion of the COD is in particulate form.

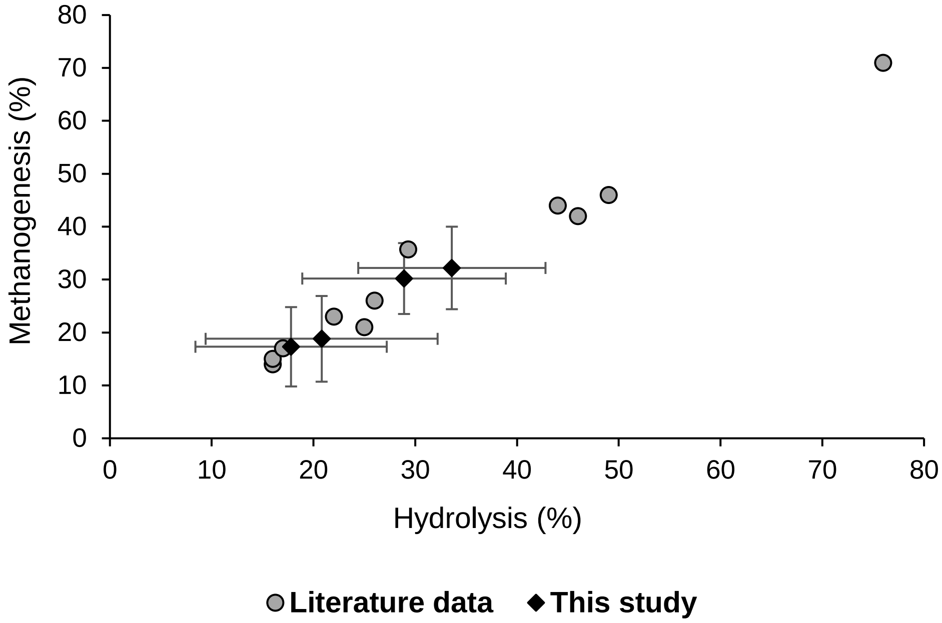

Figure 2-4 Average values of hydrolysis and methanogenesis for the two UASB reactors with standard deviation bars and comparison with previous studies.

Halalsheh et al. (2005) operated a single-stage and a two-stage UASB reactor both in winter and in summer, observing similar percentages for hydrolysis and methanogenesis, regardless of temperature, and higher percentages in summer. They observed that the disparities in the results of the two UASB configurations, were related to different hydraulic retention times; with an increase in HRT from 8-10 to 23-27 hours, solids removal and methane production increased and hydrolysis at 18°C raised from 16% to 46%. The importance of improved solids retention was also highlighted by Mahmoud et al. (2004) and Ribera-Pi et al.

(2020), who both showed higher COD conversion and solids hydrolysis when the UASB reactor was followed by a post-treatment with a recirculation of solids to the UASB reactor. Moreover, Ribera-Pi et al. (2020) hypothesized that, due to the particles size and its filtration properties, flocculent sludge showed higher efficiency of solids hydrolysis in terms of mass of solids hydrolysed per volume and time compared to granular sludge. However, lower density and settling ability could lead to biomass washout and an overall loss of activity, which could impact the hydrolysis in the reactor.

2.3.4 Evolution of sludge properties in time

The particle size distribution (PSD) of the sludge was measured at the beginning of the study and after 10 months of operation in order to evaluate the evolution of the sludge particles in time. Overall, the size at which researchers define sludge particles as anaerobic granules is not uniform. Show et al. (2004) defined granules as particles with diameter larger than 500 µm, Toja Ortega et al. (2021) used 200 µm to separate flocs from granules. However, other studies have considered granules particles with diameters as small as 100 µm, as described by Bhunia & Ghangrekar (2007). In our study, the same criteria as Toja Ortega et al. (2021) was adopted to distinguish between flocs (<200 µm) and granules (>200 µm). The inoculum of UASB-R1 was primarily (61%) constituted of granular particles >1000 µm. On the other hand, in the inoculum of UASB-R2, 41% of particles were flocs with sizes between 50 and 200 µm. However, after 10 months of operation, the fraction 50-200 µm in UASB-R1 increased from 8% to 39% becoming equivalent to the fraction of particles >200 µm (41%). The PSD of the sludge in UASB-R2 remained fairly stable during the study, with a slight decline in the fraction of particles sized >200 µm from 39% to 35% (Table 2-3). Therefore, the two sludges presented a very similar particle size distribution after 10 months of operation.

Table 2-3 Particle size distribution of sludge in UASB-R1 and UASB-R2 at the beginning and after 10 months of operation described as volumetric distribution with particle size (d) ranges from <50 to >1000 μm.

		d (μm)				
		<50	*50-200*	*200-500*	*500-1000*	*>1000*
Volume (%)	***UASB-R1 inoculum***	15	8	1	15	61
	UASB-R2 inoculum	20	41	17	14	8
	UASB-R1 - 10 months	20	39	15	13	13
	UASB-R2 - 10 months	24	41	16	13	6

The Sauter mean diameter of the sludge particles, calculated from the particle size distribution, was used as an approximation of the characteristic size of the sludge samples and correlated to the hydrolysis percentage, as it is normally used for mass transfer considerations (Haramkar et al., 2021). Hydrolysis is affected by mass transfer and therefore by the available contact area. Smaller particles have higher specific surface area, hence providing a greater area available for particle capture. Results from three sludge samples, collected during the 10th month of operation, revealed similar average Sauter mean diameter (63 μm for UASB-R1 and 52 μm for UASB-R2) and average hydrolysis (27.3% for UASB-R1 and 29.1% for UASB-R2). Moreover, as reported in Figure 2-5, the hydrolysis percentage increased with a decrease in sludge particle size (R^2=0.73 for UASB-R1 and R^2=0.82 for UASB-R2). Therefore, the similar particle size distribution achieved in both reactors led to a similar chance of solids retention within the bed and possibly to the non-significantly different hydrolysis percentages observed during the trial.

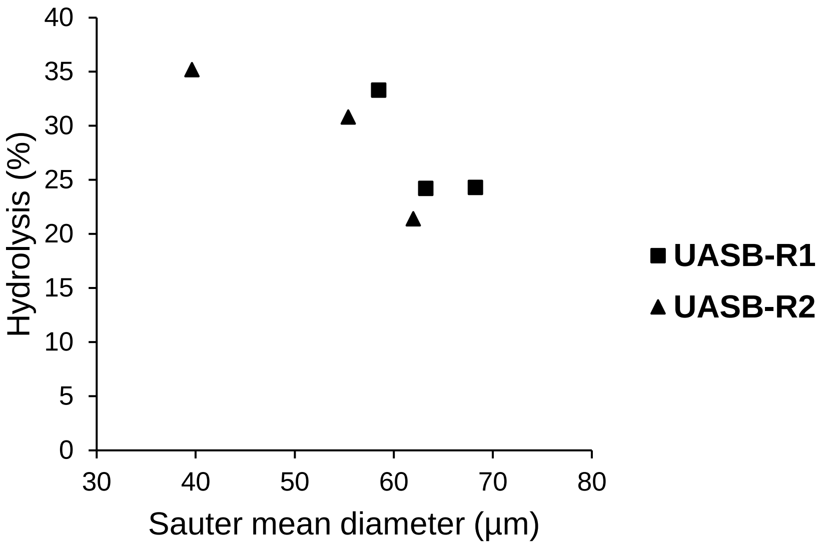

Figure 2-5 Relationship between Sauter mean diameter of the sludge particles and hydrolysis in UASB-R1 and UASB-R2 during the 10th month of operation.

Ultimately, the bacterial diversity of the sludge was analysed to identify differences in the two inocula and variations in time (Figure 2-6). It was revealed that the mesophilic industrial sludge in UASB-R1, coming from food processing wastewater treatment, presented a predominance of the order *Lactobacillales* (*Bacilli* class), also known as lactic acid bacteria, which can grow anaerobically and produce lactic acid as the main end-product of carbohydrates fermentation (Aguirre & Collins, 1993). A similar microbial composition was observed by Xing et al. (2010) in the sludge from an expanded granular sludge bed reactor treating synthetic brewery wastewater at 20°C. On the other hand, the adapted sludge in UASB-R2 was largely composed of *Eubacteriales* or *Clostridiales*, an order of bacteria belonging to the *Clostridia* class. *Clostridia* are part of the same phylum (*Firmicutes*) as *Bacilli*, nevertheless, they lack aerobic respiration abilities and are obligate anaerobes involved in organic matter degradation and fermentation (Fykse et al., 2016). *Clostridiales* was observed as the predominant order of *Firmicutes* also in the previous studies in which the sludge of UASB-R2 was utilised (Ribera-Pi et al., 2020). After 10 months of operation, both reactors experienced a shift in the bacterial community as *Proteobacteria* became the dominant phylum. Furthermore, contrary to the inoculum, the most abundant order (*Campylobacterales*) was the same for both types of sludge. The relative abundances in Figure 2-6 showed that, the bacterial community in the two types of sludge, developed after 10 months of treating municipal wastewater below 20°C, was not dependent on the origin and acclimation of the inoculum, however more samples could be necessary to confirm the evolution of the bacterial diversity in time and further analysis is necessary to determine the archaeal composition.

Regarding the archaeal population in anaerobic reactors treating municipal wastewater at low temperature, literature presents conflicting results. Some studies reported that, at low temperature, hydrogenotrophic methanogenesis may be more significant than acetoclastic methanogenesis, possibly due to an increase in hydrogen solubility and improved thermodynamic conditions for hydrogenotrophic methane formation (Connaughton et al., 2006; McKeown et al., 2009; O'Reilly et al., 2009). However, other studies observed an increase in the abundance of acetoclastic methanogens while temperature decreased (Ozgun et al., 2015; Ribera-Pi et al., 2020). This could be due to the fact that hydrogen is produced during fermentation and, since hydrolysis is reduced at lower temperatures, the limited production of hydrogen could cause a substrate limitation for hydrogenotrophic methanogenesis. Ozgun et al. (2015) found that with a reduction of temperature from 25°C to 15°C the abundance of *Methanosaeta*, acetate-utilizing methanogens, increased. Accordingly, Ribera-Pi et al. (2020), operating UASB-configured reactors at around 10°C, reported that *Methanosaeta* was the most abundant genus of methanogens. Therefore, despite hydrogenotrophic methanogenesis requiring less energy than acetoclastic methanogenesis, a coexistence of both pathways should be expected in anaerobic reactors treating municipal wastewater at low temperatures.

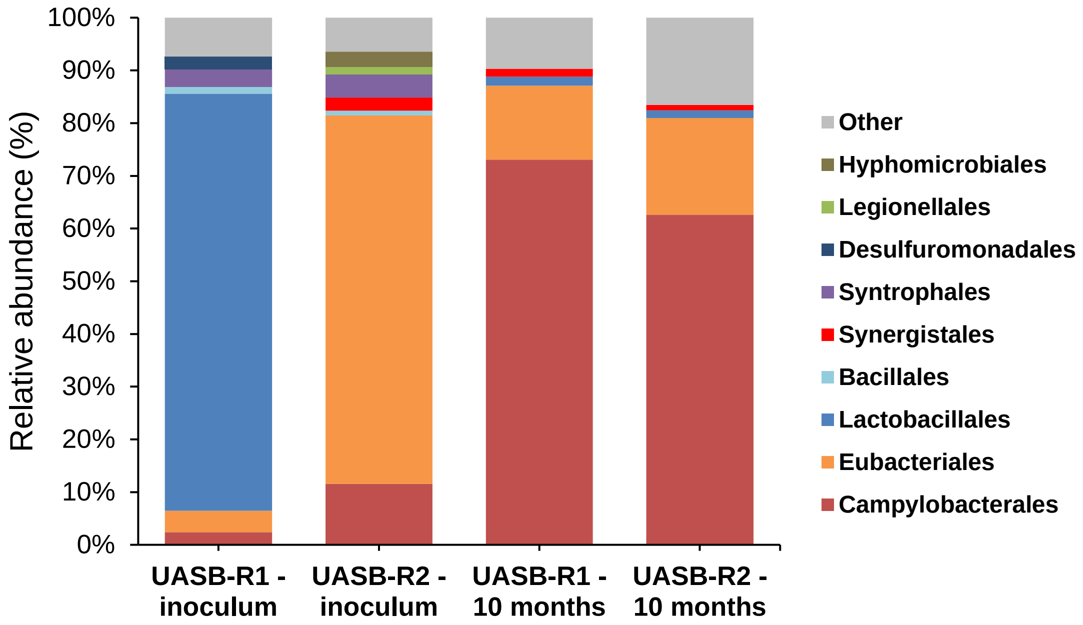

Figure 2-6 Bacterial diversity of sludge samples from the two pilot-scale UASB reactors (inoculum and after 10 months of operation), illustrated by orders (relative abundance cutoff of 1%).

2.4 Conclusions

Two pilot-scale UASB reactors were operated at low temperature (<20°C) to treat settled municipal wastewater in order to understand the effect of two different types of inoculum on the hydrolytic and methanogenic stage, solids retention and effluent quality. This study showed that the UASB reactor proved to be a robust technology, able to keep consistent removal efficiencies (around 50% COD removal and 60% TSS removal), despite variations in influent COD and TSS, and regardless of inoculum type and acclimation. Methane was mostly found dissolved in the effluent, reaching over 70% of the total production even at 19°C, and methane yields were not statistically different between the two reactors.

In both UASB reactors, methanogenesis was found to be linearly correlated to hydrolytic percentages, which in turn were affected by temperature. An increase in average temperature from 13 to 19°C caused a rise in the percentage values of around 11-13%. Furthermore, hydrolysis was linked to the particle size distribution of the sludge bed, observing that the percentage of hydrolysis was inversely correlated to the Sauter mean diameter of the sludge particles.

During the study, the two seed sludges developed similar bacterial diversity and physical properties, showing a decrease in particle size. As smaller particles tend to be washed out from the reactor due to lower settling abilities, strategies to enhance solids retention at low temperature (e.g. membrane post-treatment, more lamella settlers or a different diameter/height ratio in the UASB reactor) could be favourable to improve hydrolysis and achieve higher methane production and an effluent quality suitable for discharge and/or reuse. Moreover, special attention is also required for the recovery of dissolved methane, to prevent greenhouse gas emissions and improve the energy balance of the process.

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3 Hydrolytic enzyme activity in high-rate anaerobic reactors treating municipal wastewater in temperate climates

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Abstract

Hydrolysis of particulate matter is considered the rate-limiting step in anaerobic treatment of municipal wastewater in temperate climates. In theory, low temperatures affect the interaction between hydrolytic enzymes and complex substrates, slowing down the utilisation kinetics, although this process remains to be understood. In this study, hydrolytic enzyme activities (β-glucosidase, protease, lipase) were evaluated in two pilot-scale upflow anaerobic sludge blanket (UASB) reactors. The two reactors were inoculated with two different types of sludge (heterogeneous adapted sludge and mesophilic granular non-adapted sludge) and, after a period of stable operation, converted to anaerobic membrane bioreactors (AnMBRs). The latter aimed at investigating how the presence of an ultrafiltration membrane, which enhances solids retention, impacted the activity of hydrolytic enzymes. The two reactors presented statistically similar total methane production (2.3-2.7 L/d for UASB reactors and 5.3-5.7 L/d for AnMBRs) and rate of solids hydrolysed (0.82-0.86 g/(L·d) for UASB reactors and 1.57-1.87 g/(L·d) for AnMBRs), regardless of the seed sludge. However, a significant difference was observed between UASB reactor and AnMBR with the same sludge. For all three enzymes the highest specific activity was measured at 37°C, excluding the predominance of psychrophilic enzymes. Nevertheless, the Michaelis-Menten constant (K_m) indicated high affinity of the enzymes for the substrate at the operational temperature of the reactors (15-20°C). Further to this, the hydrolytic enzymes presented generally higher affinities in the AnMBR configuration compared with the UASB reactors. This study

demonstrates, for the first time, that different seed sludges can equally adapt, as hydrolytic enzymatic affinity to the substrate reached similar values in the two reactors at the operational temperature of 15-20°C. Most importantly, combining a UASB reactor with membrane ultrafiltration does enhance the potential for hydrolysis underpinned by a favourable Michaelis-Menten constant of the hydrolytic enzymes.

Keywords: anaerobic membrane bioreactor, glucosidase, lipase, low temperature, protease, UASB reactor

3.1 Introduction

The considerable energy demand and production of excess sludge of aerobic processes have encouraged the implementation of high-rate anaerobic reactors, especially the upflow anaerobic sludge blanket (UASB) reactor, as an alternative for municipal wastewater treatment. Interesting advantages of anaerobic wastewater treatment include energy savings, due to lack of aeration and biogas recovery, lower sludge production and the possibility of recovering nutrients from the effluent (Robles et al., 2021).

Municipal wastewater is characterised by low-strength streams, rich in particulate organic matter, such as carbohydrates, proteins and lipids. Anaerobic degradation is initiated by fermentative bacteria, whose hydrolytic enzymes break down these complex polymeric substrates into soluble molecules. In temperate climates (<20°C) hydrolysis has been identified as the rate-limiting step, causing accumulation of non- or partially-hydrolysed solids in the reactor (Ribera-Pi et al., 2020). Low temperatures can reduce diffusion rates of the substrates and increase the viscosity of the medium, leading to mass transfer limitations (Dev et al., 2019). These changes result in an increase of the kinetic requirements to overcome the activation energy barrier and a decline of the reactions rates (Dhaulaniya et al., 2019).

Psychrophiles are microorganisms inhabiting cold environments with optimal growth at 15°C (De Maayer et al., 2014). To maintain an appropriate enzymatic rate and sustain essential cellular processes, psychrophiles synthetise psychrophilic enzymes, which have developed survival mechanisms to overcome the low-temperature drawbacks (Margesin et al., 2007). To reduce the activation energy, these enzymes decrease the number of enthalpy-driven interactions that need to be disrupted during activation, leading to an increase in flexibility of the active site of the enzyme, at the expense of activity at higher temperatures. This causes a lower stability of the protein structure and a looser binding of the substrate in psychrophilic enzymes, resulting in a lower substrate affinity compared to that of mesophilic counterparts (Collins & Margesin, 2019).

Due to the higher activity at low temperatures, the use of a psychrophilic inoculum could be a valid solution to sustain hydrolysis in UASB reactors operated in temperate climates. However, the availability of this type of seed sludge is limited since it can only be sourced in extreme environments difficult to access. Moreover, temperate climates are susceptible to seasonal temperature variations, which would affect the physical properties of wastewater and could negatively impact the psychrophilic enzymes, due to their structural instability.

Another possibility is to use a mesophilic inoculum and implement different strategies to improve the kinetic rates at low temperature. Hydrolysis is a mass transfer limited process, therefore increasing the contact between the substrate and the sludge would facilitate the increase of hydrolytic efficiencies, reducing the accumulation of solids. Several studies have reported a successful use of a mesophilic inoculum at low temperatures, in particular in anaerobic membrane bioreactors (AnMBRs) (Gouveia et al., 2015; Hejnic et al., 2016; Ribera-Pi et al., 2020; Wang et al., 2019).

AnMBRs are high-rate anaerobic reactors, usually UASB-configured, in which a membrane is placed after or inside the biological reactor and allows the complete retention of solids and biomass, increasing the sludge retention time. Ribera-Pi et al., (2020) observed that the rate of solids hydrolysed increased in an AnMBR compared to a UASB reactor and overall AnMBRs were able to improve the organic removal efficiencies of UASB reactors (Ribera-Pi et al., 2020; Wang et al., 2019).

While these studies from literature have shown that the anaerobic treatment of municipal wastewater is feasible in temperate climates and even without a psychrophilic inoculum, the hydrolytic mechanisms and adaptation to low temperatures are still unclear. Only few studies have reported activities of hydrolytic enzymes in anaerobic reactors treating wastewater (Anupama et al., 2008; Berrio-Restrepo et al., 2017; Petropoulos et al., 2018). However, the variation with temperature of enzymatic activity and substrate affinity in high-rate anaerobic reactors for municipal wastewater treatment has not been measured yet. Therefore, in this study, the activity of the sludge employed in two UASB reactors, inoculated with different types of sludge, and then converted in AnMBRs, to promote hydrolysis, has been investigated at different temperatures to advance the current knowledge on the adaptation of anaerobic hydrolytic enzymes to low temperatures.

3.2 Materials and methods

3.2.1 Description and operation of the pilot-scale plants

Two 70 L cylindrical UASB reactors were operated in parallel and fed with the same settled wastewater, pumped from the bottom with peristaltic pumps (520Du, Watson Marlow, Falmouth, UK) to achieve a hydraulic retention time (HRT) of 8 hours. Each reactor was inoculated with 11 L of sludge derived from a different source. The first reactor, UASB-R1, was seeded with mesophilic granular sludge from food processing wastewater treatment, while the second reactor, UASB-R2, was inoculated with sludge coming from municipal anaerobic digestion and pulp and paper processing wastewater treatment and had been employed for over five years in the same reactor aimed at anaerobic wastewater treatment in the UK, making it already adapted to the studied conditions (Ribera-Pi et al., 2020; Wang et al., 2018a). Three lamella settlers were placed along the height of each reactor and a three-phase separator on top facilitated the collection of effluent and biogas. The effluent was then recirculated using peristaltic pumps (620Du, Watson Marlow, Falmouth, UK) to achieve an upflow velocity of 0.8 m/h.

The two UASB reactors were subsequently converted into AnMBRs with the addition of an external submerged hollow-fibre ultrafiltration membrane tank (25 L). For each AnMBR, the effluent overflowed into the membrane tank and the retentate was recycled back to the UASB reactor. The upflow velocity was kept at 0.8 m/h, while the HRT of both UASB reactors varied between 4-6 h and was constant at 1 h for both membrane tanks. Permeate was collected with a peristaltic pump (520Du, Watson Marlow, Falmouth, UK). AnMBR-R1 (originally UASB-R1) was connected to a mini C-MEM cartridge (SFC Umwelttechnik GmbH, Slazburg, Austria) with high density polyethylene fibres with pore size of 0.02 µm and membrane area of 1.4 m^2. AnMBR-R2 (originally UASB-R2) was fitted with a Zenon ZW-10 module (GE Water & Process Technologies, Oakville, Canada) with polyvinylidene fluoride (PVDF) fibres with pore size of 0.04 μm and membrane area of 0.93 m^2. Pressure transducers (PX319-030A5V, Omega, Manchester, UK) and a multichannel data logger (ADC-20, Pico Technology, St Neots, UK) were used to record the transmembrane pressure. AnMBR-R1 was equipped with a permeate tank for backwashing. Nitrogen-enriched air was used for gas sparging (NG6, Noblegen gas generator, Gateshead, UK), which was regulated by a solenoid valve (Type 6014, Burkert, Ingelfingen, Germany) connected to a timer relay (PL2R1, Crouzet, Valence, France). The two membranes were operated in pseudo dead-end filtration mode, alternating filtration with gas sparging and relaxation or backwashing. AnMBR-R1 operation included filtration for 5 min and backwashing with gas sparging for 30 s, as suggested by the supplier, while AnMBR-R2 employed cycles of filtration (9 min) followed by relaxation and gas sparging (1 min), according to the optimal operational conditions identified for this membrane module by Wang et al. (2018b). The use of different membrane modules or filtration cycles is not expected to influence the tests as the purpose of this study is to better understand the adaptation to low temperature of hydrolytic enzymes in UASB reactors and investigate how the activity is affected by the presence of an ultrafiltration membrane.

3.2.2 Wastewater and biogas analysis

Before the enzymatic tests, the UASB reactors were in operation for over 10 months at temperatures between 6-20°C, while the AnMBRs were running for more than 1 month at around 20°C, achieving in both cases stable removals. The data here analysed for effluent quality and methane production span 3 months for UASB reactors and 2 months for AnMBRs in steady-state operation, during which the samples for enzymatic analysis were collected. Wastewater samples of influent, UASB effluent and AnMBR effluent were analysed multiple times a week in duplicates. Total suspended solids (TSS), volatile suspended solids (VSS) and biochemical oxygen demand (BOD) were measured according to Standard Methods (APHA, 2012). Total and soluble chemical oxygen demand (COD, sCOD) and sulphate (SO_4) were analysed with cell test kits (Merck KGaA, Darmstadt, Germany). Wastewater samples for sCOD and SO_4 were filtered at 0.45 µm with retention membrane filters (Whatman, Cytiva, Little Chalfont, UK). Temperature and pH were measured on site with a portable Multi Meter (HQ40D, Hach Lange GmbH, Düsseldorf, Germany).

The total methane produced was calculated combining gaseous and dissolved methane. The production of biogas was measured with gas meters (TG 0.5, Ritter, Bochum, Germany) and the composition was determined with in-line CO_2 and CH_4 sensors (BCP-CO_2 and BCP-CH_4, BlueSens gas sensor GmbH, Herten, Germany). Dissolved methane in the effluent of UASB reactors was measured following the method described by Cookney et al. (2016) and the concentration was calculated according to Equation (3-1) (Souza et al., 2011):

$$[CH_4]_{diss} = \frac{([\%CH_4]_{gas}/100) \cdot [\rho \cdot V_{gas} + (P_T - P_V) \cdot K_H \cdot V_{liq}]}{V_{liq}} \quad \textbf{(3-1)}$$

where, $[CH_4]_{diss}$ is the concentration of dissolved methane (mg/L), $[\%CH_4]_{gas}$ is the concentration of methane in the headspace (%), ρ is the density of methane (651 mg/L at 20°C and 0.988 atm), V_{gas} is the volume of gas in vial (mL), P_T is the atmospheric pressure (0.988 atm at Cranfield, UK), P_V is the water vapour pressure (0.023 atm at 20°C), K_H is the Henry's constant for methane (24.9 mg L^{-1} atm^{-1} at 20°C) and V_{liq} is the volume of liquid in vial (mL). For AnMBRs, since the membrane tanks were not sealed and a direct measurement would have not been representative, the dissolved methane was estimated using Henry's Law as described in Equation (3-2):

$$\frac{C_g}{C_s} = H_u \quad \textbf{(3-2)}$$

where, C_g is the concentration of methane in the gas phase, C_s is the saturation concentration of methane in the liquid phase and H_u is the dimensionless Henry's Law constant, depending on temperature. Hydrolysis percentages were calculated according to Equation (3-3) (Elmitwalli et al., 2002):

$$Hydrolysis\ (\%) = 100 \cdot \frac{CH_4\ as\ COD + sCOD_{out} - sCOD_{in}}{COD_{in} - sCOD_{in}} \quad \textbf{(3-3)}$$

where, the subscripts “in” and “out” indicate the influent and effluent wastewater, respectively, and 0.25 L_{CH4}/g_{COD} was used for the conversion between methane and COD. The rate of solids hydrolysed in the reactor was expressed as the load of solids removed per unit of biological reactor volume (70 L), multiplied by the hydrolysis percentage (Ribera-Pi et al., 2020). Statistical tests were performed to evaluate first outliers, then normality (Shapiro-Wilk test). Welch’s t-test was applied for analysis of the means, using a 95% confidence limit for wastewater and methane data (3.3.2) and a 85% confidence limit for enzymatic data (3.3.3 and 3.3.4), where not indicated otherwise.

3.2.3 Microbial community

The microbiome of the sludge was analysed to determine the bacterial differences in UASB reactors and AnMBRs. Sludge samples, one for each reactor (UASB-R1, UASB-R2, AnMBR-R1, AnMBR-R2), used for enzymatic analysis, were kept in the cold room and shipped to an external laboratory for analysis. DNA extraction was performed with Qiagen DNeasy Powersoil Pro Kit, according to kit’s instructions. PCR analysis and sequencing were carried out with the Nanopore 16S Barcoding Kit (SQK-RAB204) and the Nanopore MinION Flowcell (R9.4.1), respectively. Despite it was possible to analyse only one sludge sample for each reactor, the use of a larger sample size for microbial analysis would increase the reliability of the results, reducing the chance of random error in sample collection and analysis.

3.2.4 Enzyme assays

Enzymatic tests were performed on sludge samples from UASB-R1, UASB-R2, AnMBR-R1 and AnMBR-R2, collected from the sampling point at the bottom of the UASB reactors. For each enzyme and protein assay, three sludge samples per reactor, collected over the course of maximum three weeks to limit microbiome and temperature variability, were analysed in triplicates. Samples were sonicated for 15 min at 30 kHz at room temperature and then centrifuged at 5000 rpm for 10 min. The supernatant was collected and analysed for protein concentration and enzymatic activity.

The protein concentration was determined with the Bradford method (Quick Start™ Bradford Protein Assay, Bio-Rad Laboratories Ltd., Watford, UK), using gamma-globulin as standard (0.125-2 mg/mL) in multiwell microplates. The absorbance of the reaction well at 595 nm, measured with a microplate reader (Infinite M200 Pro, Tecan, Austria), was corrected based on sample and reagent blanks.

To investigate the degradation of the main particulate organic components of municipal wastewater (carbohydrates, lipids and proteins), the enzymes considered in this study were β-glucosidase, lipase and protease, as they are regarded as equally important in the hydrolytic process (Whiteley & Lee, 2006). Colorimetric assays, measured with a microplate reader (Infinite M200 Pro, Tecan, Austria), were used to determine specific activity and kinetics constants for each enzyme.

Specific activity, defined as the concentration of product released in a certain amount of time divided by the protein concentration, was measured after 2 hours of incubation using a single substrate concentration and at three different temperatures (4, 20, 37°C). Samples and substrate controls were used to correct the final values. A first-order kinetics (Michaelis-Menten model) was used to describe the rate of enzymatic hydrolysis in this study (Whiteley & Lee, 2006). The Michaelis-Menten equation was solved using a non-linear regression in order to determine, the Michaelis-Menten constant (K_m), which provides an indication of substrate affinity. As for specific activity values, controls were run to assess colour development in sample and substrate blanks. The assays were carried out for β-glucosidase and lipase at different temperatures (4, 7, 15, 20, 37°C). It was not possible to measure K_m for protease as, when stopping the reaction after 15 min, the colour developed in the sample blank was too similar to the colour of the reaction wells. Table 3-1 reported a summary of the assays carried out in this study.

Table 3-1 Summary of enzymatic tests carried out in this study on sludge samples from pilot-scale UASB reactors and AnMBRs to evaluate specific activity and substrate affinity of β-glucosidase, lipase and protease.

Enzyme	*Substrate*	*Substrate concentration (mM)*	*Incubation time (min)*	*Incubation temperature (°C)*
β-glucosidase	4-nitrophenyl β-D-glucopyranoside	0.5-5	15	4, 7, 15, 20, 37
	4-nitrophenyl β-D-glucopyranoside	2	120	4, 20, 37
Lipase	4-nitrophenyl palmitate	0.1-0.5	20	4, 7, 15, 20, 37
	4-nitrophenyl palmitate	0.2	120	4, 20, 37
Protease	Casein	2 (mg/mL)	120	4, 20, 37

The assay to determine β-glucosidase kinetics parameters used 4-nitrophenyl β-D-glucopyranoside (N7006, Sigma-Aldrich, UK) as substrate, in the range 0.5-5 mM, and 4-nitrophenol (241326, Sigma-Aldrich, UK), measured at 405 nm, as standard. Sodium carbonate (1 M) was used as stopping reagent and sodium chloride (0.14 M) as buffer (Kreutz et al., 2016). Sample and substrate were placed with a ratio 1:1 (0.5 mL) in a microcentrifuge tube and incubated at the temperatures of interest. The reaction was stopped after 15 min with 0.5 mL of stopping reagent and the tubes were centrifuged according to Kreutz et al. (2016). The supernatant was transferred to a microplate (250 µL) to read the absorbance of the released product. The assay to determine specific activity followed the same procedure, stopping the reaction after 2 hours and using a concentration of substrate of 2 mM.

Lipase kinetics parameters were determined using 4-nitrophenyl palmitate (N2752, Sigma-Aldrich, UK) as substrate with concentrations ranging from 0.1-

0.5 mM. Stock and working solutions were prepared according to Gessesse et al. (2003). The assay was carried out at the different temperatures in a microplate, using 25 µL of sample and 225 µL of substrate (1:9), measuring the release of 4-nitrophenol (241326, Sigma-Aldrich, UK) at 405 nm after 20 min. The assay to determine specific activity followed the same procedure, using 0.2 mM as substrate concentration and measuring the absorbance after 2 hours.

Protease assay was performed only for the determination of specific activity at different temperatures after a 2-h incubation, according to the methodology developed by Sigma-Aldrich for protease colorimetric detection (PC0100, Sigma-Aldrich, UK) using casein (2 mg/mL) as substrate. Tyrosine, which is a product of the cleavage of casein, was used as standard and its presence was revealed with the Folin and Ciocalteu's reagent at 660 nm, after the addition of 10% w/w trichloroacetic acid.

3.3 Results and discussion

3.3.1 Microbiome characterisation

The bacterial diversity of the two UASB reactors and the two AnMBRs is reported in Figure 3-1. While the microbiomes of the two inocula were extremely different, reflecting their different origin (Chapter 2, Figure 2-6), after over 10 months of operation in the same conditions, the two UASB reactors developed similar relative abundances, although more samples would be required to ascertain the replicability of the results (Figure 3-1). During the operation of the UASB reactors, the predominant order was *Campylobacterales* (*Proteobacteria* phylum). The other bacterial orders identified in the samples are involved in the hydrolysis of particulate matter. *Lactobacillales* (*Bacilli* class, *Firmicutes* phylum) can grow anaerobically and produce lactic acid from carbohydrates fermentation (Aguirre & Collins, 1993). *Eubacteriales* or *Clostridiales* are obligate anaerobes belonging to the *Clostridia* class and *Firmicutes* phylum, which have been identified as a dominant class in the microbial community of anaerobic reactors and are able to degrade lipids, proteins and polymeric carbohydrates (Fykse et al., 2016). *Synergistales* (*Synergistetes* phylum) are syntrophic fermentative bacteria that degrade amino acids into volatile fatty acids and develop syntrophic relationships with methanogens, contributing to acidogenesis and acetogenesis (Damodara Kannan et al., 2020).

With the addition of the membrane, the overall abundance of bacteria with hydrolytic abilities, identified in the UASB reactors, increased from 17.3% to 34.3% in AnMBR-R1 and from 20.8% to 73.3% in AnMBR-R2. Moreover, the presence of other orders, involved in hydrolysis, increased. The proliferation of *Bacteroidales* (*Bacteroidota* phylum), capable of sugar fermentation, suggests an enhancement in volatile fatty acids degradation in AnMBRs (Azman, 2016). However, a codominance of *Bacteroidales* and *Clostridiales*, previously reported in similar operational conditions (Ribera-Pi et al., 2020), was not observed in this study. The combined relative abundance of *Syntrophobacterales*, *Syntrophales* and *Syntrophorhabdales*, belonging to the *Deltaproteobacteria* class and *Proteobacteria* phylum, increased in AnMBRs, reaching 9.2% in AnMBR-R1. These bacteria can degrade short-chain fatty acids, such as acetic, propionic, and butyric acid, to carbon dioxide and hydrogen in syntrophic relationships with methanogens (Nakasaki et al., 2020). However, these orders have been characterised by metabolic flexibility and can grow as sulphate-reducing bacteria or fermentative microorganisms, depending on the environmental conditions (Plugge et al., 2011). Moreover, the fluctuation in their abundance has been correlated to COD:SO_4 ratio, as it was observed that a decrease in COD:SO_4 led to a microbial shift to incomplete-oxidizing sulphate-reducing bacterial species, which could cause volatile fatty acids accumulation and reduced methane production (Lu et al., 2018).

Regarding archaeal communities, while no data was collected in this study, literature data suggest that the addition of a membrane affects to some extent the relative abundances of methanogens in the sludge. However, the evolution is not univocal. Ozgun et al. (2015) observed that after membrane addition, the genus *Methanosaeta* (acetoclastic methanogens) increased from less than 15% to 56% in relative abundance, while *Methanobacterium* (hydrogenotrophic methanogens) decreased by 45%. Similarly, Petropoulos et al. (2019) reported that *Methanosaeta* dominated the archaeal communities in the AnMBR reactor, although particularly on the surface of the membrane, while hydrogenotrophic methanogens were more abundant in the UASB reactor. On the other hand, Smith et al. (2015) found that hydrogenotrophic methanogenesis was the preferred pathway in the biofilm, but not in the suspended biomass, and Ribera-Pi et al. (2020) did not observe a marked shift in the composition of the methanogenic community between a flocculent UASB reactor and AnMBR. Therefore, analysis of the archaeal community would be necessary to investigate the impact of the membrane in this study as no clear conclusions can be drawn from literature.

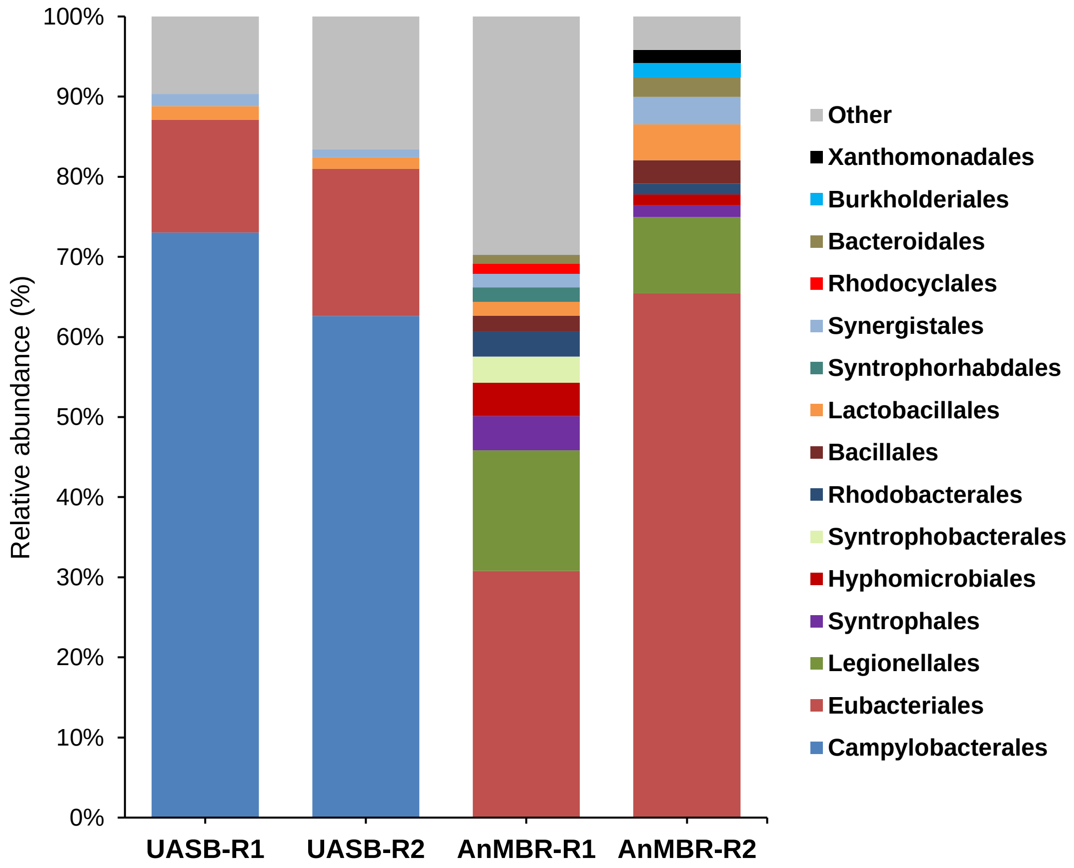

Figure 3-1 Bacterial community of sludge samples from pilot-scale UASB reactors and AnMBRs, described by orders (relative abundance cutoff of 1%).

3.3.2 Effluent quality, methane production and hydrolysis

While both UASB reactors and AnMBRs where operating in steady-state conditions when the enzymatic tests were carried out, the influent wastewater, which affects the microbiome of the sludge, was statistically different between the two periods (Table 3-2). In particular, COD and TSS concentrations increased from 243 to 350 mg/L and from 130 to 207 mg/L, respectively, when the AnMBRs were in operation. BOD:COD ratio remained stable at around 0.39 and COD was mainly in particulate form (around 80%) in both periods (Table 3-2). However, COD:SO_4 increased from 3.8 to 6.9 during AnMBR operation, which could have triggered the increase of *Deltaproteobacteria*, observed in the microbiome characterisation.

Removals for COD, TSS and SO_4 in UASB-R1 were higher than the ones in UASB-R2, respectively 57%, 74% and 36% against 49%, 61% and 24%. During the AnMBRs operation, the effluent streams were more similar, with the exception of COD removal, which was higher in AnMBR-R2 (92% against 88% in AnMBR-R1) (Table 3-2). Overall, the removal efficiencies achieved in this study in all reactors were in line with literature data for the treatment of municipal wastewater in temperate climates (9-18°C) (Gouveia et al., 2015; Ribera-Pi et al., 2020; Wang et al., 2019). Furthermore, the average total methane production was not significantly different between the two UASB reactors and the two AnMBRs.

Table 3-2 Influent and effluent wastewater characterisation, removal efficiencies and methane production of the two UASB reactors and AnMBRs. The star (*) indicates the parameters for which two reactors with the same configuration showed significantly different values. n.a.: not applicable.

		UASB influent	UASB-R1 effluent	UASB-R2 effluent	AnMBR influent	AnMBR-R1 effluent	AnMBR-R2 effluent
Characterisation							
Temperature	°C	16.1±2.8	15.2±3.0	15.3±3.0	20.6±1.0	20.0±1.7	20.0±2.1
COD	mg/L	243±61	99±24*	121±32*	350±104	37±11*	29±2*
sCOD	mg/L	46±11	37±9	35±9	58±8	34±9	26±4
BOD	mg/L	94±26	44±10	53±10	139±40	15±5	16±1
TSS	mg/L	130±30	35±8*	46±10*	207±55	5±3	4±2
VSS	mg/L	110±28	32±7*	42±9*	186±51	4±2	3±2
SO_4	mg/L	64±8	40±13*	49±11*	51±4	19±7	19±7
$COD:SO_4$		3.8	n.a.	n.a.	6.9	n.a.	n.a.
Removals							
COD	%	n.a.	57±12*	49±10*	n.a.	88±4*	92±2*
sCOD	%	n.a.	18±12	23±13	n.a.	43±14	54±4
BOD	%	n.a.	50±12	43±11	n.a.	89±5	87±2
TSS	%	n.a.	74±5*	61±11*	n.a.	98±2	98±1
SO_4	%	n.a.	36±20*	24±13*	n.a.	70±9	60±15
Methane							
Total CH_4 production	L/d	n.a.	2.7±1.1	2.3±0.6	n.a.	5.3±1.7	5.7±0.7

To better understand how the removal efficiencies translated into hydrolytic efficiencies, hydrolysis and solids hydrolysed were calculated for each reactor (Table 3-3). The two UASB reactors and the two AnMBRs did not display statistical differences in these parameters. It was observed that UASB-R1 presented marginally higher hydrolysis and rate of solids hydrolysed than UASB-R2, which could be linked to higher COD and TSS removals observed in Table 3-2, while, during AnMBR operation, AnMBR-R2 was performing slightly better in terms of hydrolytic efficiencies, in line with COD removals (Table 3-2).

In AnMBRs, the hydrolysis percentage resulted lower than in UASB reactors, as previously observed by Ribera-Pi et al. (2020), which could seem to contradict the hypothesis that the use of a membrane would promote hydrolysis due to higher solids retention in the reactor. However, when the hydrolysis percentages were applied to the solids removed, the rate of solids hydrolysed clearly showed that the AnMBR allowed for better solids hydrolysis. The results from Table 3-3 suggest that enzymatic activities of hydrolases in this study would be expected to be different between UASB reactor and AnMBR, but not between the two reactors with the same configuration, but different seed sludge.

Table 3-3 Average of hydrolysis percentages and rates of solids hydrolysed in the UASB reactors and AnMBRs operated during this study.

		UASB-R1	***UASB-R2***	***AnMBR-R1***	***AnMBR-R2***
Hydrolysis	%	28.3±9.5	24.7±9.2	18.2±9.5	21.4±12.0
Rate of solids hydrolysed	g/(L·d)	0.86±0.29	0.82±0.28	1.57±0.35	1.87±0.41

3.3.3 Protein concentration and specific enzymatic activity

The protein concentration of the sludge samples collected for enzymatic tests from UASB reactors and AnMBRs was measured to obtain a first indication of enzymes content in the reactors. UASB-R2 displayed higher average concentration (584 mg/L) compared to UASB-R1 (447 mg/L), while AnMBR-R2 concentration (434 mg/L) was slightly lower than AnMBR-R1 (451 mg/L) (Figure 3-2). Overall, neither the addition of the membrane nor the type of sludge did have a marked effect on the protein concentration of sludge, as all the observed series of data were not statistically different from one another ($p>0.05$).

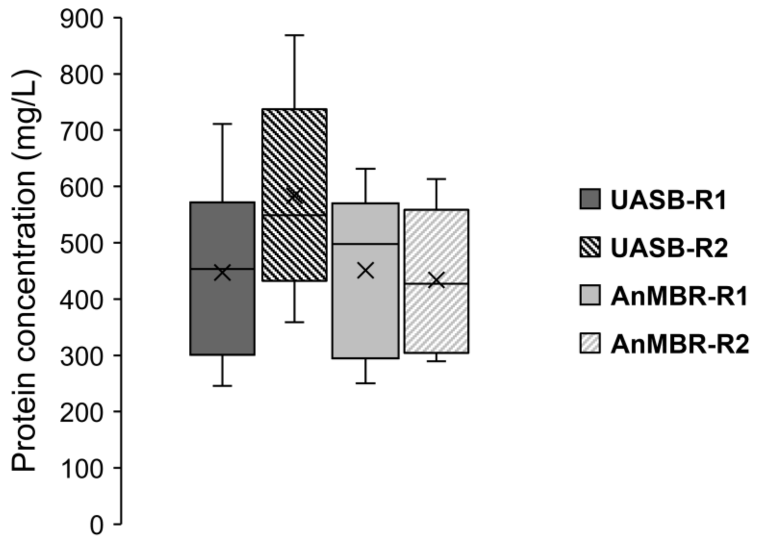

Figure 3-2 Protein concentration in the sludge samples from UASB reactors and AnMBRs used for enzymatic assays.

The protein concentration was used to determine the specific enzymatic activity (SEA) of the enzymes at different temperatures (4, 20, 37°C) (Figure 3-3). In all configurations, the activity of each enzyme increased with temperature, especially when incremented to 37°C. AnMBRs presented higher activities compared to UASB reactors in all cases, except for β-glucosidase at 20°C (0.17±0.02 nmol/(mg·min) for UASB-R1 and 0.16±0.08 nmol/(mg·min) for AnMBR-R1 ($p>0.15$)) and protease at 4°C (6.84±2.02 nmol/(mg·min) for UASB-R1 and 4.73±1.81 nmol/(mg·min) for AnMBR-R1 ($p>0.15$)), suggesting that overall the presence of the membrane helped increasing hydrolytic activity. Statistical differences ($p<0.15$) between UASB reactors and AnMBRs were identified particularly for protease in both setups and all temperatures, except for UASB/AnMBR-R1 at 4°C. The highest specific activity observed for β-glucosidase was 0.52 nmol/(mg·min) in AnMBR-R1 and 0.55 nmol/(mg·min) in AnMBR-R2. Regarding lipase, specific activity reached 1.33 nmol/(mg·min) in AnMBR-R1 and 2.02 nmol/(mg·min) in AnMBR-R2. Ultimately, protease presented the highest values for specific activity, achieving 135 nmol/(mg·min) in AnMBR-R1 and 196 nmol/(mg·min) in AnMBR-R2 (Figure 3-3). Between AnMBRs, the specific enzymatic activity of AnMBR-R2 was slightly higher than AnMBR-R1, matching the trend in hydrolysis percentages (Table 3-3), however, no significant difference was revealed with statistical tests, except for protease at 4 and 20°C, with AnMBR-R2 performing better. Similarly, the two UASB reactors did not display statistically different enzymatic activity at the same temperature, except for β-glucosidase at 20°C and protease at 4°C, with UASB-R1 achieving higher activities in those assays.

Assays at temperatures >37°C would allow to determine which is the optimal temperature for enzymatic activity of the sludge used in this study, corresponding to the maximum of specific activity observed. The fact that the enzymes displayed higher activities at 37°C suggests that the operation for over a year at temperatures <20°C did not cause the development of psychrophilic microorganisms that secreted psychrophilic enzymes, which present peak activity between 20-30°C (Feller & Gerday, 2003). However, the temperature for maximum enzyme activity could not always match the optimal growth temperature of the microorganism (Brenchley, 1996). In particular, it was reported that the enzymes activity could be less well adapted to cold compared to microbial growth and present higher activity at temperatures beyond the growth range of the bacteria (Huston et al., 2000). Petropoulos et al. (2018) reported that lipase activity in the sludge from batch reactors treating municipal wastewater decreased with temperature, as observed in this study, and adaptation of the sludge at different temperatures (4 and 15°C) did not have a significant impact when measuring activity at 37°C. Therefore, to investigate the adaptation of the sludge samples to the operational conditions of this study (<20°C), the affinity of the sludge, expressed in terms of K_m, the half-saturation constant of the Michaelis-Menten model, was measured for different substrates at different temperatures.

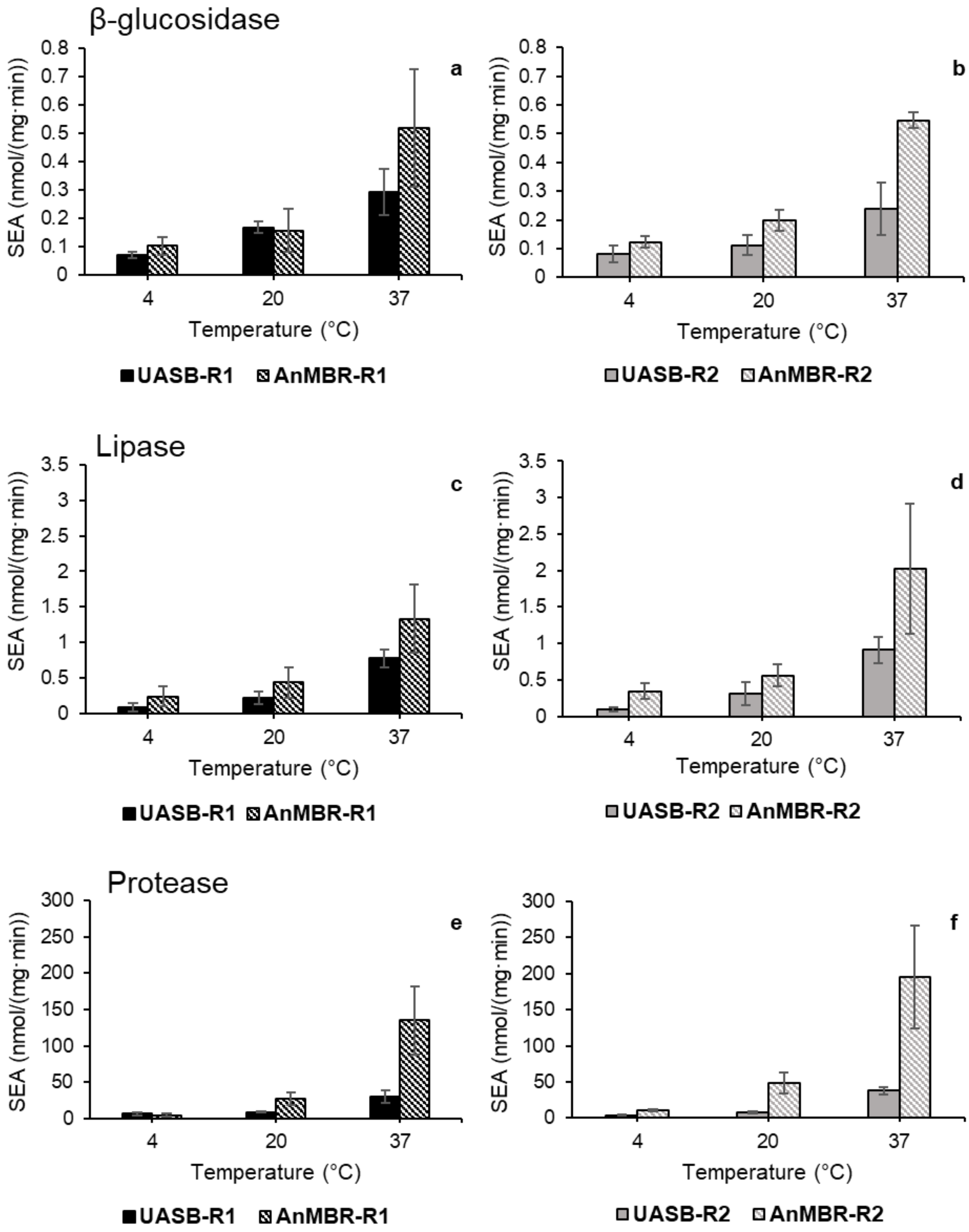

Figure 3-3 Specific enzymatic activity of β-glucosidase (3-3a, 3-3b), lipase (3-3c, 3-3d) and protease (3-3e, 3-3f) in the different sludge samples (UASB-R1, UASB-R2, AnMBR-R1 and AnMBR-R2) at 4, 20 and 37°C.

3.3.4 Enzymes substrate affinity at different temperatures

Results from Figure 3-4 revealed that β-glucosidase and lipase exhibited lowest K_m values at around 15-20°C, corresponding to the average operational temperature of the reactors. The trend did not change when UASB reactors were converted in AnMBRs. Overall, the enzymes from AnMBRs showed lower K_m values compared to UASB reactors, except for lipase at 20°C in UASB/AnMBR-R2 (Figure 3-4). No statistical difference was observed in the two AnMBRs, as well as in the two UASB reactors, with the exception of lipase at 15 and 37°C ($p<0.15$). Only a few cases tested presented a significantly lower K_m after the addition of the membrane (β-glucosidase at 7°C in UASB/AnMBR-R2, lipase at 15°C in UASB/AnMBR-R1 and lipase at 37°C in UASB/AnMBR-R2). Lowest K_m values for β-glucosidase and lipase were 0.37 mM in AnMBR-R1 and 0.52 mM in AnMBR-R2, both at 15°C, and 0.26 mM in AnMBR-R1 and 0.20 mM in AnMBR-R2, both at 15°C, respectively (Figure 3-4).

The highest K_m values were normally observed at the highest and lowest temperatures assayed, therefore 4-7°C and 37°C. This was expected since these temperatures are outside the operational range measured at pilot-scale (13-21°C). However, the UASB reactors, before the period here analysed, experienced also temperatures down to 6°C and, from Figure 3-4, lipase in UASB reactors seemed to reflect that, since K_m values were lower at 4°C than at 37°C (0.84 mM at 4°C and 1.21 mM at 37°C in UASB-R1 and 0.93 mM at 4°C and 1.87 mM at 37°C in UASB-R2) (Figure 3-4).

It was shown that enzymes display lowest K_m values at the physiological temperature of the producing microorganism (Kulakova et al., 2004). Kulakova et al. (2004) observed that the optimal temperature for a psychrophilic esterase was around 35°C. However, lowest K_m values were found at 25°C, which was the optimal growth temperature of the bacteria producing the enzyme. Therefore, the trend in Figure 3-4 suggests that the physiological temperature of the hydrolytic bacteria in this study was 15-20°C, showing sludge adaption to the operational temperature.

There is no indication in literature that the assays for enzymatic activity developed for activated sludge are influenced by the presence of oxygen (Coelmont et al., 2023), therefore, the specific activity of anaerobic sludge can be measured in aerated conditions. In this study, the assays were adapted from literature for activated sludge. However, despite the clear trend, the high standard deviations in Figure 3-4 and the difficulty in carrying out the short-term enzymatic test for protease suggest that more replicates may be needed to define the K_m values and modifications to the protease assay could be considered to try to reduce the colour development in the sludge sample blank. A different substrate (azocasein) was tested, following the procedure described by Gessesse et al. (2003), with a similar outcome. Possible solutions could include a different procedure for the preparation of the sludge sample (concentration, extraction, purification etc.), the use of a different type of assay (e.g. fluorescence-based assays) or the investigation of a related enzyme, like amino-peptidase.

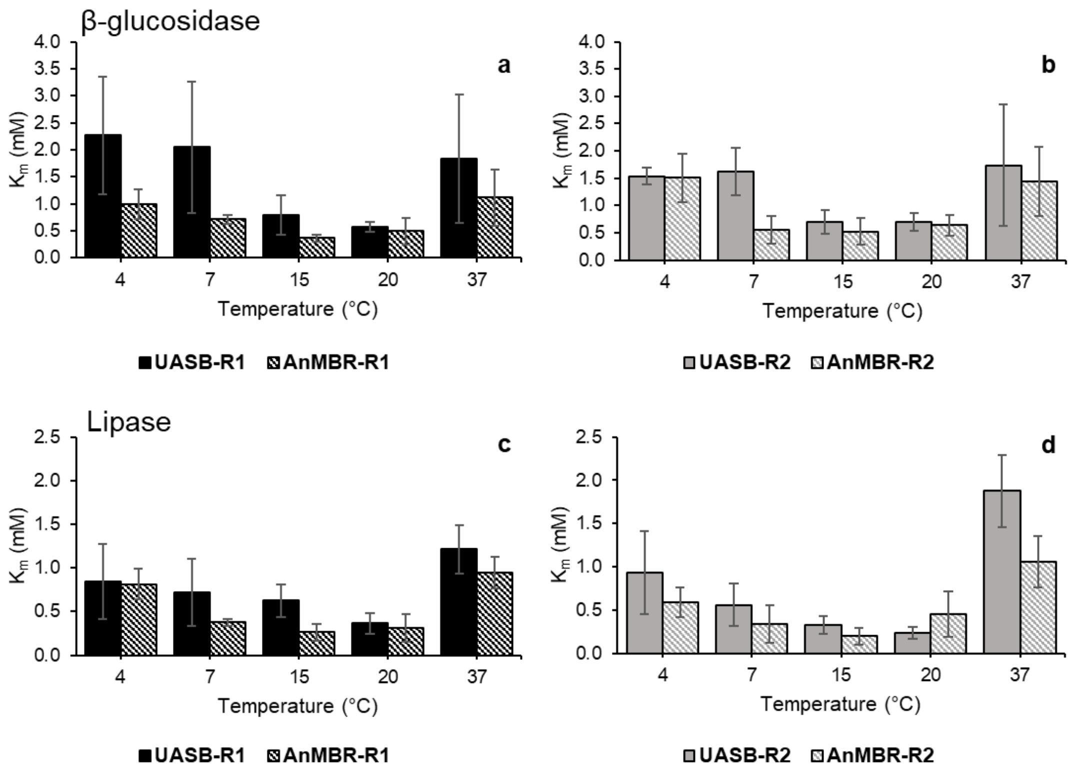

Figure 3-4 Michaelis-Mentent constant (K_m) for β-glucosidase (3-4a, 3-4b) and lipase (3-4c, 3-4d) at different temperatures (4, 7, 15, 20, 37°C), measured in sludge samples from UASB reactors and AnMBRs.

3.4 Conclusions

Two pilot-scale UASB reactors, subsequently converted in AnMBRs, were seeded with two different types of mesophilic sludge and their operation, to treat municipal wastewater in temperate climates (<20°C), was assessed in terms of effluent quality, methane production and hydrolysis percentages. Removals of COD and TSS were around 50% and 60% in UASB reactors and 90% and 98% in AnMBRs, respectively, and production of methane and hydrolysis percentages were not statistically different between the two UASB reactors and the two AnMBRs.

To better understand the adaptation of the sludge to low temperature, sludge samples were collected from the two reactors and in the two configurations to measure the activity of hydrolytic enzymes (β-glucosidase, protease and lipase). The specific activity increased with temperature (from 4°C to 37°C) for all three enzymes, implying that the operation at low temperatures did not cause the development of psychrophilic enzymes in the mesophilic sludge. However, the Michaelis-Menten constant (K_m) revealed that the enzymes, in all configurations, displayed higher affinity, and therefore lower K_m, at the operational temperature of the reactors (15-20°C). The hydrolytic enzymes present lower K_m values at the physiological temperature of the producing bacteria. Therefore, the results from this study suggest that both inocula adapted successfully to the low-temperature municipal wastewater treatment, matching the temperature for optimal bacterial growth to the operational one, while the hydrolytic enzymes maintained higher activities at mesophilic temperatures (37°C).

3.5 Acknowledgements

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4 Impact of influent characteristics on demonstration-scale anaerobic membrane bioreactor for municipal wastewater treatment in temperate climates

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Abstract

Anaerobic membrane bioreactors (AnMBRs) constitute a promising solution for the anaerobic treatment of municipal wastewater in temperate climates due to the improved solids retention provided by the membrane. The scalability of the technology for this application is currently being investigated as the performance of the treatment needs to be correlated with site-specific characteristics. In this study, a demonstration-scale AnMBR, treating 200 m^3/d of settled municipal wastewater from a full-scale municipal wastewater treatment plant (WWTP), was operated for over 120 days at temperatures <21°C. The influent wastewater was characterised by numerous fluctuations over the trial period, mainly of chemical oxygen demand (COD) (165-351 mg/L) as well as the ratio COD to sulphate (SO_4) concentration (COD:SO_4 between 1.4-3.7). The COD fluctuations were expected, however the dewatering liquors originating from centrifugation of the sludge digestate were recirculated to the head of the WWTP, adding further variability to the influent, especially in relation to oxidation-reduction potential (ORP). When the AnMBR was fed with an organic loading rate >0.25 g_{COD}/(L·d), COD and total suspended solids (TSS) removals were over 70% and 94%, respectively. SO_4 removal increased from 19 to 85%, resulting in the influent COD being in part utilised by sulphate-reducing bacteria (SRB) (up to 25%). As consequence, a reduction in methane yield from 0.17 to 0.09 $L_{CH4}/g_{COD,rem}$ was observed. The competition between SRB and methanogens was further analysed

to better understand its relationship with influent parameters, revealing the influence of oxidation-reduction potential on SO_4 removal. This study demonstrates how the influent characteristics, in particular the presence of sulphate, have a great impact on the successful scalability of AnMBRs for municipal wastewater treatment in temperate climates and how site-specific interventions become necessary to ensure a continuous stabilised performance.

Keywords: anaerobic treatment, biogas, large-scale, low temperature, membrane bioreactor, sulphate-reducing bacteria

4.1 Introduction

The requirement to reduce energy demand and greenhouse gas emissions in wastewater treatment plants (WWTPs) has heightened the interest in anaerobic-based technologies as an alternative to conventional aerobic systems. The attractiveness of anaerobic processes for municipal wastewater treatment lies in several key aspects, but importantly these do not require aeration and have the potential to be energy-neutral due to the degradation of organic matter into methane-rich biogas. Furthermore, anaerobic reactors are compact, robust and simple technologies, implying low capital and operational costs, and produce a smaller amount of waste sludge compared to aerobic processes (Stazi & Tomei, 2018).

Despite being an established technology for municipal wastewater treatment in countries like Brazil and India (Chernicharo et al., 2015), few challenges arise when applied in temperate climates. Hydrolysis of particulate matter is the rate-limiting step due to slower kinetics when temperature declines (Daud et al., 2018). This leads to the accumulation of suspended solids in the reactor and progressive reduction of effluent quality and biogas production, if improperly managed. Moreover, due to an increase in methane solubility at lower temperatures, the fraction of methane dissolved in the effluent can constitute a substantial portion of the total methane production (around 40%) (Li et al., 2021). This fraction, if not recovered, can rise the direct process greenhouse gas (GHG) emissions and impair the energy balance of the system.

Modifications to the configuration of anaerobic reactors to enhance solids retention and hydrolysis rates are important for the successful application of anaerobic technologies at low temperature. The anaerobic membrane bioreactor (AnMBR), which combines an anaerobic process (such as an upflow anaerobic sludge blanket (UASB) reactor) with membrane filtration, has been shown to enable improved solids management in temperate climates (Ribera-Pi et al., 2020; Wang et al., 2019). AnMBRs, due to the presence of the membrane, can provide complete retention of solids and biomass. This increases the sludge retention time and therefore the contact between biomass and wastewater (i.e.

substrate) and leads to improved hydrolysis, higher methane production and an overall high-quality effluent free of solids and pathogens (Stazi & Tomei, 2018). Moreover, the clean AnMBR effluent, rich in nutrients, can be of interest for fertigation or nutrient-based product recovery opportunities and ultimately water reuse (Robles et al., 2021).

To date, there are several AnMBR full-scale plants treating industrial wastewater, while for municipal wastewater the majority of literature results come from pilot-scale studies (Robles et al., 2021; Shin & Bae, 2018). Long-term operation of a pilot-scale municipal AnMBR was studied by Gouveia et al. (2015). The AnMBR, operated stably for over three years at around 18°C, consisted of a 160 L UASB reactor and a 150 L membrane tank. The total chemical oxygen demand (COD) removal achieved in this study was 87% and the methane yield varied between 0.18-0.23 $L_{CH4}/g_{COD,rem}$. With an increase in AnMBR scale to 5 m^3, Kong et al. (2021) demonstrated at 25°C a COD removal over 90% and methane yield around 0.18-0.23 $L_{CH4}/g_{COD,rem}$. Ultimately, the long-term operation of a semi-industrial scale AnMBR (40 m^3), treating high-loaded sulphate-rich municipal wastewater, was evaluated at temperatures between 10-27°C (Robles et al., 2022). In this study, COD removal was on average 87% and methane yield ranged between 0.07-0.17 $L_{CH4}/g_{COD,in}$ (as influent COD). Moreover, interesting reviews analysed the energy balance of pilot-scale AnMBR trials treating municipal wastewater in temperate climates and demonstrated energy neutrality or positivity for several studies (Lei et al., 2018; Shin & Bae, 2018).

Some technical challenges still need to be addressed to successfully scale up the technology and demonstrate its potential in full-scale applications. Current research is mainly focusing on membrane fouling (Charfi et al., 2012; Lin et al., 2013; Aslam et al., 2017; Shin & Bae, 2018; Anjum et al., 2021), since its mitigating and control strategy represents the primary operational cost in AnMBRs (Vinardell et al., 2020). However, considering that, due to the smaller footprint and high valorisation of the organic matter, AnMBR presents itself as a competitive alternative for the substitution of ageing and energy-intensive conventional plants, other parameters need to be considered when discussing scalability issues. These are particularly related to site-specific wastewater characteristics, like organic loading rate, temperature and sulphate concentration, and the operation and management of pre-existing treatment plants connected to the AnMBR.

The low organic strength of municipal wastewater constitutes a challenge for anaerobic treatment at low temperature (<20°C) due to lower methane production per volume of treated wastewater and higher dissolved methane loss in the effluent (Vinardell et al., 2020). Several techniques have been applied to increment the organic loading rate, including co-digestion, concentration through biosorption-sedimentation and membrane-based processes, such as direct membrane filtration, dynamic membrane filtration and forward osmosis (Vinardell et al., 2020; Aslam et al., 2022), but the ability of implementing these is also site-specific.

The presence of sulphate (SO_4) in the influent wastewater can have a great impact on the feasibility of the AnMBR technology in treating municipal wastewater, due to the competition between methanogens and sulphate-reducing bacteria (SRB) for the same substrates. Additionally, the reduced sulphur species (sulphide and hydrogen sulphide) can cause corrosion, bad odours, precipitation of metals and eventually, inhibition of the anaerobic process (Lei et al., 2018). Therefore, the closed monitoring of site operations and hence influent characteristics represents a key aspect for the successful implementation of anaerobic treatment at large-scale in temperate climates.

This study describes the effect of sudden variations in influent characteristics, including COD concentration and COD:SO_4 ratio, on effluent quality and methane production in a demonstration-scale AnMBR treating settled municipal wastewater at 14-21°C, identifying critical aspects for the successful scalability in temperate climates.

4.2 Materials and methods

4.2.1 Description of the demonstration-scale plant

The demonstration-scale anaerobic membrane bioreactor (AnMBR) plant was installed in the 92,000 population-equivalent municipal WWTP, serving the town of Redditch (UK). It consisted primarily of a UASB reactor and an ultrafiltration (UF) membrane reactor (Figure 4-1). Pictures of the plant are reported in Appendix C (Figure C-1, Figure C-2). Storage tanks were installed before and after each process stage to allow for possible flow variations in future experiments and ensure balanced loads as requested in this study. Settled wastewater, coming from the full-scale on-site primary sedimentation tank of the WWTP, was fed by a pumping station into a 200 m^3 mixed buffer tank, usually half full, with a hydraulic retention time (HRT) of around 12 hours. A constant flow rate of 200 m^3/d of influent settled wastewater was pumped into the UASB reactor by a dry-installed centrifugal pump (KSB Sewatec, UK), monitored by flow and pressure meters (Promag 400 and PMC-531, Endress & Hauser Ltd, UK) installed on the line.

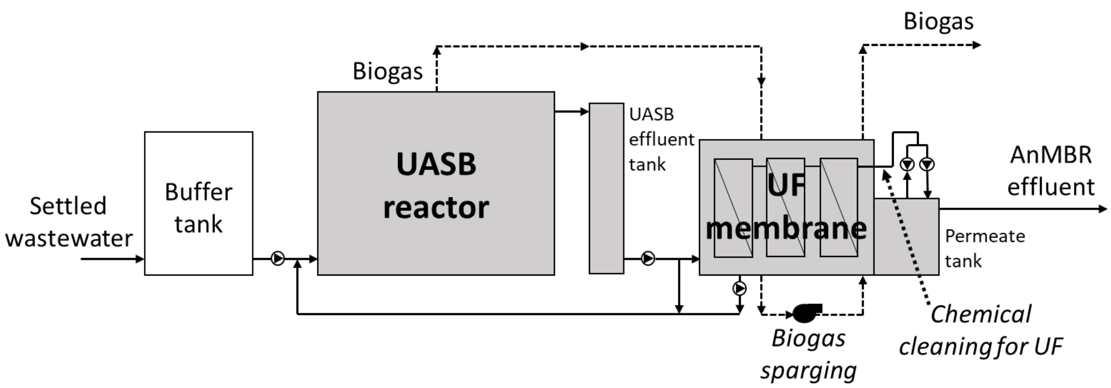

Figure 4-1 Schematic representation of the demonstration-scale AnMBR.

The UASB reactor (Waterleau, Wespelaar, Belgium) was a rectangular reactor with a total water height of 6.7 m and useful volume of 124 m^3. Influent wastewater was distributed at the bottom through two pipes fitted with holes over the whole length of the reactor. A three-phase separator placed at the top helped separate the UASB effluent from biogas and sludge. The UASB reactor was seeded with 28 m^3 of industrial mesophilic granular sludge (64 g/L of volatile solids, VS) coming from a UASB reactor treating food processing wastewater. Effluent from the UASB reactor overflowed in a 7.6 m^3 effluent tank. The HRT of the UASB reactor was 15 hours. Recirculation of 292 m^3/d of wastewater back to the UASB feeding line, necessary to sustain an upflow velocity of 0.7 m/h, was mainly performed with UF membrane retentate with a progressive cavity pump (Seepex SK32, Germany). However, in case of UF plant shutdown, the UASB effluent could be redirected and fed for recirculation by the UF dry-installed centrifugal feeding pump (KSB Sewatec, UK).

The UF membrane tank (20 m^3) was composed of three modules of 64 hollow-fibre (HF) C-MEM™ cartridges (SFC Umwelttechnik GmbH, Salzburg, Austria) each, for a total membrane area of 1074 m^2. The fibres in each cartridge were coiled around the central carrier and had a pore size of 0.02 µm and outer diameter of 0.4 mm. The cartridge, provided with a housing shell, had a cylindrical shape with a height of 410 mm and diameter of 164 mm. The water level inside the UF tank was kept around 1.5 m. A permeate tank of 6.3 m^3 was placed adjacent to the membrane reactor. Permeate filtration was performed for 300 s (Grundfos PN-SNB pump, UK), followed by 30 s of backwashing (Grundfos PN-SNB pump, UK) and gas sparging with biogas coming from the UASB reactor (Utile 90/5 gas blower, UK), achieving a net flux of 7 $L/(m^2 \cdot h)$. Fouling control through backwashing and gas sparging was performed in sequence in order to always have two modules filtrating, while the third one was being backwashed with 14.9 m^3/h of permeate and sparged with 362 Nm^3/h of biogas. Flow meters (Sitrans FM MAG 6000 & MAG 5100W, Siemens, UK) and pressure sensors (ACK30, IFM, UK) were used to monitor the membrane filtration performance and transmembrane pressure (TMP) and regulate chemical cleaning, performed with sodium hydroxide, sodium hypochlorite and citric acid, according to manufacturer's instructions using three different dosing pumps (ProMinent, UK).

4.2.2 Operation of the demonstration-scale plant

This study analyses the first 130 days of operation of the AnMBR. The operation of the AnMBR was divided into 5 periods based on the variation of the influent characteristics. Period I (days 1-15) refers to the plant start-up, as the sludge was adapting to municipal wastewater and temperature, reaching COD and biochemical oxygen demand (BOD) removals of 60% and 80%, respectively. Period II (days 16-30) describes the steady-state operation of the plant, when removals stabilised together with biogas production. This Period ended due to the start of disturbances and variations in the influent. These changes were caused by on-site perturbations before the AnMBR, mainly due to the recirculation of dewatering liquors to the head of the WWTP and variation in the desludging frequency of the primary sedimentation tanks. Period III and IV (days 31-80 and days 81-110) were affected by changes in carbon and solids content in the influent, while Period V (days 111-130), compared to Periods II-III-IV, was characterised by a sharp decrease in influent wastewater temperature.

Wastewater samples of AnMBR influent and effluent were collected manually and analysed multiple times a week. Biogas from the three-phase separator of the UASB reactor was used to sparge the hollow-fibre membrane and analysed after the UF membrane tank. Sludge samples from inside the UASB reactor at different heights (0.5, 1, 1.5, 3, 4.5 m) were collected every week to monitor the sludge blanket.

4.2.3 Analytical methods

Temperature and pH were analysed on site with a transmitter (Liquiline CM444, Endress & Hauser Ltd, UK), while oxidation-reduction potential (ORP) was measured with a field electrode (Intellical MTC101, Hach Lange GmbH, Düsseldorf, Germany). The wastewater samples were analysed by an external laboratory. Total suspended solids (TSS), volatile suspended solids (VSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), alkalinity, electrical conductivity, ammonium (NH_4-N), phosphate (PO_4-P), sulphate (SO_4), total iron and sulphide were analysed according to standard methods (HMSO, 1981). Soluble COD (sCOD) was determined according to the COD procedure after filtration of the wastewater sample at 0.45 µm. Volume and composition of biogas were determined via grab samples with a portable gas analyser (Gas Data GFM 436, Coventry, United Kingdom) multiple times a week. Dissolved methane concentration was continuously determined in the AnMBR effluent with an in-line sensor (Solu-Blu™ CH4, Pro-Oceanus, Bridgewater, Canada), allowing the observation of daily and seasonal variations in the concentration of methane in the liquid phase. Methane yield was determined considering methane produced both in dissolved and gas phases and excluding the fraction of COD removed by the sulphate-reducing bacteria (SRB) from the total COD removed (0.67 g of O_2 consumed per g SO_4 removed).

Sludge samples from each sampling point were collected every week and analysed to determine total solids (TS) and volatile solids (VS) content. TS and VS were measured according to Standard Methods (APHA, 2012). In the COD mass balance, the fraction of COD accumulated as sludge was determined according to Lobato et al. (2012), with sludge yield assumed as 0.08 g_{VS}/g_{COD}, while the wasted sludge was indicated as the difference between influent COD and the other outputs, obtained from samples analysis and calculations. The conversion of methane into COD was done stoichiometrically. Statistical analysis was performed to evaluate first outliers, then normality through Shapiro-Wilk tests. Welch's t-test was applied for normal distributions, while Mann–Whitney U test was used in the other cases. The analysis was based on a 95% confidence limit.

4.3 Results and discussion

4.3.1 Influent wastewater

The characterisation of the influent wastewater over the course of the experimental period is reported in Table 4-1. The influent wastewater at the plant start-up (Period I) was characterised by low concentrations of COD and TSS, 166 mg/L and 44 mg/L, respectively, and pH of 7.8 that increased to 8.3 in Period II. However, in Period III, pH decreased to 7.3 and COD and TSS increased to 351 mg/L and 124 mg/L, respectively (Table 4-1). Moreover, as reported in Figure 4-2, the soluble fraction of COD (sCOD:COD) decreased from an average of 53% to 33% in Period III, meaning that most of the carbon in the influent was in particulate form. In Period IV, influent COD declined to 262 mg/L (Table 4-1) and sCOD:COD remained stable at around 36% (Figure 4-2). In Period V, besides a marked decrease in temperature compared to the previous operational Periods (from 18-21°C to 14°C), the concentration of BOD increased to 130 mg/L (Table 4-1) and the average BOD:COD ratio, previously ranging between 26-35%, increased to 45% (Figure 4-2). Sulphate concentration in the influent did not show abrupt variations during the AnMBR operation (96-131 mg/L) and, therefore, the ratio COD:SO_4 grew according to the concentration of influent COD, ranging from 1.4 to 3.7 (Table 4-1). The concentrations of ammonium and phosphate increased slightly at the onset of Period III from an average of 22.8 mg/L to 33.6 mg/L for NH_4-N and from 1.8 mg/L to 5.9 mg/L for PO_4-P, but the observed rise is within the common range of concentrations in municipal wastewater (Kehrein et al., 2020).

Table 4-1 Influent characterisation divided into 5 selected operational periods, defined as Period I (days 1-15), Period II (days 16-30), Period III (days 31-80), Period IV (days 81-110) and Period V (days 111-130). OLR: organic loading rate.

		Period I	*Period II*	*Period III*	*Period IV*	*Period V*
OLR	g_{COD}/(L·d)	0.163±0.016	0.162±0.022	0.344±0.054	0.257±0.036	0.278±0.043
Temperature	°C	17.8±0.8	17.5±0.2	20.8±0.9	19.3±0.6	14.4±1.7
pH		7.84±0.21	8.31±0.48	7.32±0.08	7.40±0.06	7.47±0.05
COD	mg/L	166±16	165±23	351±55	262±36	284±44
sCOD	mg/L	88±8	95±8	117±26	92±14	97±23
BOD	mg/L	47±17	43±6	124±34	84±24	130±34
TSS	mg/L	44±7	44±13	124±19	96±5	114±12
VSS	mg/L	40±6	36±11	107±17	84±3	96±13
Conductivity	mS/cm	974±47	928±48	1075±39	982±106	1068±48
NH_4-N	mg/L	25.1±2.1	22.8±1.7	33.6±1.2	36.9±7.3	34.4±0.8
PO_4-P	mg/L	2.0±0.2	1.8±0.2	5.9±1.0	4.2±1.5	4.3±0.4
SO_4	mg/L	131±5	120±4	96±9	117±16	130±9
Alkalinity	mg_{CaCO3}/L	313±15	303±13	361±21	321±37	343±19
sCOD:COD	%	53±8	58±6	33±6	36±4	34±6
BOD:COD	%	28±7	26±1	35±8	27±14	45±8
COD:SO_4		1.4±0.3	1.4±0.2	3.7±0.8	2.3±0.1	2.2±0.5

The ORP in the primary sedimentation tank, from which the influent to the AnMBR originated from, decreased sharply around day 30 from positive values (over 200 mV) to negative values below -100 mV (Figure 4-2). This could be due to the redirection into the primary sedimentation tank of dewatering liquors, originated from the anaerobic digestion of sludge, which are characterised by a highly reductive environment.

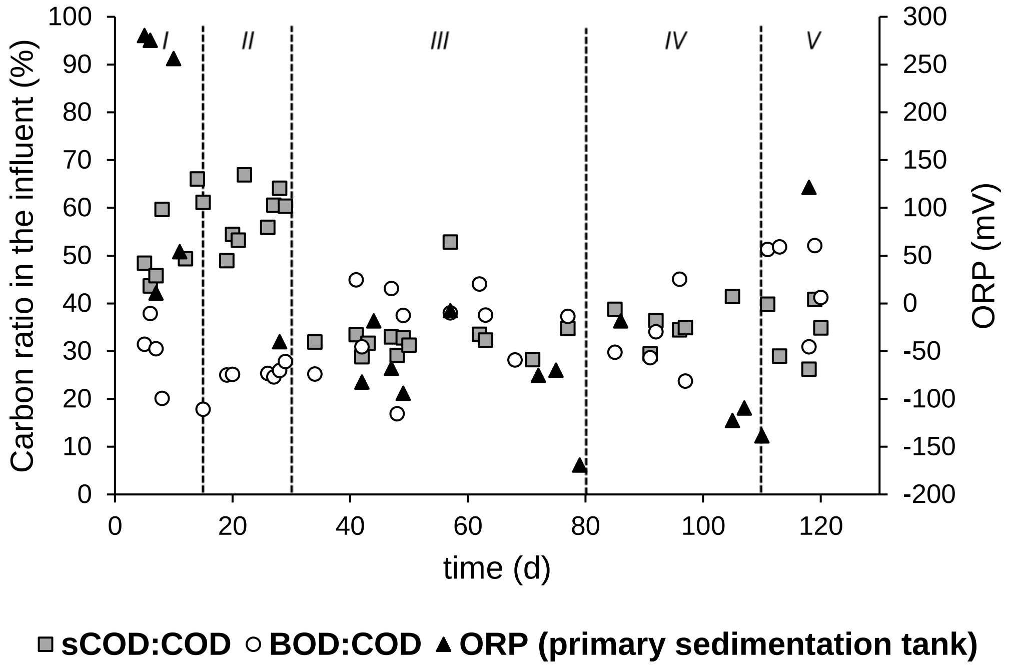

Figure 4-2 Variations in the AnMBR influent sCOD:COD and BOD:COD ratios and ORP of the primary settling tank during AnMBR operation (Periods I, II, III, IV, V).

4.3.2 Effluent quality and AnMBR removals

The AnMBR effluent quality and removals are described in Table 4-2. The average COD concentration in the effluent wastewater was 67 mg/L during steady-state (Period II) and around 80 mg/L in the subsequent Periods (III, IV, V), with soluble COD accounting for the highest fraction (75-85%, Table 4-2). BOD values in Periods III-IV-V were above the discharge limit of 25 mg/L. Total suspended solids concentrations in AnMBR effluent were slightly higher than expected after a membrane treatment (between 5.0-7.2 mg/L). Similar AnMBR studies, operating at 25°C, starting from around 200-250 mg/L of TSS, did not detect any solids in the effluent (Ji et al., 2020; Kong et al., 2021; Rong et al., 2022). Possible causes of the presence of solids in the AnMBR effluent are related to the integrity of the membrane fibres and, in this particular case, a possible leak from the membrane reactor to the permeate tank since the two were adjacent. Moreover, AnMBR effluents could be susceptible to metal sulphide precipitation. However, due to the complexity of the installations, these options are still under investigation. The quantity of sulphate in the effluent drastically decreased at the onset of Period III, declining from 98 mg/L to 12 mg/L and reaching an average of 7 mg/L at the end of the trial (Table 4-2). The effluent concentration of nutrients, nitrogen and phosphorus, ranged from 34.5 to 46.4 mg/L for NH_4-N and between 3.9 and 6.8 mg/L for PO_4-P. The increase in nutrients concentration after anaerobic wastewater treatment is expected because, within the hydrolytic stage, organic nitrogen is converted into ammonium during protein degradation, while organic phosphorus is solubilised into phosphate, increasing the concentration of these ions in solution (Von Sperling & Chernicharo, 2005). These concentrations make the AnMBR effluent suitable for fertigation or nutrient recovery processes, like ion exchange or membrane-based technologies (Robles et al., 2021).

Table 4-2 AnMBR effluent characterisation and removals divided into the selected operational Periods (I, II, III, IV, V). n.a.: not available.

		Period I	*Period II*	*Period III*	*Period IV*	*Period V*
Effluent quality						
pH		7.99±0.28	8.23±0.28	7.59±0.10	7.68±0.04	7.70±0.08
COD	mg/L	67±9	67±6	81±15	79±3	81±15
sCOD	mg/L	52±6	56±5	57±9	65±7	72±11
sCOD:COD	%	78±5	83±7	75±15	85±9	85±9
BOD	mg/L	10±3	11±1	34±4	31±6	37.3
TSS	mg/L	n.a.	n.a.	7.2±4.3	5.0±1.5	6.2±2.2
VSS	mg/L	n.a.	n.a.	4.1±2.8	2.6±0.5	3.2±2.2
Conductivity	mS/cm	1040±49	1016±17	1146±78	1042±87	1092±29
NH_4-N	mg/L	34.5±2.2	35.1±0.8	46.4±4.1	37.8	42.3±3.0
PO_4-P	mg/L	3.9±0.3	4.0±0.7	6.8±0.5	4.0	5.5±0.2
SO_4	mg/L	105±6	98±3	12±8	16±10	7±4
Removals						
COD	%	61.1±6.5	59.1±4.6	77.0±2.7	71.0±3.1	71.4±1.9
sCOD	%	40.2±7.7	42.9±1.9	48.9±12.0	25.1±10.8	22.2±10.4
BOD	%	80.4±3.7	72.8±2.7	72.8±5.6	60.7±13.6	69.3±8.2
TSS	%	n.a.	n.a.	94.3±2.3	95.0±1.6	94.5±2.0
SO_4	%	19.4±2.6	18.6±2.4	87.8±8.3	84.5±7.2	94.3±2.9

COD removal during the entire trial was around 60-70% (Figure 4-3), increasing slightly from Period II to Period III. Similarly, BOD removal after start-up was around 60-70%, while soluble COD removal decreased from around 40-50% (Periods II, III) to 20-25% (Periods IV, V). However, as the study progressed, the standard deviation on average BOD and sCOD removal increased, suggesting a slight process instability. COD and BOD removals were lower than the ones found in similar studies treating raw wastewater at ambient temperature. Kong et al. (2021) observed, at 25°C in a 5 m^3 AnMBR, COD and BOD removals over 90% and 95%, respectively, starting from influent COD concentrations between 368-482 mg/L and BOD concentrations between 147-246 mg/L. Lim et al. (2019) obtained in a 1.4 m^3 AnMBR removals of 88% for both BOD and COD, with average BOD concentrations of 250 mg/L and COD concentrations of 610 mg/L, at temperatures ranging from 12.7 to 31.5°C.

Figure 4-3 clearly shows that at the onset of Period III, coinciding with an increase in influent COD and COD:SO_4 ratio, sulphate removal rose sharply from 20 to 85-95%, suggesting an increase in SRB activity. Similarly, other AnMBR studies, treating sulphate-rich municipal wastewater (SO_4 around 300-360 mg/L) obtained a complete removal of sulphate and conversion in sulphide (Giménez et al., 2012; Seco et al., 2018).

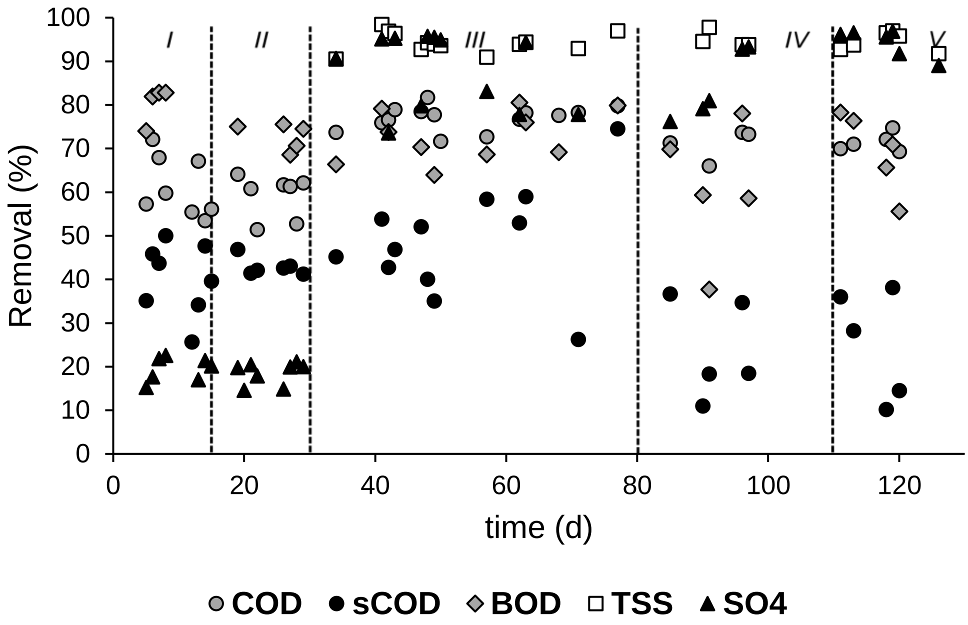

Figure 4-3 Variations in AnMBR removals during the plant operation (Periods I, II, III, IV, V).

4.3.3 Methane production

Biogas production throughout the operation was monitored and reported in Figure 4-4, alongside its methane content. In steady-state (Period II) the methane production stabilised with a methane concentration in the biogas around 45%. The increase in influent COD from 165 mg/L (Period II) to 351 mg/L (Period III) (Table 4-1), contributed to an increase in methane content in the biogas up to 60% (Period III, Figure 4-4). However, around 10 days after the start of Period III, biogas production abruptly decreased from around 50 L/h to 35 L/h (Figure 4-4). This could be linked to different possible changes in the biological process and substrate utilisation path inside the UASB reactor in Period III. The abrupt increase in SO_4 removal could be due to a proliferation of SRB, which, removing organic matter available for methanogens, reduce methane production (Seco et al., 2018). This hypothesis could be confirmed with microbial analysis of the sludge during the different periods of operation. Moreover, despite the increase in total influent COD, the drop in biogas production could be related to a reduction in the biodegradability of the substrate. The increase in the particulate fraction of COD may suggest an influent more difficult to biodegrade. However, COD fractionation tests would allow a better understanding of the quantity of organic matter actually available for methane production.

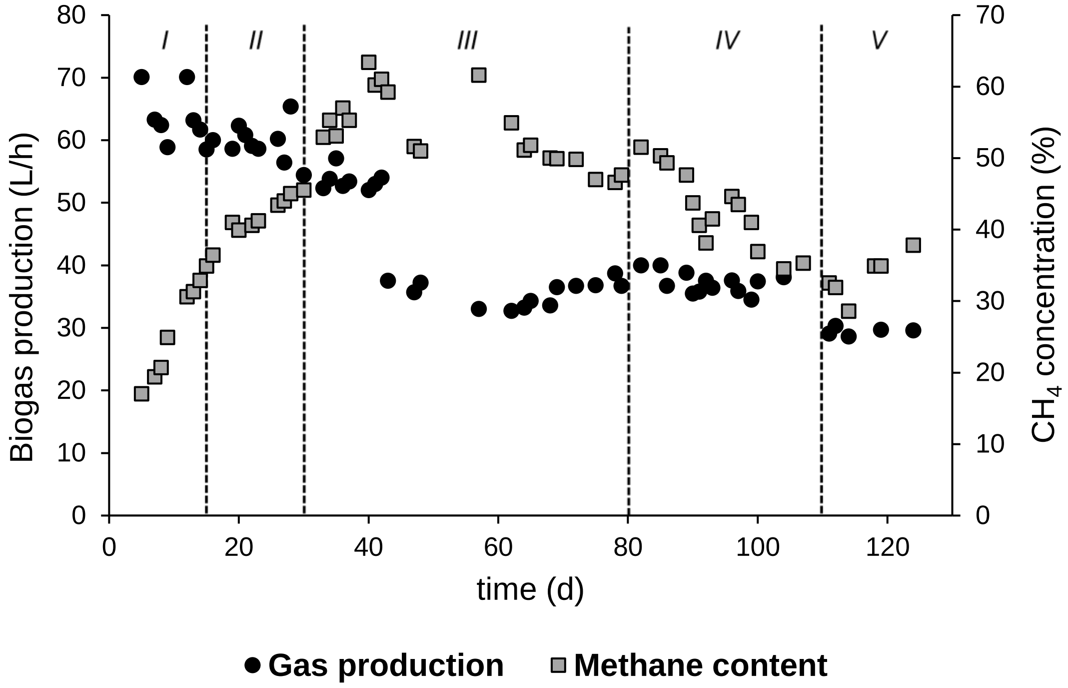

Figure 4-4 Biogas production from AnMBR and methane content in the biogas during the plant operation (Periods I, II, III, IV, V).

With a decline in influent COD at the beginning of Period IV, while biogas production remained stable, the methane content decreased from 50% to 35% (Figure 4-4). This is in accordance with the Cakir & Stenstrom (2005) model, developed at 20°C, which relates influent COD to methane content in biogas. The values from this study (excluding Period V due to a significant different temperature) followed the trend described by the model (Figure 4-5).

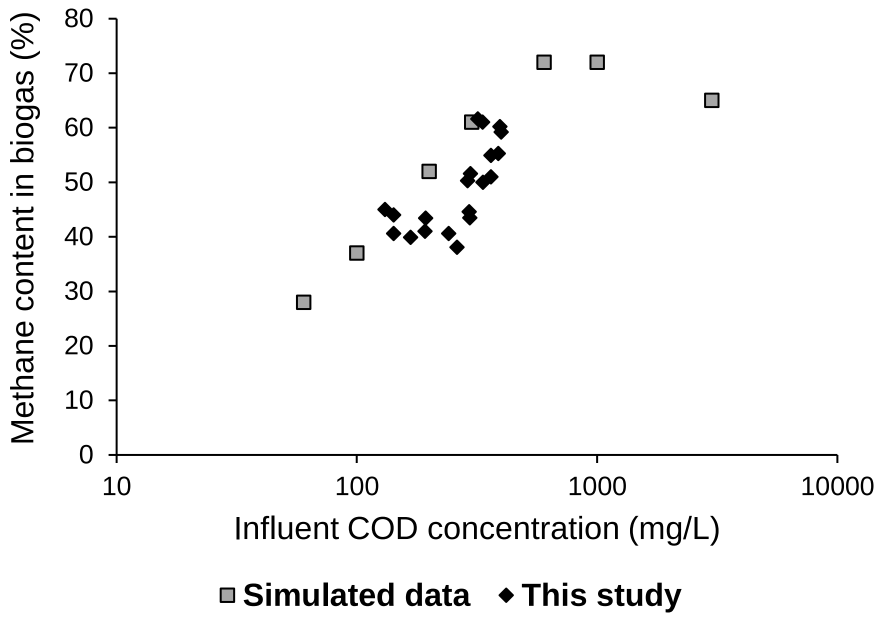

Figure 4-5 Relationship between methane content in the biogas and influent COD concentration during the study (Periods II, III, IV), compared with simulation data extracted from Cakir & Stenstrom (2005).

Dissolved methane in AnMBR effluent was constantly monitored with in-line sensors and average concentrations are reported in Table 4-3. While the concentration remained stable during Periods II, III and IV, around saturation, regardless of changes in COD and SO_4 removals, the significant decrease in temperature in Period V determined an increase in the daily average dissolved methane concentration in the AnMBR effluent from 8 mg/L (Period IV) to 16 mg/L (Period V), reaching a saturation degree of 1.71, in line with data from literature. Supersaturation is mainly observed in UASB reactors (reporting saturation degrees from 1.4 to 6.9), due to entrapment of biogas bubbles within the sludge bed, while in anaerobic membrane bioreactors the degree is usually lower, between 1-1.5, thanks to higher biomass retention (Crone et al., 2016).

The sensors, installed on the AnMBR effluent line, allowed to observe daily variations in the dissolved methane concentration, which were likely related to daily temperature fluctuations. Therefore, in Table 4-3, it was also reported the average of the daily maximum methane values reached in the AnMBR effluent (12-24 mg/L). This is to highlight the increase the dissolved CH_4 concentration is subjected to during the day and how a constant monitoring is necessary to successfully manage its discharge and a possible subsequent recovery treatment. Several technologies have been employed for dissolved methane removal and recovery, including aeration, gas stripping, membrane processes and biological oxidation. In the latter, methane cannot be recovered as it is transformed by methane oxidising bacteria in hydrocarbons and CO_2 (Rongwong et al., 2018). Aeration is a simple solution that allows for high mass transfer area, but it is difficult to scale up and operate and it could lead to potential explosion hazards due to the presence of a mixture of methane and oxygen. On the other hand, gas stripping could be easily scaled up, but it has a relatively low mass transfer area, provided by packing materials, and could cause flooding and foaming problems (Stazi & Tomei, 2021). Membrane contactors, which allow gas-liquid mass transfer without dispersion of one phase into the other, are able to avoid flooding and foaming. Desorption through the membrane is driven by sweeping gas and/or vacuum. More research is needed for their application in the recovery of dissolved methane in anaerobic effluents at full-scale due to wetting, fouling and clogging phenomena. Current strategies to control these issues are energy-intensive and may negatively affect the economic and environmental assessment for the implementation of this technology at full-scale (Stazi & Tomei, 2021). Moreover, the variation of the inlet dissolved methane concentration may have an impact on the net energy production of the degassing system as a higher concentration would affect the membrane area required for the recovery. Therefore, improving the mass transfer mechanism and transferring a larger amount of methane from the liquid to the gas phase would require providing more electricity to the vacuum pump and liquid pump (Kalakech et al., 2022).

Overall, during this study, dissolved methane accounted for 74-92% of total methane produced (Table 4-3). Therefore, in this case, a recovery process would likely be needed to reach energy neutrality and avoid greenhouse gas emissions from the effluent.

Table 4-3 Dissolved methane concentration in AnMBR effluent during the study (Periods II, III, IV, V).

		Period II	*Period III*	*Period IV*	*Period V*
Average dissolved methane (daily average)	mg/L	7.70±2.08	7.73±1.41	8.13±2.11	15.58±6.74
Average dissolved methane (daily max)	mg/L	15.07±7.44	12.37±4.79	14.86±6.69	24.20±5.93
Average dissolved CH_4/ total CH_4	%	74±5	76±6	80±4	92±4

Methane yield, based on methane produced both in liquid and gas phases, is reported in Figure 4-6, alongside the overall methane production. Higher total methane production was observed in Periods II and V (average between 2500 and 3500 L/d, Figure 4-6). In Period II, corresponding to a stable biogas production of 60 L/h (Figure 4-4), methane yield was on average 0.17 $L_{CH4}/g_{COD,rem}$. Subsequently, due to a decrease in methane gas production and growth of the fraction of COD removed by sulphate-reducing bacteria, methane yield decreased to 0.06-0.09 $L_{CH4}/g_{COD,rem}$ (Periods III and IV). However, in Period V, with an increase in the dissolved methane contribution (Table 4-3), the methane yield improved to 0.13 $L_{CH4}/g_{COD,rem}$. These values, while lower than the theoretical one, match the methane yield obtained in similar studies with a high fraction of influent particulate COD (Peña et al., 2019) and high sulphate concentration (Seco et al., 2018).

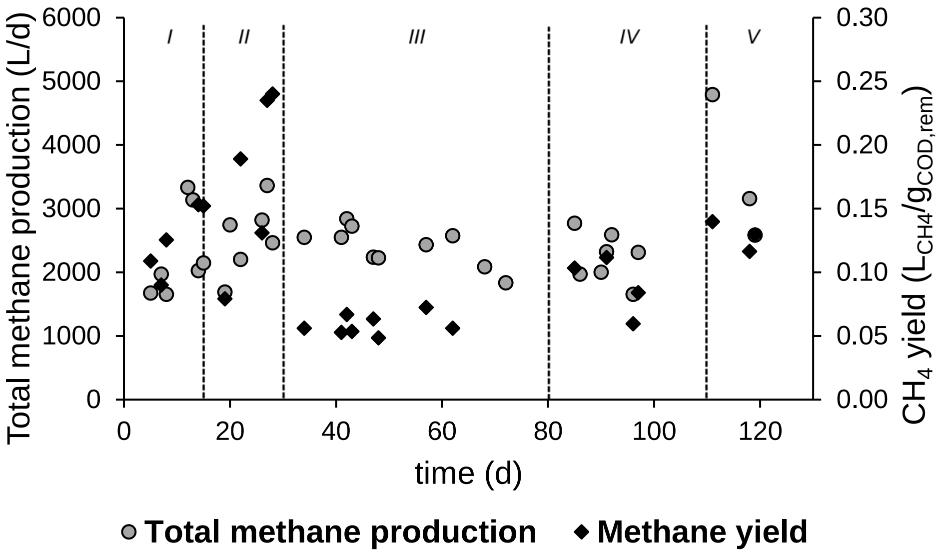

Figure 4-6 Methane yield and overall methane production in the demonstration-scale AnMBR during the study (Periods I, II, III, IV, V).

4.3.4 Mass balance

A COD mass balance was calculated for each period of operation (Figure 4-7). Each element of the graph represents a fraction of influent COD. During steady-state (Period II), over 40% of influent COD remained in the effluent and around 23% was converted into methane (Figure 4-7). Moreover, sulphate reduction did not prevail over methanogenesis. Following the increase in total COD (Period III), COD removal improved (Table 4-2), but the fraction of COD utilised by methanogens did not increase (Figure 4-7), suggesting COD was mainly removed by SRB and more importantly by membrane retention and not biodegradation. As previously observed in Figure 4-2, the predominant influent COD fraction in Periods III, IV and V was particulate, which could be more difficult to biodegrade.

During Period IV, the COD removed by sulphate reduction was more than double the amount of COD used for methane production. A similar proportion was observed by Robles et al. (2022) during the operation of a large-scale AnMBR at 19°C and COD:SO_4 of 2.4. The increase in methane production from Period IV to V, mainly due to improved dissolved methane production could be linked to the increase in influent BOD from 84 mg/L to 130 mg/L, characterising Period V.

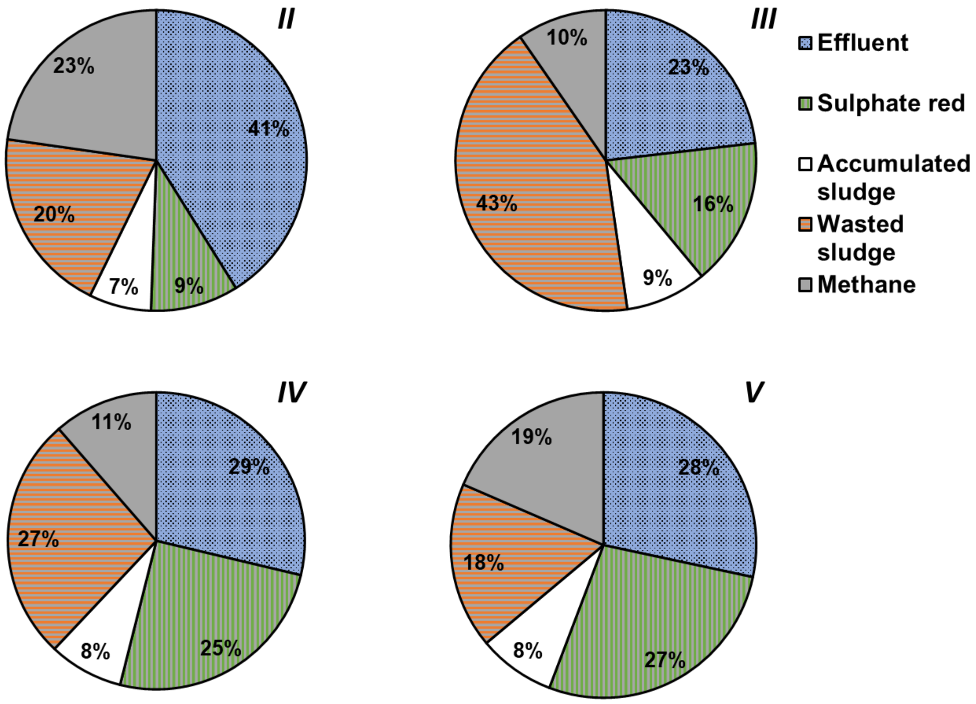

Figure 4-7 COD mass balance of the different Periods of the trial (II, III, IV, V).

4.3.5 Competition between SRB and methanogens

The abrupt change in sulphate removal between Period II and III (Figure 4-3) was further investigated to better understand the competition between sulphate-reducing bacteria and methanogens for the same substrate in the anaerobic reactor due to influent variation. Sulphate-reducing bacteria are anaerobic bacteria that oxidise hydrogen and organic compounds using sulphate as terminal electron acceptor (O'Flaherty et al., 2006). Autotrophic SRB compete with hydrogenotrophic methanogens for hydrogen, while heterotrophic SRB compete with acetoclastic methanogens mainly for acetate (Robles et al., 2021). The conversion of these two compounds coupled with sulphate reduction has a thermodynamical advantage over methanogenesis (O'Flaherty et al., 2006). Moreover, the Michaelis-Menten kinetics constant for SRB and methanogens, defining substrate affinity, can help determine the outcome of the competition (Table 4-4).

Table 4-4 Selected kinetics data of sulphate-reducing bacteria and methanogens based on substrate utilisation (adapted from Oude Elferink et al. (1994)). K_m: Michaelis-Menten constant.

Bacterial strain	K_m
Hydrogen-utilising (μM)	
Sulphate-reducers	
Desulfovibrio	0.7-4.0
Methanogens	
Methanospirillum	2.5-5
Methanobacterium	2-14
Methanosarcina	13
Acetate-utilising (mM)	
Sulphate-reducers	
Desulfobacter	0.07-0.23
Methanogens	
Methanosaeta	0.39-1.2
Methanosarcina	3.0

From Table 4-4 it can be established that SRB have higher affinity for hydrogen than methanogens. Therefore, while hydrogenotrophic methanogens are still present in the sludge, it is suggested that hydrogenotrophic SRB have a lower threshold for hydrogen and outcompete hydrogen-utilising methanogens (Oude Elferink et al., 1994). However, in case of acetate utilisation, the competition is not well defined as differences in kinetics properties between acetoclastic methanogens and acetate-utilising SRB are smaller (Table 4-4). Paulo et al. (2015) reported several cases in literature in which acetoclastic methanogens were not outcompeted by SRB. This is because other factors, besides affinity, can affect the competition, particularly the type of substrate and the COD:SO_4 ratio.

In this study, influent COD:SO_4 ratio ranged from a minimum of 1.4 (Period II) to a maximum of 3.7 (Period III) (Table 4-1) and, exceeding 0.67, it was theoretically always enough for a complete reduction of sulphate and simultaneous methane production (Lens et al., 1998). However, these values were not high enough to predict the outcome of the competition. A COD:SO_4 over at least 10 is necessary to ensure methanogenesis is not impacted by sulphate reduction (Paulo et al., 2015). In this study, unlike the fraction of COD removed by SRB, the fraction of COD removed by methanogens, was positively correlated to COD:SO_4 (Appendix C, Figure C-3).

Observing the mass balance results (Figure 4-7), in Periods III and IV, COD was mainly removed by SRB, suggesting favourable conditions for higher SRB growth rates and reduction of methane production. In addition to reducing the biodegradable fraction of available substrate for methanogens, the sulphate reduction causes the production of sulphide. Sulphide concentration in the AnMBR effluent was measured during Periods IV and V and was around 26.5 mg/L. Considering influent SO_4 was above 100 mg/L (Table 4-1) and total iron in the wastewater decreased from 2.3 mg/L to 0.6 mg/L, it was suggested that part of the produced sulphide could have precipitated as insoluble metal sulphide. While sulphide concentration during the trial was not high enough to cause toxicity (Oude Elferink et al., 1994), the precipitation and therefore loss of trace metals, which act as enzyme cofactors for methanogens, could indirectly jeopardise methanogenesis (Paulo et al., 2015). The requirement of nutrients and trace metals in an anaerobic reactor depends on the type and relative abundance of the species present, substrate type, operational temperature and reactor configuration. However, all methanogens were found to require iron, cobalt and nickel, albeit at different stimulating concentrations (Zandvoort et al., 2006). Thanh et al. (2016) provided a literature review of required concentration of essential metals (iron, nickel, cobalt, zinc, molybdenum, selenium and tungsten) in anaerobic reactors. Among those metals, iron is especially important because it is a fundamental component of key functional proteins involved in energy metabolism (Baek et al, 2019). In UASB reactors, operating at mesophilic temperature with a OLR between 2.6-7.8 $g_{COD}/(L{\cdot}d)$, the required iron concentration varied based on the substrate type and was reported to be 0.55 mg/L when treating methanol and 10 mg/L for distillery effluent (Thanh et al., 2016). Therefore, although municipal wastewater is believed to present all appropriate nutrients and trace metals for anaerobic degradation (Chernicharo, 2007), it is difficult to provide the exact requirements since they change case by case.

Despite the presence of low COD:SO_4 ratio, in Period II sulphate reduction was not prevailing, and removal was around 19% (Table 4-2). This was probably caused by an overall lower substrate availability (only 43 mg/L of influent BOD, Table 4-1), which could have led to a lack of electron donors for sulphate reduction. Moreover, the higher value of the ORP of the feed wastewater could have played a role in facilitating the growth of methanogens. In fact, studies showed that ORP values above -100 mV inhibit SRB (Zhang et al., 2022). As suggested by Figure 4-8, at the beginning of the study (Periods I and II), when SO_4 removal was low, the ORP in the buffer tank was >-100 mV, while, with the decrease in ORP to values around -150 mV, due to a change in the influent composition, SO_4 removal started to increase and SRB became predominant due to supposed higher growth rates.

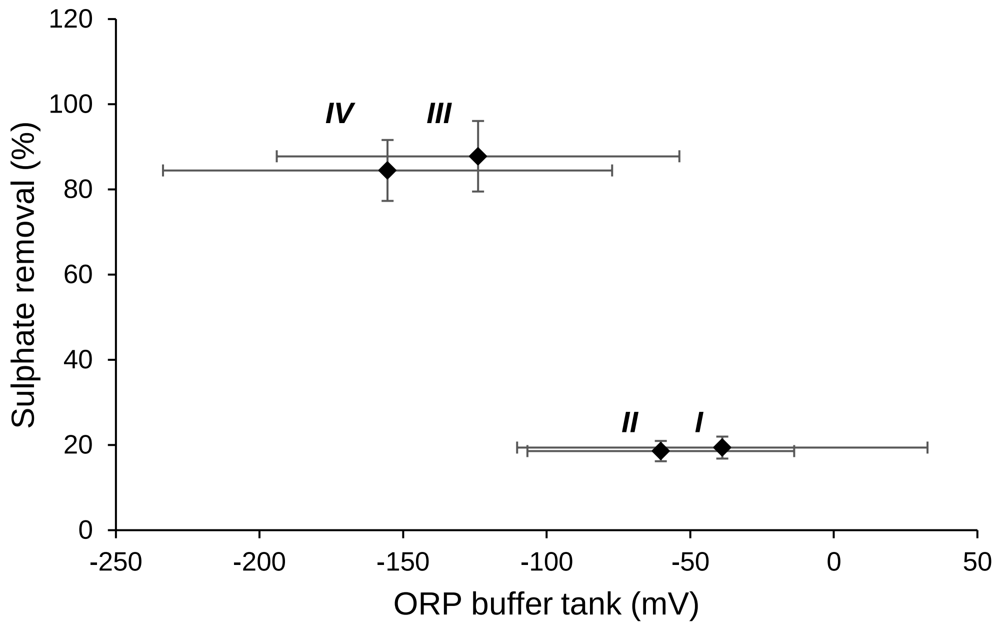

Figure 4-8 Relationship between AnMBR removal of sulphate and ORP of influent wastewater during the study (Periods I, II, III, IV). Data for ORP in buffer tank during Period V were not collected.

The use of techniques to control ORP constitutes a possible solution to manage sulphate reduction in anaerobic reactors. Khanal & Huang (2003) demonstrated that an ORP increase of 50 mV, from the initial value of -280 mV, was enough to reduce SO_4 removal and remove dissolved and gaseous sulphide, without inhibiting methanogenesis. However, they also noted how the target ORP depends on the influent sulphate. Therefore, a site-specific strategy is needed.

Other possible solutions to lower the activity of SRB include the change of operational parameters or the addition of inhibitors (Liu et al., 2018). In addition, another possibility to reduce the consumption of COD by SRB, which could be more applicable in AnMBRs, would be to exploit the shape and size of sulphate-reducing bacteria compared to methanogenic granules. Since SRB are lighter and mainly in form of flocs, increasing the upflow velocity of the UASB above 1.5 m/h could help wash out the SRB and reduce their activity (Shayegan et al., 2005).

4.4 Conclusions

A demonstration-scale anaerobic membrane bioreactor was fed with settled municipal wastewater combined with sludge dewatering liquors in order to understand the impact of influent variation on the effluent quality and methane yield, in temperate climates (<21°C). The AnMBR achieved up to 77% and 73% of COD and BOD removal, respectively, with a maximum methane yield of 0.17 $L_{CH4}/g_{COD,rem}$ at steady state. Methane was mostly produced in dissolved phase (74-92% of total methane production), implying a recovery process is necessary to avoid greenhouse gas emissions and improve net energy efficiency of the process.

Low influent COD:SO_4 ratio throughout the study led to a predominance of sulphate reduction over methanogenesis, causing the methane yield to decrease to 0.06-0.09 $L_{CH4}/g_{COD,rem}$. Moreover, the presence of dissolved sulphide was detected, which can hinder the biological process as it is harmful to microorganisms and cause corrosion and bad odours. Control strategies to reduce SRB activity result to be necessary to obtain a stable performance within the anaerobic reactor and prevent sulphide nuisance downstream. Possible solutions involve ORP control to inhibit sulphate-reduction and/or increase in upflow velocity to wash out sulphate-reducing bacteria.

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5 Investigating the impact of horizontally orientated hollow fibres in pilot- and demonstration-scale anaerobic membrane bioreactors

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Abstract

Municipal wastewater treatment with anaerobic membrane bioreactors (AnMBRs) achieves high quality effluent and reduces the energy demand of secondary wastewater treatment processes. Among submerged membrane technologies, the hollow-fibre (HF) configuration, in which the fibres can be arranged vertically or horizontally, is widely employed, with a preference for vertical fibres. In this study, two different pilot-scale HF membrane modules, one with horizontal fibres (AnMBR-HZ) and the other with vertical fibres (AnMBR-VERT), were operated in short-term tests at different fluxes and sparging rates to evaluate differences in fouling rates and energy consumption. At a net flux of around 7 L/(m^2·h), AnMBR-VERT was able to maintain fouling rates below 1 mbar/h over 24 h with a specific gas demand per membrane area (SGD_m) of 1.2 m^3/(m^2·h), while AnMBR-HZ achieved a fouling rate of 1 mbar/h only at 1.8 m^3/(m^2·h). Higher sparging rates were necessary for the horizontally orientated module mainly due to its coiled and highly packed configuration. However, the limited height of the horizontal module allowed for lower energy consumption (0.07 kWh/m^3 for AnMBR-HZ and 0.10 kWh/m^3 for AnMBR-VERT) in the same conditions. The horizontal module was then tested at demonstration-scale with an increased membrane area in a 200 m^3/d AnMBR treating settled wastewater. Scaling up, the energy consumption of AnMBR increased to 0.257 kWh/m^3, for a net flux of 9 L/(m^2·h) and SGD_m of 1.0 m^3/(m^2·h), with gas sparging accounting for the highest fraction, while fouling rates were slightly lower, suggesting that the pilot-scale studies provided a conservative assessment of the membrane performance at higher scale. This study evaluates the differences in membrane performance of horizontal and vertical fibres in AnMBR for municipal wastewater treatment and provides a direct comparison of the same horizontal membrane module at different scales, highlighting the need for the optimisation of the gas sparging cycle to ensure sustainable operation at higher fluxes, accounting for the different hydrodynamic environment created by the horizontal fibres.

Keywords: AnMBR, energy, fouling rate, horizontal, municipal, scalability

5.1 Introduction

The use of ultrafiltration membranes for wastewater treatment is increasingly appealing due to the possibility of achieving high quality effluent, free of solids and pathogens, in a limited footprint. The membranes, usually combined with a biological treatment (aerobic or anaerobic), can be placed inside or outside a tank. Submerged membrane bioreactors (MBRs), which operate by either suction on the permeate side and/or water head pressure on the retentate side, are favoured as they consume much less energy than external MBRs (Yang et al., 2006; Judd, 2016).

Among submerged MBR technologies, the hollow-fibre (HF) configuration is currently the most widely employed, based on m^2 of membrane area sold/used. The original HF product was the horizontally orientated low-density polyethylene (LDPE) fibre from Mitsubishi Rayon in 1992, followed a year later by the vertically orientated polyvinylidene fluoride (PVDF) fibre from Zenon (now Veolia) (Buer & Cumin, 2010). The fibres have diameters between 0.4 and 2.6 mm and pore sizes between 0.03 and 0.4 µm (Judd, 2016). Currently, all but one HF commercial MBR products are configured in a vertical orientation to alleviate issues observed with the original horizontal module concerning clogging and its control (Hai et al., 2005); including the current Mitsubishi product (Sterapore). The exception is the relatively recent product C-MEM, developed by SFC Umwelttechnik. The module contains 0.4 mm outer diameter, high density polyethylene (HDPE) fibres with a pore size of 0.02 µm combined into bundles and wound up around a cartridge carrier, allowing reduced space requirements and lower differences in the hydrostatic pressures along the cartridge. Importantly, the fibres are encased within a shell to enhance backwashing and gas sparging impacts. Currently there are over 100 operational sites for industrial and municipal wastewater treatment as well as development work coupling the membranes to non-thermal plasma for removal of pharmaceuticals (Back et al., 2018a) and arsenic (Back et al., 2018b). Other horizontal orientated products exist, such as the Zeeweed 1000, but are not used in MBR applications due to concerns about clogging.

In submerged HF membrane modules, the permeate normally flows from the outside to the inside of the fibres and, independently of the mode of operation (cross-flow or dead-end), a certain degree of membrane fouling, caused by the deposition of particles and colloids on or inside the membrane surface, always occurs (Akhondi et al., 2017). Fouling is the main drawback in membrane processes as it requires cleaning and management strategies, reduces productivity and increases energy consumption. A number of interesting reviews have described the different types of fouling based on foulants and their interaction with the membrane (Chang et al., 2002a; Meng et al., 2009; Drews, 2010; Wang et al., 2018b).

In submerged systems, the use of relaxation, which is a stop in filtration, backwashing, applied through reversing the permeate flow, and gas sparging constitute common strategies to control fouling. In particular, gas sparging or bubbling is commonly applied where the rising bubbles induce shear transients and eddies on the fibre surface generating fibre vibration and back lift of particles (Akhondi et al., 2017). The effectiveness of bubbling is influenced by the rate and frequency of the sparge, bubble size (2-5 mm), module configuration (e.g. membrane spacing and fibre looseness [1-5%]) and tank geometry (Böhm et al., 2012; Cui et al., 2003). The most significant is the gas sparging regime, which can be continuous, intermittent or pseudo dead-end (Wang et al., 2018a). Intermittent or cyclic gas sparging (e.g. 10 s on, 10 s off) was first reported by Guibert et al. (2002), who were able to reduce the extent of fouling and the associated costs in an immersed HF membrane in a bentonite suspension and has been applied to anaerobic MBRs (Martin Garcia et al., 2013). Maximum efficiency is provided by pseudo dead-end mode which involves sequential cycles of filtration without gas sparging and backwashing with gas sparging to further reduce energy consumption (Wang et al., 2018a).

Considering the current need of reducing energy demand of wastewater treatment processes, anaerobic membrane bioreactors (AnMBRs) represent an interesting alternative to conventional activated sludge systems to treat municipal wastewater. Similarly to aerobic MBRs, AnMBRs provide high quality effluent, which is suitable after further processing for water reuse (municipal and industrial) and as a feed for hydrogen production through electrolysis, lower sludge production and emit no nitrous oxide (Hillis et al., 2022). Importantly, AnMBRs do not require energy for biological aeration and instead produce biogas, which can be used to generate electricity, enabling a route to achieve energy self-sufficiency. However, it is crucial to optimise the fouling control system as it is the biggest contribution to energy consumption and can range from 0.038 to 5.68 kWh/m^3 (Martin et al., 2011). To illustrate, Shin & Bae (2018) observed that the average energy demand for gas sparging of pilot-scale AnMBRs was 0.21 kWh/m^3 accounting for around 30% of the energy demand of municipal aerobic MBRs (0.7-0.9 kWh/m^3) as reported by Krzeminski et al. (2012). Minimum energy demand for sustainable operation, defined as a fouling rate of less than 1 mbar/h, has been shown to occur under pseudo dead-end sparging at 0.14 kWh/m^3 for a net flux of 13.5 $L/(m^2{\cdot}h)$ (LMH) and a specific gas demand per membrane area (SGD_m) of 2.0 $m^3/(m^2{\cdot}h)$ (Wang et al., 2018a). Lower energy demands can be achieved when operating at lower fluxes, e.g. 6 LMH, but require much larger total membrane surface area and so are not economically favourable.

Research into fouling control and energy reduction approaches in AnMBRs remains a topic of considerable interest in terms of optimisation of gas sparging, alternative scouring agents, rotation, vibration, dynamic membranes as well as coupling the AnMBR to forward osmosis or electrochemical cells (Anjum et al., 2021). In addition, a number of studies have compared the impact of membrane geometry (Hai et al., 2005; Martin Garcia et al., 2011). However, there is a paucity of research on the comparison of fibre orientation. Previous work used fibres in open tanks and showed that vertical orientation reduces flux decline by 5-15% compared to horizontally orientated fibres under equivalent conditions (Chang et al., 2002b). Furthermore, it is reported that bubbles can get trapped between groups of fibres so for two-phase flows, like MBRs, vertically orientated membranes are preferred (Chang et al., 2002b; Fane et al., 2002). Computational fluid dynamics analysis of the impacts of fibre orientation revealed that vertically orientated fibres experience 25% more membrane surface shear compared to horizontally orientated fibres at the same aeration intensity (Liu et al., 2016). In the study, horizontal orientated fibres were reported to increase the resistance to fluid flow and pressure drop and as such reduce liquid velocity through the membrane fibres. To the authors' best knowledge, the only reported investigation into the use of horizontally orientated hollow fibres (Mitsubishi Rayon membranes) in granular AnMBR (open tank system), as well as comparison to other membrane geometries, treated municipal wastewater under temperate conditions (Fawehinmi, 2006; Fawehinmi et al., 2007). A fouling rate of 0.4 mbar/h was reported at a flux of 9 LMH and a temperature of 12ºC. At this temperature, the pilot plant delivered a consistent COD removal between 88-91% and a biomass growth rate of 0.003 $g_{MLVSS}/(g_{COD}\cdot d)$. No other studies have reported the performance of the current horizontally orientated hollow-fibre system, which is encased in a shell structure as opposed to be within open tanks, or a direct comparison between horizontally and vertically orientated hollow fibres.

To resolve this knowledge gap, the current study has run two different pilot-scale membrane modules, one with vertically orientated fibres (ZW-10) and the other with horizontally orientated fibres encased in a shell (C-MEM) for anaerobic treatment of municipal wastewater under temperate conditions. The comparison included treatment performance, fouling and energy demand, across different fluxes and sparging rates. In addition, the horizontally orientated fibre system was compared to the data generated from a parallel study using multiple C-MEM cartridges for a 200 m^3/d AnMBR. The aim is to understand how the orientation of the hollow fibres impacts the sustainability of membrane operation and the energy consumption of an AnMBR treating municipal wastewater.

5.2 Materials and methods

5.2.1 Description and operation of the pilot-scale anaerobic membrane bioreactors

Two pilot-scale anaerobic membrane bioreactors (AnMBR-HZ and AnMBR-VERT) were operated in parallel to treat municipal wastewater (Figure 5-1). Each AnMBR comprised a 70 L cylindrical upflow anaerobic sludge blanket (UASB) reactor and an external hollow-fibre ultrafiltration membrane tank. Both UASB reactors were fed with municipal wastewater from the bottom (520Du, Watson Marlow, Falmouth, UK) and employed acclimatised sludge during the trial. Sludge settling was aided by three lamella settlers, placed along the height of the reactor, and a three-phase separator on top. The effluent overflowed from the top into a 25 L membrane tank. The retentate of the membrane tank was recycled back to the UASB reactor (620Du, Watson Marlow, Falmouth, UK) to sustain an upflow velocity of 0.8 m/h. The hydraulic retention time (HRT) of the UASB reactors and membrane tanks was always the same for both reactors and, during the tests, varied between 4-6 h for the UASB reactors, while it was kept at 1 h for the membrane tanks. An overflow was placed on AnMBR-VERT for wastewater flow balance. Biogas production was measured with gas meters (TG 0.5, Ritter, Bochum, Germany) and the composition was determined with in-line carbon dioxide (CO_2) and methane (CH_4) sensors (BCP-CO_2 and BCP-CH_4, BlueSens gas sensor GmbH, Herten, Germany).

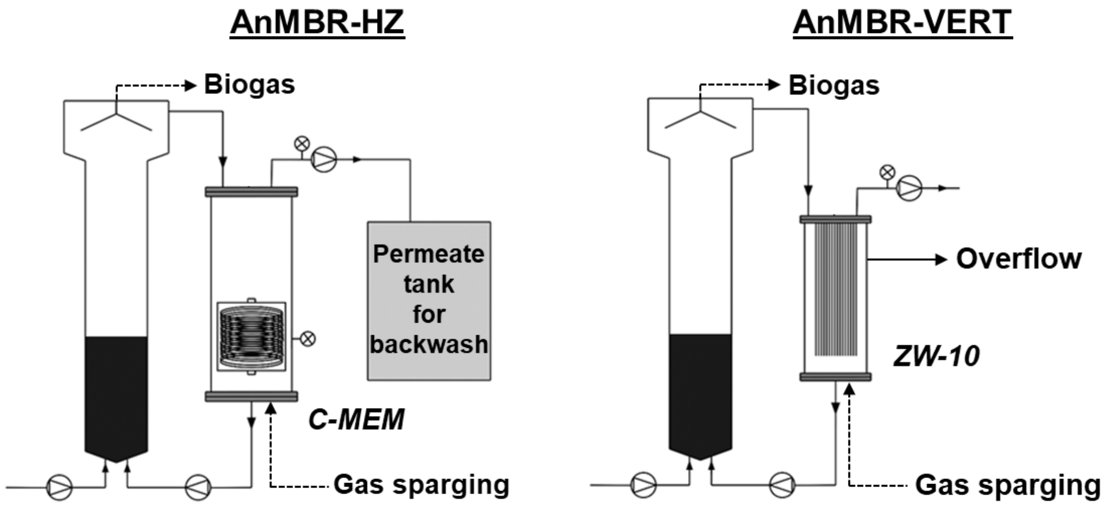

Figure 5-1 Schematic representation of the two pilot-scale anaerobic membrane bioreactors.

The two AnMBRs were characterised by different membrane modules and fibre orientations (Figure 5-2). The AnMBR-HZ was fitted with a horizontally orientated mini C-MEM cartridge (SFC Umwelttechnik GmbH, Salzburg, Austria), while AnMBR-VERT was operated with a vertically orientated Zenon ZW-10 module (Veolia Water Technologies & Solutions, Trevose, USA).

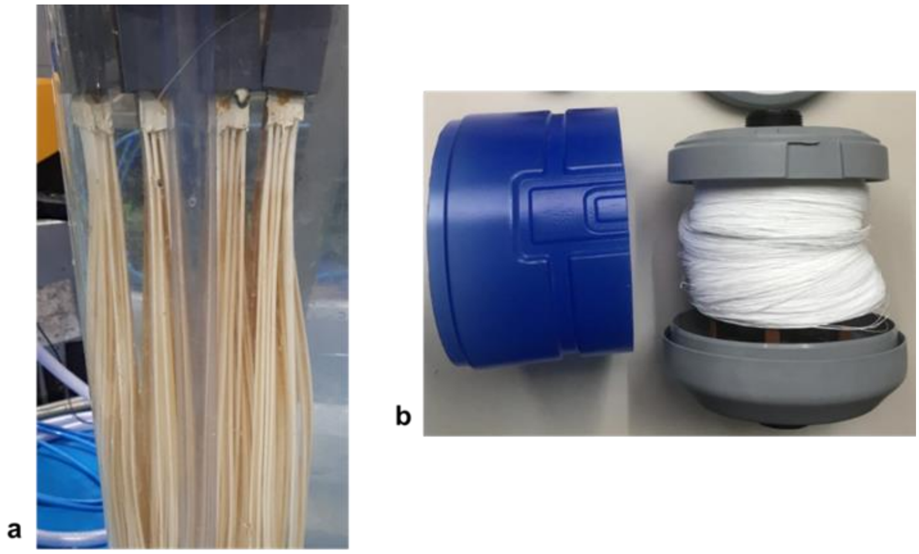

Figure 5-2 Details of the fibres of the two membrane modules. a: ZW-10 module with vertically orientated PVDF fibres; b: mini C-MEM module (with the cover removed) with horizontally orientated and coiled HDPE fibres (picture reported with permission from manufacturer).

The main differences in the two membranes lie in the material, fibre orientation and age of the membranes (Table 5-1). The C-MEM module was new, while the ZW-10 module has been previously used, but comprehensively chemically cleaned prior to use. Permeate was collected with a peristaltic pump (520Du, Watson Marlow, Falmouth, UK). Pressure transducers (PX319-030A5V, Omega, Manchester, UK) were placed on the permeate line and on the membrane tank side at the height corresponding to the middle of the membrane module length to determine the transmembrane pressure (TMP). TMP data were acquired continuously with a multichannel data logger (ADC-20, Pico Technology, St Neots, UK). AnMBR-HZ was equipped with a permeate tank for backwashing. Gas sparging was performed with nitrogen (NG6, Noblegen gas generator, Gateshead, UK) and regulated by a solenoid valve (Type 6014, Burkert, Ingelfingen, Germany) connected to a timer relay (PL2R1, Crouzet, Valence, France).

Table 5-1 Physical characteristics of the two pilot-scale membrane modules.

		Zenon ZW-10	***SFC-U C-MEM (mini)***
Material		Polyvinylidene fluoride (PVDF)	High-density polyethylene (HDPE)
Fibre orientation		Vertical	Horizontal
Fibre configuration		Curtain bundle	Wound up around the cartridge carrier
Flow path		Outside-in	Outside-in
Housing shell		No	Yes
Diameter of a cartridge	mm	/	164
Height of a cartridge	mm	/	200
Pore size	µm	0.04	0.02
Fibre length	m	0.5	0.74
Fibre outer diameter	mm	1.9	0.4
Fibre internal diameter	mm	0.8	0.3
Membrane area	m^2	0.93	1.4

The two membranes were operated in pseudo dead-end filtration mode. The operation of AnMBR-VERT was based on previous pilot studies, alternating filtration (9 min) with relaxation combined with gas sparging (1 min) (Wang et al., 2018a). The filtration/backwash cycle for AnMBR-HZ was suggested by the supplier and included an alternation between filtration (5 min) and backwashing of the module combined with gas sparging (30 s). Critical flux (J_c) was determined with the flux step method (Le Clech et al., 2003), using flux steps of 3 LMH, with a step duration of 10 min. The tests were conducted in batch with recirculation of the permeate back to the tank, without gas sparging and performed in triplicates.

Short-term tests (24 h) were performed with the two membranes under the same conditions to determine the differences in fouling rate (dTMP/dt) over a range of fluxes (J_{20}) and specific gas demands per membrane area (SGD_m) (Table 5-2). Based on cycles duration the net values of flux ($J_{20,net}$) and SGD_m ($SGD_{m,net}$) were calculated (Table 5-2). The test fluxes were chosen based on critical flux results, while the $SGD_{m,net}$ values were decided in order to have the SGD_m varying roughly between 0.8-2 $m^3/(m^2 \cdot h)$, to compare results with previous pilot studies (Wang et al., 2018a). The threshold for sustainable membrane operation was fixed at a fouling rate <1 mbar/h over 24 h (Wang et al., 2018a). After each test, the membrane was physically cleaned and clean water permeability was assessed to ensure the same starting point in terms of membrane permeability for each test. Over the duration of the study, clean water permeability varied by less than 10% for both membranes. Previous studies by Wang et al. (2018a) showed high replicability of short-term fouling rate tests, which in this study was confirmed by a low standard deviation error (<2.5%) for the triplicates of the fouling rate of each membrane module, for case A2, the first case to be tested (Table 5-2).

Table 5-2 Description of short-term fouling rate tests.

			Cases					
			A1	***A2***	***A3***	***B1***	***B2***	***B3***
AnMBR-HZ (C-MEM)	J_{20}	LMH	8	8	8	12	12	12
	$J_{20,net}$	LMH	7.3	7.3	7.3	10.9	10.9	10.9
	SGD_m	$m^3/(m^2 \cdot h)$	0.9	1.3	1.8	0.9	1.3	1.8
	$SGD_{m,net}$	$m^3/(m^2 \cdot h)$	0.08	0.12	0.16	0.08	0.12	0.16
AnMBR-VERT (ZW-10)	J_{20}	LMH	8	8	8	12	12	12
	$J_{20,net}$	LMH	7.2	7.2	7.2	10.8	10.8	10.8
	SGD_m	$m^3/(m^2 \cdot h)$	0.8	1.2	1.6	0.8	1.2	1.6
	$SGD_{m,net}$	$m^3/(m^2 \cdot h)$	0.08	0.12	0.16	0.08	0.12	0.16

5.2.2 Description and operation of the demonstration-scale anaerobic membrane bioreactor

The demonstration-scale anaerobic membrane bioreactor was installed in the 92,000 population-equivalent municipal wastewater treatment plant of Redditch (UK) and treated 200 m^3/d of settled wastewater. It consisted primarily of a UASB reactor, seeded with sludge obtained from the same source as AnMBR-HZ, and an ultrafiltration membrane tank, provided with three modules of 64 hollow-fibre C-MEM cartridges (height of 410 mm and diameter of 164 mm) each, for a total membrane area of 1074 m^2. The water height in the tank was kept at around 1.5 m. As in the pilot-scale C-MEM plant, permeate filtration was performed for 5 min (Grundfos PN – SNB pump, UK), followed by 30 s of backwashing (Grundfos PN – SNB pump, UK) and gas sparging (Utile 90/5 gas blower, UK) with biogas coming from the UASB reactor, maintaining an SGD_m of 1 $m^3/(m^2 \cdot h)$. Flow meters

(Sitrans FM MAG 6000 & MAG 5100W, Siemens, UK) and pressure sensors (ACK30, IFM, UK) were used to monitor the membrane filtration performance and TMP. Chemical cleaning with sodium hydroxide, sodium hypochlorite and citric acid was performed according to manufacturer's instructions using three different dosing pumps (ProMinent, UK), when filtration TMP reached 0.7 bar. Sodium hydroxide and sodium hypochlorite were dosed together to perform a caustic/chlorine wash (pH=12 and 5000 mg/L max total chlorine), while the acid wash was performed with citric acid (pH=2.5). The cleaning of each module required 1.5 hours.

5.2.3 Analytical methods

Wastewater samples of pilot-scale AnMBR influent and effluent were collected multiple times a week and the analyses were performed in duplicates. Temperature and pH were measured right after sampling with a portable Multi Meter (HQ40D, Hach Lange GmbH, Düsseldorf, Germany) on site. Biochemical oxygen demand (BOD), total suspended solids (TSS), volatile suspended solids (VSS), and alkalinity were measured according to Standard Methods (APHA, 2012). Total and soluble chemical oxygen demand (COD, sCOD), sulphate (SO_4), ammonium (NH_4-N) and phosphate (PO_4-P) were analysed with cell test kits (Merck KGaA, Darmstadt, Germany). Wastewater samples for cell test analysis of SO_4, NH_4-N, PO_4-P and sCOD were filtered at 0.45 µm with retention membrane filters (Whatman, Cytiva, Little Chalfont, UK) before analysis.

The total methane produced was calculated combining gaseous and dissolved methane. In this study, the gaseous production was directly measured with gas meters (TG 0.5, Ritter, Bochum, Germany) and CO_2 and CH_4 sensors (BCP-CO_2 and BCP-CH_4, BlueSens gas sensor GmbH, Herten, Germany), while the dissolved methane was estimated using Henry's Law as described in Equation (3-1):

$$\frac{C_g}{C_s} = H_u \qquad \textbf{(5-1)}$$

where, C_g is the concentration of methane in the gas phase, C_s is the saturation concentration of methane in the liquid phase and H_u is the dimensionless Henry's Law constant, depending on temperature. Since the AnMBRs were not sealed, it was not possible to collect representative wastewater samples to determine the dissolved methane concentration. However, literature data suggest that AnMBRs can achieve dissolved methane concentrations closer to the thermodynamic equilibrium (Crone et al., 2016). Therefore, for lack of direct measurements, Henry's law was used to provide a first approximation of the concentration of methane produced in the liquid phase.

Methane yield was expressed as a ratio between methane produced in both phases and the COD removed by methanogens, which was determined excluding the fraction of COD removed by the sulphate-reducing bacteria (SRB) (0.67 g of O_2 consumed per g SO_4 removed). Statistical tests were performed to evaluate first outliers, then normality (Shapiro-Wilk test). Welch's t-test was applied for normal distributions, while Mann–Whitney U test was used in the other cases. The analysis was based on a 95% confidence limit.

The energy consumption of feeding and recirculation pumps was determined according to Equation (5-2), while for the permeate and backwashing pump Equation (5-3) was used.

$$E\ (kW) = \frac{\rho g H Q}{\eta} \quad \textbf{(5-2)}$$

$$E\ (kW) = \frac{TMP_e Q}{\eta} \quad \textbf{(5-3)}$$

where, ρ is the density of wastewater, assumed 1000 kg/m^3, g is the gravitational acceleration (9.81 m/s^2), H is the water head acting on the feeding and recirculation pumps, Q is the flow rate, TMP_e is the effective pressure on permeate and backwashing pumps, calculated considering both the transmembrane pressure and the water head and η is the efficiency of the pumps, assumed 80%. The specific energy consumption (kWh/m^3) for each element was obtained using the net permeate production, which is affected by the filtration cycle duration. The specific energy consumption of the blower was determined according to Equation (5-4) (adapted from Shin & Bae, 2018):

$$E\left(\frac{kWh}{m^3}\right) = \frac{k \cdot SGD_{m,net}}{J_{20,net} \cdot 3600}, \qquad k = \frac{P_{A,1} T_{A,1} \lambda}{2.73 \cdot 10^5 \eta_b (\lambda - 1)} \left[\left(\frac{P_{A,2}}{P_{A,1}} \right)^{(1-(1/\lambda))} - 1 \right] \tag{5-4}$$

where $P_{A,1}$ is the inlet (atmospheric) absolute pressure, $P_{A,2}$ is the outlet absolute pressure based on the water head at the bottom of the membrane module, $T_{A,1}$ is the absolute temperature of the gas (nitrogen or biogas), λ is the heat capacity ratio (1.3 for biogas and 1.4 for nitrogen), η_b is the blower efficiency, assumed 60%, $SGD_{m,net}$ is the net specific gas demand per membrane area and $J_{20,net}$ is the net flux, based on net permeate production.

5.3 Results and discussion

5.3.1 Effluent quality and methane production

During the duration of the study, the operation of the two anaerobic membrane bioreactors was assessed in terms of effluent quality and methane production (Table 5-3, Table 5-4). The influent temperature was approximately 20°C for the entire duration of the trial. The influent COD was on average 350 mg/L and was mainly in particulate form since soluble COD was around 58 mg/L, while TSS concentration reached 207 mg/L (Table 5-3). The COD and BOD concentration in the permeate was 37 mg/L and 15 mg/L, respectively, for AnMBR-HZ and 29 mg/L and 16 mg/L, respectively, for AnMBR-VERT, achieving values comparable to previous studies operated on the same wastewater (Wang et al., 2018a). Removal efficiencies were similar in both reactors, reaching BOD and TSS removals of around 87% and 98%, respectively (Table 5-3). The only statistical difference between the two AnMBRs was observed in the permeate COD, and therefore COD removal (88% for AnMBR-HZ and 92% for AnMBR-VERT). However, the removals achieved in both reactors are consistent with the ones obtained from other pilot-scale AnMBR studies treating municipal wastewater with either orientation of membrane and revealed no important impact of fibre orientation with respect to treatment performance, as expected (Shin & Bae, 2018; Fawehinmi et al., 2006).

Table 5-3 Influent and effluent wastewater characterisation and removal efficiencies for pilot-scale AnMBRs during the duration of the study. The star (*) indicates the parameters with a statistical difference between the two reactors.

		Influent	*AnMBR-HZ effluent*	*AnMBR-VERT effluent*	*AnMBR-HZ removals (%)*	*AnMBR-VERT removals (%)*
pH		7.8±0.3	7.8±0.1	7.8±0.2		
Temperature	°C	20.6±1.0	20.0±1.7	20.0±2.1		
COD	mg/L	350±104	37±11*	29±2*	88±4*	92±2*
sCOD	mg/L	58±8	34±9	26±4	43±14	54±4
BOD	mg/L	139±40	15±5	16±1	89±5	87±2
TSS	mg/L	207±55	5±3	4±2	98±2	98±1
VSS	mg/L	186±51	4±2	3±2	98±2	98±1
Alkalinity	mg_{CaCO3}/L	339±54	270±19	266±23		
NH_4-N	mg/L	27.0±3.4	34.4±5.1	32.5±4.9		
PO_4-P	mg/L	5.9±0.7	6.7±0.7	6.4±0.8		
SO_4	mg/L	51±4	19±7	19±7	63±13	62±13

Total methane production was around 5 L/d for both reactors, with dissolved methane accounting for 70-77% of the total (Table 5-4). The methane yields achieved were 0.15±0.09 $L_{CH4}/g_{COD,rem}$ for AnMBR-HZ and 0.16±0.10 $L_{CH4}/g_{COD,rem}$ for AnMBR-VERT and were not statistically different. The methane yield was below the theoretical value reflecting the high content of particulate COD in the influent wastewater, which is physically removed by the membrane and not biodegraded, as previously observed by Peña et al. (2019). Moreover, the membrane tanks were not sealed and the headspace gas was not collected and measured during the trial. Therefore, a fraction of methane is likely to have been lost in the tank through the membrane, due to pressure differences during operation. Comparing with the performance of UASB reactors from previous studies (Chapter 2), employing the same sludge and wastewater temperature, while methane production increased in AnMBRs, the methane yields were not significantly different, because of the high fraction of particulate COD removed by the membrane (Table 5-4). However, methane yields obtained in the AnMBRs in this study resulted in line with literature data, which ranged between 0.09-0.23

$L_{CH4}/g_{COD,rem}$ (Gouveia et al., 2015; Kong et al., 2021a; Peña et al., 2019; Seco et al., 2018).

Table 5-4 Methane production and methane yield in the two pilot-scale AnMBRs during the duration of the study. UASB reactors results report the values before the addition of the membrane. UASB-R1 is AnMBR-HZ without membrane, while UASB-R2 is AnMBR-VERT without membrane.

	Gas CH_4 (L/d)	***Dissolved CH_4 (L/d)***	***Total CH_4 (L/d)***	***CH_4 yield ($L_{CH4}/g_{COD,rem}$)***
UASB-R1	0.76±0.43	2.93±1.01	3.70±1.37	0.15±0.04
UASB-R2	0.74±0.29	2.14±0.52	2.89±0.56	0.17±0.05
AnMBR-HZ	1.59±0.83	3.71±0.98	5.30±1.67	0.15±0.09
AnMBR-VERT	1.31±0.63	4.38±0.33	5.69±0.73	0.16±0.10

5.3.2 Fouling rate during short-term tests

The critical flux, defined as a threshold fouling rate of 0.1 mbar/min without sparging, of the horizontally orientated membrane was 30 LMH, which was substantially higher than that observed for the vertically orientated membrane at 9 LMH (Appendix D, Figure D-1and Figure D-2). Previous data for the vertically orientated membrane reported similar values of critical flux of 3-14 LMH without sparging (Martin Garcia et al., 2011). The impact of different gas sparging flow rates was studied at sub- (8 LMH) and supra- (12 LMH) critical conditions for the vertically orientated membrane based on the observed critical flux test. In comparison, the clean water permeability of the two membranes was 306 LMH/bar for the horizontally orientated membrane, corresponding to the suggested value provided by the supplier, and 438 LMH/bar for the vertically orientated membrane, similar to results obtained in other studies with the same membrane module after an enhanced chemical clean (Nascimento et al., 2017).

Sustainable operation, defined as a threshold fouling rate of 1 mbar/h, only occurred at a J_{20} of 8 LMH and $SGD_{m,net}$ of 0.16 $m^3/(m^2 \cdot h)$ for both orientations of the membrane fibres (Figure 5-3). In all cases, the fouling rate for AnMBR-HZ was higher than AnMBR-VERT and increased as the gas sparging rate decreased. To illustrate, in the case of AnMBR-HZ at a flux of 8 LMH, decreasing the $SGD_{m,net}$ from 0.16 to 0.08 $m^3/(m^2{\cdot}h)$ caused a five-fold increase in the fouling rate up to 5 mbar/h and at 12 LMH a three-fold increase up to 9 mbar/h. The exception to this was the AnMBR-VERT operated under supra critical fluxes (12 LMH; net flux of 10.8 LMH) where the fouling rate was 1.44 mbar/h and 1.36 mbar/h at $SGD_{m,net}$ of 0.12 $m^3/(m^2{\cdot}h)$ and 0.16 $m^3/(m^2{\cdot}h)$, respectively (Figure 5-3). Overall, this suggested optimal operational conditions at a minimum SGD_m of 1.2 $m^3/(m^2{\cdot}h)$ and a sustainable flux of 8 LMH (net flux of 7.2 LMH). Comparison to a previous study using the same vertically orientated membrane revealed fouling rates below 1 mbar/h at a net flux of 10 LMH when the SGD_m was above 1.0 $m^3/(m^2{\cdot}h)$ and a net flux of 13.5 LMH when the SGD_m was 2.0 $m^3/(m^2{\cdot}h)$ (Wang et al., 2018a). The reduced performance reflects potential ageing impacts (Lye et al., 2021), but fouling rate typically vary based on differences in the feed water and seed material characteristics (Akhondi et al., 2017). Since the clean water permeabilities at the beginning of each test varied less than 10% (Appendix D, Figure D-3 and Figure D-4), and therefore no deterioration was observed, this suggests that the influent wastewater characteristics had a greater impact.

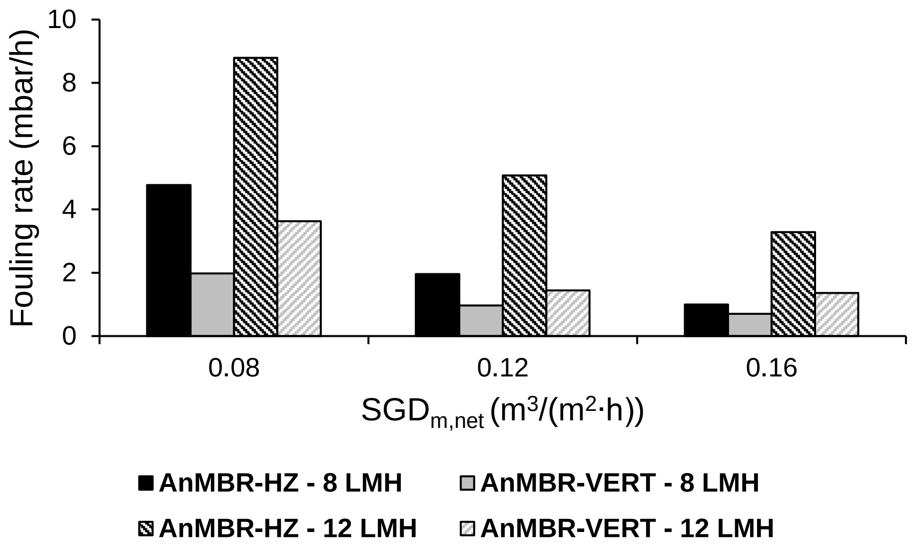

Figure 5-3 Impact of net specific gas demand per membrane area ($SGD_{m,net}$) on fouling rate at different fluxes during short-term (24 h) tests.

The horizontally orientated membrane always generated higher fouling rates compared to those of the vertically orientated membrane at the same flux and $SGD_{m,net}$ (Figure 5-3). For instance, to achieve a similar fouling rate at a flux of 12 LMH, the horizontally orientated membrane required more than double the sparging rate despite operating substantially below the critical flux. Interestingly, the fouling rate in horizontally orientated membrane did not reach a plateau with an increase in gas sparging rate, but it kept decreasing. The implication is that sustainable operation could actually be achieved for higher fluxes by increasing the SGD_m over 1.8 $m^3/(m^2 \cdot h)$.

The difference in fouling observed for the two fibre orientations is consistent with previous findings concerning the relative effectiveness of gas sparging for both (Liu et al., 2016). Importantly, the orientation impacts the hydrodynamic environment generated around the fibres (Chang & Fane, 2000). In the case of vertically orientated fibres, the coarse gas bubbles rise along the length of the fibres and form a pseudo slug flow generating multiple points of dynamic shear and fibre vibration. Support for this view can be taken from the fact that the effectiveness of the sparge is known to reduce when operated for prolonged periods with the maximum impact occurring within the first few seconds. In contrast, in horizontally orientated fibres, the gas bubbles can be trapped between the fibres, reducing the effective sparging efficiency. Moreover, Abdullah et al. (2015) observed that, in a horizontally orientated densely packed membrane modules, most of the shear stresses induced by gas sparging were located on the outside plane of the module, suggesting that the greatest contribution to fouling would likely be confined to the fibres within the inner parts of the fibre bundle. The implication is that whilst the presence of a shell in the C-MEM module improves the contact between coarse bubbles and the fibres, the coiling of the fibres potentially reduces the physical cleaning of a portion of the internal fibres, leading to higher overall fouling rates at fixed sparging rates. It should also be noted that the two membranes were made of different materials, had different pore sizes and optimisation of backwashing is likely to be different.

Akhondi et al. (2017b) reported that cyclic filtration and backwashing at a constant flux can help remove foulants from larger membrane pores, but an increase of the backwashing flux could also remove foulants from smaller pores. However, some drawbacks include the possibility of enlarging the pores, and, if backwashing is performed too frequently, the loss of the cake layer employed as a secondary protection from internal fouling can allow macromolecules to enter the pores (Akhondi et al., 2017). Pore size is, in fact, another parameter that can affect fouling rate. While larger pores are more prone to mechanisms of internal adsorption and pore clogging, smaller pores are expected to reject more particles leading to higher resistance of the cake layer (Le-Clech et al., 2006). However, this type of fouling is believed to be reversible and easily removable. Fouling could also be influenced by the membrane material. While both membranes were hydrophilic, different polymers have different affinities with some fractions of organic matter, which may cause a diversity of the material accumulated on and within the membrane surface (Yamato et al., 2006).

Whilst these membrane parameters are known to also influence fouling behaviour, the impact is expected to be less than that of sparging, which is the principal way to manipulate the hydrodynamics of submerged systems to mitigate fouling (Akhondi et al., 2017). In particular, in this study, the interaction between the bubbles released by gas sparging and the membrane surface is primarily affected by the module configuration and the fibre orientation.

5.3.3 Energy consumption

The energy consumption of the AnMBRs under sustainable operating conditions (J_{20} of 8 LMH and $SGD_{m,net}$ of 0.16 $m^3/(m^2{\cdot}h)$) were 0.07 kWh/m^3 for AnMBR-HZ and 0.10 kWh/m^3 for AnMBR-VERT (Figure 5-4). The consumption for the AnMBR-VERT was in line with previous pilot studies employing the same filtration cycle (Wang et al., 2018a). For comparison, typically reported ranges for the energy demand of membrane operation when treating settled wastewater are 0.16 to 0.88 kWh/m^3 and 0.1 to 2.3 kWh/m^3 when treating crude wastewater (Wang et al., 2018b). Further, analysis of reported granular AnMBRs estimated energy demands between 0.05 and 1.66 kWh/m^3 with an average of 0.39 kWh/m^3 (Anjum et al., 2021).

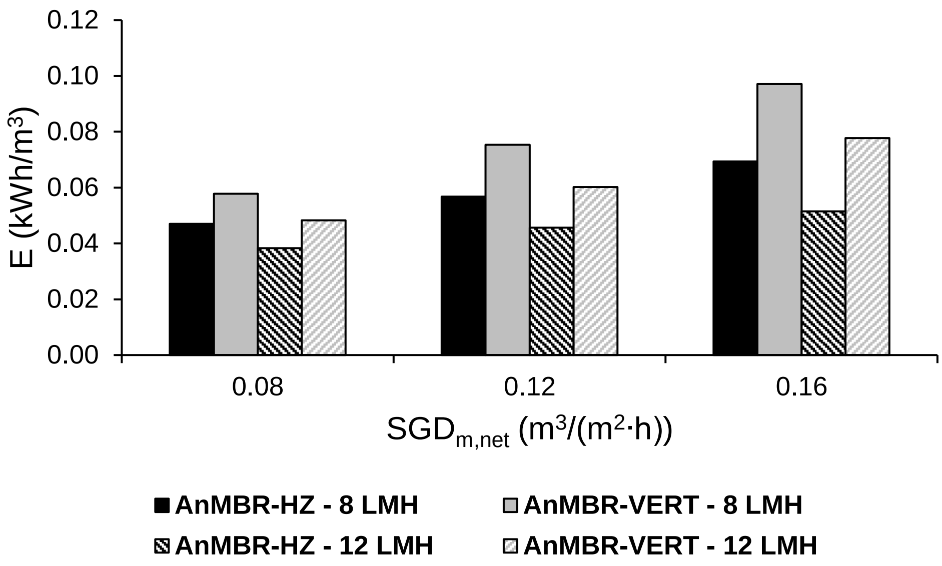

Figure 5-4 Energy consumption for the two pilot-scale AnMBRs for each case tested during the short-term (24 h) trials.

The highest contribution to total energy consumption was ascribed to gas sparging, reaching up to 73% of the total for AnMBR-HZ and up to 82% for AnMBR-VERT (Figure 5-5). This supports previous reports for AnMBRs treating municipal wastewater and reveals the significance of optimisation of the gas sparging operation (Pretel et al., 2014; Shin & Bae, 2018; Shin et al., 2021). The difference between the two systems was based on the water head inside the membrane tank which was 40 cm for AnMBR-HZ and 75 cm for AnMBR-VERT. While this value is specific to the pilot configuration used in the current study, it suggests that the limited size of the C-MEM cartridges in a full-scale application would enable operation under lower water height in the membrane tank, offering an advantage of horizontally over vertically orientated fibre bundles. The energy demand for the permeate pump and backwashing pump accounted for a total of 9% for AnMBR-HZ and 4% for AnMBR-VERT (only permeate pump).

The overall energy balance was calculated considering all methane produced both in liquid and gas phases used for electricity production (with 35.8 MJ/m^3 as methane lower heating value and assuming 32% as the conversion efficiency of an engine to electricity). The balance was positive for both systems reaching 0.88 kWh/y (0.011 kWh/m^3 with 80.3 m^3/y of effective permeate production) for AnMBR-HZ and 0.94 kWh/y (0.016 kWh/m^3 with 58.7 m^3/y of effective permeate production) for AnMBR-VERT (Figure 5-5). Overall, the work demonstrates the importance of optimisation of the gas sparging in delivering an energy positive outcome. It should be noted that these values are based on pseudo dead-end operation where the gas sparge occurs 9-10% of the total time. Operation with more intense gas rates or for longer time periods will significantly impact the overall energy balance. To illustrate, both systems described in Figure 5-5 would become net zero energy if the sparge duration or SGD_m was increased of 20%.

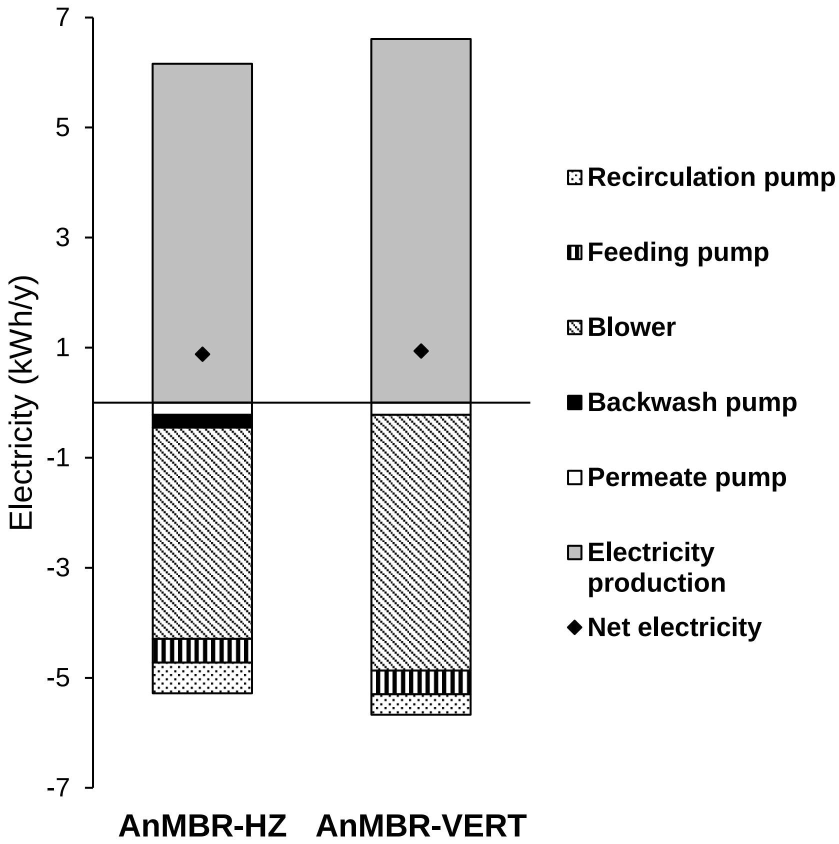

Figure 5-5 Electricity balance for the two pilot-scale AnMBRs. The energy consumption reported is the highest observed during the short-term trials (case A3) corresponding to J_{20} = 8 LMH and $SGD_{m,net}$ = 0.16 $m^3/(m^2{\cdot}h)$. Effective permeate production was 80.3 m^3/y for AnMBR-HZ and 58.7 m^3/y for AnMBR-VERT.

5.3.4 Comparison with demonstration-scale plant

The horizontally orientated fibre module was utilised in a 200 m^3/d demonstration-scale AnMBR plant, enabling comparison between pilot- and full-scale systems. The plant was operated in pseudo dead-end filtration mode at a net flux of 9 LMH and a SDG_m of 1.0 $m^3/(m^2 \cdot h)$. The fouling rate observed over two periods of stable operation was 2.6 mbar/h (Period I) and 2.2 mbar/h (Period II), after a chemical clean (Figure 5-6), indicating that the chemical clean was effective at maintaining operation. The comparative fouling rate in the pilot trials at a lower net flux of 7.3 LMH and a similar SGD_m (0.9 $m^3/(m^2 \cdot h)$) was 4.8 mbar/h, revealing that the smaller pilot-scale membrane module could provide a conservative assessment of the performance data of the full-scale system.

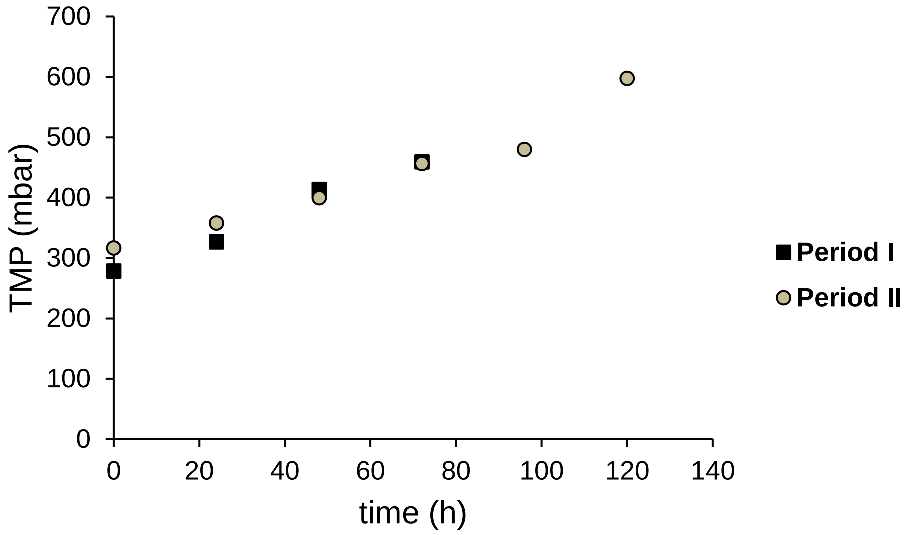

Figure 5-6 Variation in time of maximum daily TMP values during the operation of the demonstration-scale AnMBR (C-MEM) at $J_{20,net}$ of 9 LMH and SGD_m of 1.0 $m^3/(m^2 \cdot h)$. Between the two periods (Period I and Period II) a chemical clean, according to manufacturer's instructions, was performed.

The overall energy consumption of the demonstration-scale AnMBR during the operation at 9 LMH was 0.257 kWh/m^3 (Figure 5-7), which, compared to the values of 0.03-0.07 kWh/m^3 observed at the pilot-scale, indicated a scale factor for the energy demand of membrane operation between pilot- and full-scale of between 3.9-7.6. This is due particularly to higher water head pressures in the full-scale plant and higher backwashing flow rate (14.9 m^3/h per module). This scale factor was above the calculated value of 2.5 reported for a vertically orientated hollow-fibre system treating municipal wastewater at 25 ºC, obtaining a total of 0.315 kWh/m^3 (Kong et al*.,* 2021b). For reference, full-scale aerobic MBRs report energy demands for membrane operation of between 0.19 and 0.7 kWh/m^3 for vertically orientated membranes operated under different sparging regimes (Judd, 2006). The lower end reflects pseudo dead-end and intermittent sparging, albeit at much higher fluxes (15-25 LMH).

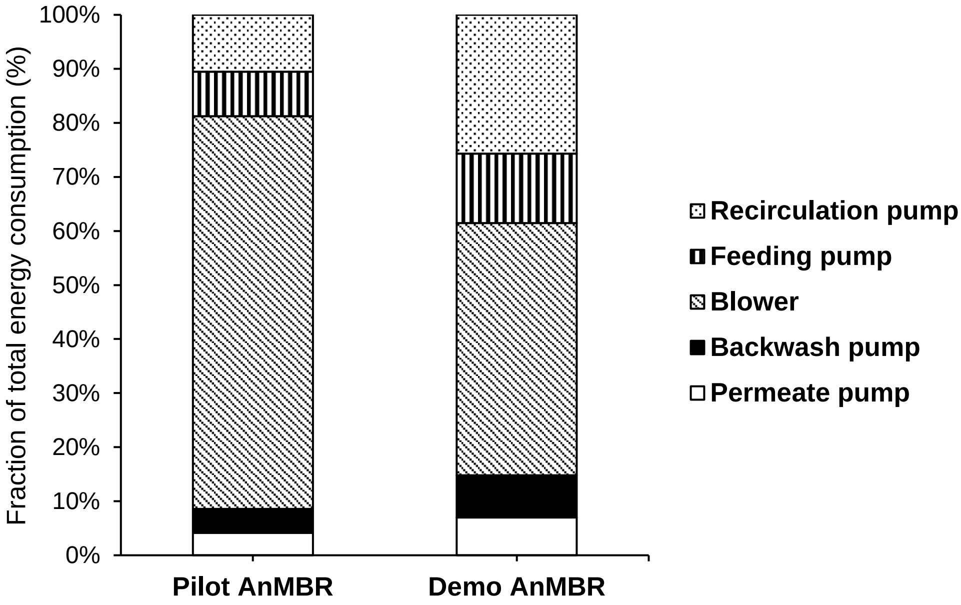

Figure 5-7 Contribution to the total energy consumption in pilot-scale and demonstration-scale AnMBRs, fitted with a horizontally orientated membrane module (C-MEM). Pilot-scale plant was operated at a net flux of 7.3 LMH and SGD_m of 1.8 $m^3/(m^2 \cdot h)$ and demonstration-scale plant was operated at a net flux of 9 LMH and SGD_m of 1.0 $m^3/(m^2 \cdot h)$.

Interestingly, despite the enhanced backwashing, the fouling rates achieved in the demonstration plant were similar to the ones at pilot-scale and still above 1 mbar/h, suggesting the necessity of increasing gas sparging rates above 1 $m^3/(m^2 \cdot h)$ or the duration to reach a sustainable operation at net fluxes over 7 LMH, as observed at pilot-scale. The latter reflects that the presence of the shell could hinder the removal of foulants from within the module, therefore, a longer period of gas sparging could help reduce the solids build-up and improve the scouring of the fibres even at lower gas rates. Importantly, the fouling rates in the two membrane modules did not follow the same trend with increasing gas sparging rates such that it is hypothesised that the different fibre orientations require a different optimisation strategy for fouling control, considering the different hydrodynamic environment around the fibres.

5.4 Conclusions

The operation of a horizontally orientated hollow-fibre membrane module (C-MEM) was compared with a vertically orientated hollow-fibre membrane module (ZW-10) at pilot-scale to anaerobically treat municipal wastewater. The horizontally orientated membrane required higher gas sparging rates (1.8 $m^3/(m^2 \cdot h)$) to reach fouling rates below 1 mbar/h at 7.3 LMH due to the difficulty in physically cleaning the fibres present within the module. However, the reduced height of the horizontally orientated membrane module, enabled operation under a lower water head, reducing the energy demand associated with gas sparging (0.05 kWh/m^3 for AnMBR-HZ and 0.08 kWh/m^3 for AnMBR-VERT). Overall, the net energy production for operation at 7.3 LMH and $SGD_{m,net}$ of 0.16 $m^3/(m^2 \cdot h)$ was 0.88 kWh/y for the horizontally orientated membrane system compared to 0.94 kWh/y for the vertically orientated membrane system.

At larger scale, the C-MEM module performed slightly better to the pilot-scale trials, in terms of fouling rates, suggesting that pilot-scale studies could be more conservative on membrane performance. The energy consumption of the AnMBR was 0.257 kWh/m^3, with gas sparging accounting for the biggest fraction (around 50%). However, as an increase in backwashing flux did not improve fouling rates, it is suggested that the net gas sparging rate would need to be increased to reach a sustainable membrane operation, leading to higher energy consumption. Therefore, developing an optimised targeted strategy for fouling control in horizontally orientated hollow-fibre membranes is key for ensuring the competitiveness and successful application of the process at larger scale.

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6 Overall discussion

This PhD thesis aims at providing a robust scientific assessment of anaerobic membrane bioreactors (AnMBRs), configured as upflow anaerobic sludge blanket (UASB) reactors, for municipal wastewater treatment in temperate climates. To achieve that, the pilot- and demonstration-scale studies carried out were designed to better understand the impact of adaptation of seed sludge, hydrolytic activity, influent wastewater variability and membrane design on the scalability of the process.

6.1 Influent wastewater

Anaerobic treatment for municipal wastewater has been historically deemed as unfavourable due to the low organic strength (<500 mg/L of chemical oxygen demand (COD)) of the influent stream. While many pilot- and full-scale studies proved otherwise (Chernicharo et al., 2015; Ribera-Pi et al., 2020; Robles et al., 2022; Shin & Bae, 2018), this thesis highlighted how prior assessment and careful monitoring of the influent and previous treatment units are crucial for a successful implementation, particularly in temperate climates (<20°C). The critical parameters that have been identified, besides the aforementioned COD, are sulphate (SO_4) and temperature.

During this study, average influent COD in municipal settled wastewater varied between 214-350 mg/L in Chapters 2-5 (Table 2-1, Table 5-3), at pilot-scale, and between 165-351 mg/L in Chapter 4 (Table 4-1), at a full-scale wastewater treatment plant (WWTP). Influent COD was linked to methane content in the biogas, as a direct correlation, simulated by Cakir & Stenstrom (2005), was confirmed both at pilot- and demonstration-scale (Figure 2-3, Figure 4-5). As observed in other studies found in literature (Giménez et al., 2012), the low strength of municipal wastewater led to high nitrogen content in the biogas and therefore less methane (between 24-45% in Chapter 2 and between 33-54% in Chapter 4). During these trials, the major fraction of influent COD was particulate, around 80% at pilot-scale (Table 2-1) and 65% in demonstration-scale (Figure 4-2), which is more difficult to biodegrade, and the biochemical oxygen demand

(BOD) was around 30-40% of COD (Figure 4-2). However, another ratio proved to be more instrumental on the outcome of the anaerobic treatment, i.e. $COD:SO_4$.

Sulphate are commonly present in municipal wastewater and, during anaerobic degradation, sulphate-reducing bacteria (SRB) use sulphate as an electron acceptor for the oxidation of the organic matter, producing sulphide. SRB are therefore in competition for the biodegradable substrate with methanogens and normally outcompete them due to higher substrate affinity (Liu et al., 2018). However, the outcome of this competition at low $COD:SO_4$ (<10) is still not completely understood, as it depends on multiple operational parameters (organic loading rate, pH, oxidation-reduction potential (ORP)). Despite similar $COD:SO_4$ ratios observed in Chapters 2 and 4, ranging between 2-4 (Table 2-1, Table 4-1), sulphate removals observed at pilot-scale (26-38%) were significantly lower than the ones at demonstration-scale (up to 94%), suggesting that the impact of influent sulphate on the overall reactor performance is site-specific, and therefore not applicable for scalability considerations. Moreover, at demonstration-scale, as the SO_4 removal increased from 19% to 85%, the biogas production sharply decreased from 60 L/h to 37 L/h, without significant temperature variations (Figure 4-4). This suggests that the high SRB activity impacted methanogenesis and it was observed that the fraction of influent COD consumed by SRB was more than double the fraction consumed by methanogens (Figure 4-7).

A key parameter in this competition was ORP, identified in Chapter 4. During one of the operational periods, despite a $COD:SO_4$ ratio of 1.4, sulphate removal was only 19% (Table 4-2). Possible causes are low substrate availability (<50 mg_{BOD}/L) and higher values of influent ORP (>-100 mV), as it was observed that an increase in ORP could reduce the activity of SRB (Khanal & Huang, 2003). Therefore, using a fresh non-septic influent could favour the growth and activity of methanogens even with the presence of SRB. If a buffer tank is used to homogenise the flow, like in Chapter 4, decreasing the hydraulic retention time and improving mixing would avoid the development of a reductive environment before the anaerobic reactor. In case the SRB take over, it has been suggested that an increase in the upflow velocity could help washing out these bacteria, as they are lighter than methanogens, while keeping the methanogens inside (Shayegan et al., 2005).

In this study, it was confirmed that organic matter removal and methane production in anaerobic reactors increase with temperature (Figure 2-2, Table 2-2), probably through the direct impact on hydrolysis. This is well known since low temperatures can reduce substrate diffusion rates and increase viscosity of the medium, leading to mass transfer limitations (Dev et al., 2019). Considering the high fraction of particulate COD in municipal wastewater and mass transfer limitations caused by low influent temperatures, a pre-treatment to promote hydrolysis could be helpful, especially at larger scale, to facilitate methane production, reducing the accumulation of non-hydrolysed solids within the reactor, and therefore increase the net energy balance.

A number of pre-treatments have been studied and applied to anaerobic reactors. Fortification of influent wastewater with pre-hydrolysed sludge allows to increase the influent organic strength. While this leads to an increase in methane production in the anaerobic reactor, this pre-treatment can cause the reduction of particle size, hence lower particle retention and solids washout, which affects the effluent quality (Lester et al., 2013). A two-stage anaerobic process, with pre-hydrolysis of the influent particulate organic matter before the UASB reactor, would separate the rate-limiting step from the other stages and improve the performance of the UASB reactor. Nevertheless, the operational conditions of the pre-treatment need to be carefully monitored in order to avoid losing carbon as methane in the hydrolysis unit (Álvarez et al., 2008; Rajagopal et al., 2019). Forward osmosis (FO) can be used to successfully concentrate wastewater and improve the organic loading rate of anaerobic systems for biogas production. However, FO technology is still under development and some issues may arise with the integration of an anaerobic treatment to a FO membrane, besides membrane fouling and salinity accumulation, such as inorganic salt inhibition, ammonia toxicity and phosphorus precipitation (Ansari et al., 2017; Vinardell et al., 2020).

6.2 Upflow anaerobic sludge blanket reactor

The upflow anaerobic sludge blanket reactor is one of the most widely used high-rate anaerobic reactors for municipal wastewater treatment. While its application has been clearly demonstrated at full-scale in tropical climates (Chernicharo et al., 2015), in this study we investigated its performance at low temperatures (<20°C) and its scalability. Since mesophilic sludge, which is normally employed in these installations, present lower activities at low temperatures, the choice of the seed sludge to use in temperate climates is still debated. This study showed that a mesophilic industrial sludge is able to achieve the same (or higher) removal efficiencies, hydrolytic percentages and methane production of an adapted sludge (Chapter 2). Moreover, the UASB reactor proved to be a robust technology since removals of COD and total suspended solids (TSS) were quite stable during the entire trial (Figure 2-2), though affected by temperature. However, the concentrations of a UASB reactor effluent do not allow direct discharge, as they are above limits, making a post-treatment necessary.

The UASB reactor was able to achieve hydrolysis percentages of around 19% at 13°C and 30% at 19°C. A linear correlation between hydrolysis and methanogenesis was found, indicating that hydrolysis was the limiting step of the anaerobic degradation (Figure 2-4). Moreover, it was found that the size of sludge particles could impact hydrolysis, as smaller particles, providing a higher specific surface area, led to higher hydrolytic efficiencies (Figure 2-5). Analysis of the bacterial community in the sludge showed that, after 10 months of operation, despite a very different starting composition, the major orders and their relative abundances in the two types of sludge converged (Figure 2-6). Furthermore, enzymatic tests on β-glucosidase, lipase and protease revealed that both sludges did not develop psychrophilic enzymes (Figure 3-3), characterised with highest activity between 15-20°C, but the optimal microbial growth temperature, corresponding to the enzymes highest substrate affinity (Kulakova et al., 2004), matched the operational temperature of the reactors (15-20°C), suggesting adaptation of the mesophilic inoculum to low temperature (Figure 3-4).

Regarding methane production, the majority of methane was found dissolved in the effluent (around 74-89% at pilot scale and 74-92% at demonstration-scale, at temperatures <21°C). In Chapter 2, methane yield of methane in gas phase was on average only 0.03 $L_{CH4}/g_{COD,rem}$, while in the liquid phase achieved 0.11 $L_{CH4}/g_{COD,rem}$ (Table 2-2). At pilot-scale, biogas production did not display sudden changes, as COD concentration and SO_4 removal stayed mostly constant. However, at demonstration-scale, the abrupt increase in SO_4 removal caused a sharp decrease in biogas production, which was registered starting from approximately 10 days after the change in influent wastewater (Figure 4-4). Moreover, the presence of dissolved methane in-line sensors allowed to see daily variations, which were likely related to daily temperature fluctuations, reaching concentrations up to 24 mg/L. Therefore, the recovery of this fraction is important for the energy balance and to avoid greenhouse gas emissions of the system and appropriate monitoring would help maximise its recovery.

The scale-up of the UASB reactor to demonstration-scale (200 m^3/d), using the same non-adapted inoculum employed at pilot-scale, which showed to be able to treat municipal wastewater as well as an adapted inoculum, allowed to make few observations on the scalability of the system. The demonstration-scale UASB reactor used in this study was a rectangular tank, designed for industrial wastewater treatment. The influent was distributed with two holed pipes placed lengthwise at the bottom. However, for municipal wastewater application, this could be upgraded following the designs of full-scale plants operated in tropical climates (Chernicharo et al., 2015), to help promote sludge blanket development and mass transfer, alongside an internal recirculation in the digestion compartment. In this study, the sludge blanket resulted not very well developed during the months of operations since sludge was mainly found in the bottom quarter of the reactor (Appendix E, Figure E-1), suggesting the formation of preferential pathways and hydraulic short circuits, which would have decreased the hydrolytic efficiency.

6.3 Anaerobic membrane bioreactor

Anaerobic membrane bioreactors are one of most promising technologies to improve hydrolysis of particulate matter at low temperature, increasing the sludge retention time and contact between biomass and substrate, and reduce effluent concentrations below discharge limits, using ultrafiltration membrane modules. In this study, a horizontally orientated coiled polyethylene hollow-fibre membrane cartridge (C-MEM) was compared with a vertically orientated polyvinylidene fluoride (PVDF) hollow-fibre membrane module (Z-10), in terms of treatment performance, fouling rates and energy consumption in pilot-scale AnMBRs (Figure 5-2).

The application of horizontal membrane modules is not currently very common, as original horizontal modules presented issues of clogging (Hai et al., 2005). However, the C-MEM module, being combined into bundles and wound up around a cartridge carrier, allows for reduced space requirements and lower differences in the hydrostatic pressures along the cartridge, which led to lower energy consumption (Chapter 5). Moreover, the possibility of using an alternative material to PVDF might be interesting since the production and disposal of PVDF membranes is currently under threat due to a proposed restriction on PFAS-containing products, submitted to the European Chemicals Agency in January 2023 and currently under consultation at the time of the writing of this thesis (ECHA, 2023).

Fouling, the deposition of particles and colloids on or inside the membrane surface, is the main drawback in membrane processes, as it requires cleaning and management strategies, reduces permeate productivity and increases energy consumption. At a net flux of around 7 LMH, AnMBR-VERT, using ZW-10, was able to maintain fouling rates below 1 mbar/h with a specific gas demand per membrane area (SGD_m) of 1.2 $m^3/(m^2{\cdot}h)$, while AnMBR-HZ, using C-MEM, achieved a fouling rate of 1 mbar/h only at 1.8 $m^3/(m^2{\cdot}h)$ (Figure 5-3). Therefore, higher sparging rates were necessary for the horizontally orientated module, mainly due to its highly packed configuration, which prevents the internal fibres to be thoroughly cleaned. However, in the same sustainable conditions, the limited height of the horizontal module allowed for lower energy consumption (0.07 kWh/m^3 for AnMBR-HZ and 0.10 kWh/m^3 for AnMBR-VERT) (Figure 5-4).

At larger scale, the C-MEM module performed slightly better than the pilot-scale trials, in terms of fouling rates, suggesting pilot studies provide a conservative assessment of membrane performance. However, the energy consumption increased to 0.257 kWh/m^3, with gas sparging accounting for the biggest fraction (Figure 5-7). Since an increase in backwashing flux did not improve fouling rates, it is suggested that the net gas sparging rate would need to be increased to reach a sustainable membrane operation, leading to higher energy consumption. Therefore, the use of a horizontal module, which proved to be competitive to a vertical module in terms of energy demand, needs to be paired with a targeted strategy for gas sparging optimisation, accounting for the hydrodynamic environment created by the horizontal fibres, in order to limit the energy consumption. Overall, anaerobic membrane bioreactors can help achieve the target of Net Zero carbon emissions, as they produce lower greenhouse gas emissions, by saving energy consumption, and have a smaller carbon footprint, due to lower waste sludge produced, than aerobic processes. Moreover, without ammonium removal, nitrous oxide, a potent greenhouse gas, is not produced.

The use of a membrane in combination with a UASB reactor allowed to increase the rate of solids hydrolysed in the biological reactor from 0.82-0.86 g/(L·d) to 1.57-1.87 g/(L·d) (Table 3-3). Moreover, enzymatic tests on β-glucosidase, lipase and protease reported higher specific activities and slightly higher substrate affinities in an AnMBR compared to a UASB reactor (Figure 3-3, Figure 3-4).

Permeate of a demonstration-scale AnMBR was characterised by low BOD and TSS concentrations, between 11-37 mg/L and 5-7 mg/L, respectively (Table 4-2). However, dissolved sulphide was around 26 mg/L, dissolved methane ranged between 7-15 mg/L and concentrations of nutrients reached 46 mg/L for ammonium and 7 mg/L for phosphate. Therefore, post-treatments on AnMBR effluent should focus on the removal of sulphide to prevent corrosion and bad odours, removal and recovery of dissolved methane to avoid greenhouse gas emissions and optimise the energy balance and removal and recovery of nutrients for water reuse applications. If nutrients are left in the effluent, possible applications of the AnMBR effluent would be fertigation, which is a simultaneous reuse of nutrients and water, struvite crystallisation and microalgae cultivation.

In anaerobic treatment of municipal wastewater, due to the high solubility of hydrogen sulphide at low temperatures, the highest fraction of sulphide is expected to be found dissolved in the effluent. As presented in Chapter 4, few controlling methods are available to inhibit the formation of sulphide, acting mainly on operational parameters, however, others focus on the on-site elimination of the generated sulphide. These include addition of iron or the use of trace silver and iron nanoparticles for precipitation of sulphide (Liu et al., 2018). Another interesting application involve the electrochemical sulphide oxidation into sulphur using metal-based electrodes (Pikaar et al., 2011; Sergienko & Radjenovic, 2021). However, the currently available technologies for sulphide removal from aqueous solutions have still a low technology readiness level and more research is needed to be able to apply them at full-scale.

In the demonstration-scale plant of this study, the presence of dissolved sulphide in the AnMBR effluent caused the deposition of a sulphur-based material on the walls of the pipes following the membrane tank (Appendix E, Table E-1). These pipes were part of a degassing system for the removal of dissolved methane (Appendix E, Figure E-2). The degassing unit included three polypropylene hollow-fibre membrane modules with four contactors each for a total membrane area of 212 m^2. Of the three modules, two were operated in series while one was always in stand-by. The first module was operated in vacuum mode, to collect as much methane as possible from the stream. The second one employed both vacuum and nitrogen as sweep gas (1.3 Nm^3/h) to polish the effluent, ensuring the dissolved methane was below discharge limits. Methane content in the gas from the first module of the degassing unit, which was mixed with the AnMBR biogas, reached up to 40-50%, making it suitable, prior an impurities assessment, for utilisation in combined heat and power systems. Other possible solutions for removal of dissolved methane include gas stripping, biological oxidation and vacuum degassing. Furthermore, biological conversion of dissolved methane into value-added products, specifically methanol, while still at research stage, is gaining traction because methanol can be used as a carbon source in denitrification, reducing the external carbon demand of WWTPs, or as substrate in microbial fuel cells for electricity production (Li et al., 2021).

Following the degassing system, the permeate was fed to ion exchange columns, which is a promising technology for the removal of nutrients (nitrogen and phosphorus). Zeolite-N was used as media for the removal of nitrogen (N), while an iron-based hybrid anion exchanger was used for phosphorus (P). Regeneration of each media was performed separately with a sodium hydroxide solution, which, once saturated, was further processed for the recovery of N and P from the solution (Guida et al., 2021a; Guida et al., 2021b). Recovered nutrients are mainly applied in the fertiliser industry, with the aim of reducing the impact of the industrial production processes and the extraction of phosphorus rock, identified by the European Union (EU) as a critical raw material in 2017 (Jiménez-Benítez et al., 2020). The effluent of the ion exchange presented BOD of 4.1 mg/L, turbidity of 0.3 NTU and absence of suspended solids and *E. coli*, adhering to the latest EU regulation (2020/741 Minimum Requirements for Water Reuse) on water reuse for irrigation, which came into place in June 2023.

In conclusion, the possibility of recovering useful resources, such as water, energy and nutrients, from wastewater, using an AnMBR and following a circular economy approach, can help the water sector contribute to the national and international Sustainable Development Goals (SDGs). In particular, it can address SDG 2 (Zero hunger), SDG 6 (Clean water and sanitation) and SDG 7 (Affordable and clean energy).

6.4 Contribution to knowledge of the thesis

This thesis contributed to advance the knowledge on the application of anaerobic membrane bioreactors for municipal wastewater treatment, identifying critical parameters for the enhancement of hydrolytic activity and methane production and for a successful scale-up in temperate climates. The contribution to knowledge is presented in Table 6-1 and graphically summarised in Figure 6-1.

Table 6-1 Summary of the contribution to knowledge of the different chapters of the thesis divided in knowledge (including theoretical and empirical) and methodology.

Knowledge		
What has been confirmed?	**What has been developed?**	**What has been found and is new?**
In UASB reactors, removal of COD and TSS, methane production and hydrolysis percentages increased with temperature (Chapter 2).	In UASB reactors, the relationship between influent COD concentration and methane content in the biogas was linear (Chapters 2-4).	A UASB reactor seeded with a non-adapted inoculum achieved non-significantly different removal efficiencies and methane yields to a UASB reactor seeded with an adapted inoculum and operated at the same conditions (Chapters 2-3).
Methane produced in UASB reactors was found mostly dissolved in the effluent (74-89% at pilot-scale and 74-92% at demonstration-scale) (Chapters 2-4).	A linear correlation between hydrolysis and methanogenesis in anaerobic reactors was found (Chapter 2).	Hydrolytic enzymes in UASB reactors and AnMBRs displayed higher substrate affinity at the operational temperature of the reactor (Chapter 3).
Removal efficiencies, methane production and hydrolytic enzyme activities increased with the addition of a membrane to a UASB reactor (Chapter 3).	Hydrolysis percentages were inversely proportional to the Sauter mean diameter of the sludge particles in UASB reactors (Chapter 2).	After more than a year of operation at temperature below 20°C, hydrolytic enzymes in anaerobic sludge did not present a psychrophilic nature (Chapter 3).
The rate of solids hydrolysed increased with the addition of a membrane to a UASB reactor (Chapter 3).	The bacterial community of two different types of anaerobic sludge converged towards the same orders after 10 months of UASB operation at the same conditions (Chapter 2) and, with the addition of a	An AnMBR fitted with a horizontal hollow-fibre coiled membrane module achieved the same removal efficiencies of an AnMBR fitted with a vertical hollow-fibre

	membrane, the overall abundance of bacteria with hydrolytic abilities increased (Chapter 3).	membrane module operated at the same conditions (Chapter 5).
Gas sparging is the biggest contributor (50-80%) to the overall energy consumption of an AnMBR (Chapter 5).	An increase in sulphate removal in an AnMBR was concomitant to a decrease in biogas production and a decrease in methane yield (Chapter 4).	Horizontal hollow-fibre coiled membranes required higher sparging rates (1.8 $m^3/(m^2·h)$) than vertically orientated modules (1.2 $m^3/(m^2·h)$) to reach a fouling rate of 1 mbar/h at 7.3 LMH in pilot-scale AnMBRs (Chapter 5).
Pilot-scale studies on membrane modules could provide a conservative assessment of the performance of the full-scale system (Chapter 5).	Low substrate availability (<50 mg_{BOD}/L) and higher values of influent ORP (>-100 mV) determined a low SO_4 removal (<20%) in a demonstration-scale AnMBR (Chapter 4).	A pilot-scale horizontal hollow-fibre coiled membrane module consumed less energy than a vertical module for the operation of AnMBRs at 8 LMH and $SGD_{m,net}$ of 0.16 $m^3/(m^2·h)$ (Chapter 5).

Methodology

What has been confirmed?	What has been developed?	What has been found and is new?
	Enzymatic assays developed for activated sludge have been adapted to anaerobic sludge, however further method development is needed (Chapter 3).	The application of a C-MEM module for anaerobic treatment of municipal wastewater requires a targeted strategy for fouling control to ensure sustainability of operation at larger scale (Chapter 5).

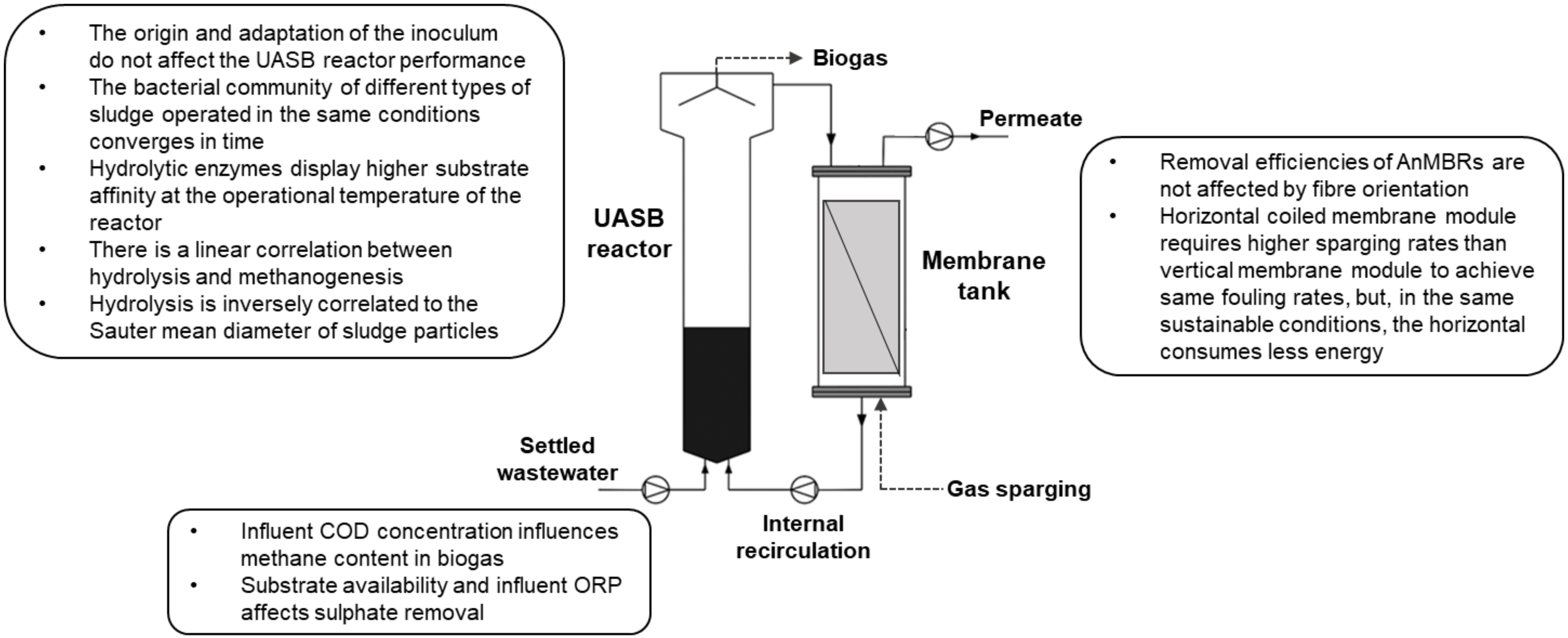

Figure 6-1 Graphical summary of the contribution to knowledge of the thesis.

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7 Conclusions and future work

7.1 Conclusions

This study demonstrated the suitability of anaerobic membrane bioreactors (AnMBRs) for municipal wastewater treatment at low temperature, identifying how critical parameters, like the seed sludge and influent characteristics or membrane design, affect the process and how site-specific control strategies to manage fouling and sulphate concentration are necessary to ensure the successful application at full-scale in temperate climates. Key findings from the thesis are presented according to the initial objectives.

Objective 1: To understand the role of physical and microbiological properties of the seed sludge on the treatment of municipal wastewater in a pilot-scale upflow anaerobic sludge blanket (UASB) reactor at low temperatures (<20°C), in terms of hydrolytic efficiency, effluent quality and methane production.

- The physical and microbiological properties of the inoculum did not impact the treatment of municipal wastewater in a UASB reactor. A fresh industrial mesophilic granular sludge was able to achieve the same removal efficiencies and methane yields of an adapted inoculum. Further to this, after 10 months of operation the two sludges converged towards the same bacterial orders and relative abundances and to similar particle size distributions.
- Removal efficiencies of carbon and solids, hydrolysis percentages and methane production improved with an increase in temperature. Methanogenesis was linearly correlated to hydrolysis, which was inversely correlated to the Sauter mean diameter of the sludge particles.
- Methane content in biogas was correlated to the influent chemical oxygen demand (COD) concentration.
- Methane was found mostly dissolved in the effluent, achieving around 74-89% of the total methane production.

Objective 2: To understand the impact of membrane ultrafiltration in pilot-scale UASB reactors on the activity of key hydrolytic enzymes and their substrate affinities at different temperatures.

- The conversion of UASB reactors to anaerobic membrane bioreactors (AnMBRs) with the addition of a membrane ultrafiltration process improved removal efficiencies (from 49-57% to 88-92% COD removal), rate of solids hydrolysed (from 0.82-0.86 g/(L·d) to 1.57-1.87 g/(L·d)) and methane production (from 2.3-2.7 L/d to 5.3-5.7 L/d). In AnMBRs the overall abundance of bacteria with hydrolytic abilities, identified in the UASB reactors, increased.
- The specific activity of β-glucosidase, protease and lipase increased with temperature (from 4°C to 37°C), implying that the operation at low temperature did not cause the development of psychrophilic enzymes in mesophilic sludge.
- The Michaelis-Menten constant of β-glucosidase and lipase was lower at 15-20°C and higher at 4-37°C, suggesting higher substrate affinity, and therefore temperature for optimal bacterial growth, at the operational temperature of the reactors, which ranged around 16-20°C.
- The AnMBRs presented higher specific enzymatic activities and substrate affinities than UASB reactors.

Objective 3: To investigate the impact of influent wastewater fluctuations of chemical oxygen demand (COD), sulphate (SO_4) and oxidation-reduction potential (ORP) on effluent quality, methane production, competition between sulphate-reducing bacteria (SRB) and methanogens and scalability of a demonstration-scale AnMBRs for municipal wastewater treatment at low temperatures.

- At influent COD concentrations of 351 mg/L (124 mg/L of biochemical oxygen demand (BOD)) and COD:SO_4 ratio of 3.7, sulphate removal was 88% and methane yield was 0.06 $L_{CH4}/g_{COD,rem}$. COD consumed by sulphate-reducing bacteria was 16% of influent COD, while COD used for methane production was 10%.

- Lower substrate availability (43 mg/L of BOD) and higher influent ORP (>-100 mV) led to a lower SO_4 removal (19%) and improved methane yield (0.17 $L_{CH4}/g_{COD,rem}$). COD consumed by sulphate-reducing bacteria was 9% of influent COD, while COD used for methane production was 23%.
- Careful monitoring of site operations and influent characteristics and control strategies to reduce SRB activity resulted to be necessary to obtain a stable performance within the anaerobic reactor and prevent sulphide formation at demonstration-scale.

Objective 4: To understand the impact of fibre orientation (vertical or horizontal) and arrangement (coiled or not) in hollow-fibre membrane modules on fouling control, and therefore energy consumption, of AnMBRs treating municipal wastewater at different scales in temperate climates.

- The orientation and arrangement of membrane fibres did not affect the performance of pilot-scale AnMBRs, in terms of effluent quality and methane production.
- The horizontally orientated membrane module required higher gas sparging rates (1.8 $m^3/(m^2 \cdot h)$) to reach fouling rates below 1 mbar/h at 7.3 $L/(m^2 \cdot h)$ compared to the vertically orientated module due to the difficulty in removing fouling from the fibres present within the module.
- The reduced height of the horizontally orientated membrane module enabled lower energy demand associated with gas sparging (0.05 kWh/m^3 for the horizontal module and 0.08 kWh/m^3 for the vertical one for a $SGD_{m,net}$ of 0.16 $m^3/(m^2 \cdot h)$).
- At demonstration-scale, fouling rates were lower compared to pilot-scale trials, but energy consumption increased to 0.257 kWh/m^3, 4-8 times higher than the pilot-scale results, due to higher water head pressures and backwashing flow rate.

7.2 Future work

Several areas requiring further research have been identified during this thesis.

- To advance the knowledge on hydrolytic enzyme activity in high-rate anaerobic reactors treating municipal wastewater at low temperature, the assays methodology can be further developed, including different methods for sample preparation or enzyme purification and extraction and different substrates.
- Long-term trials are needed at demonstration-scale to evaluate operation of AnMBRs for municipal wastewater treatment with variable influent flow, in order to investigate a typical wastewater treatment plant scenario.
- A different design of the UASB reactor at demonstration-scale for municipal wastewater treatment in temperate climates could be developed to ensure a more uniform distribution of the influent flow and better contact between biomass and substrate.
- Further research is needed to determine how to optimise gas sparging in horizontally orientated membrane module to limit energy consumption in AnMBR applications.
- Methods to control sulphate-reducing bacteria activity in AnMBRs will need to be tested at full-scale to better understand the competition between SRB and methanogens at low COD:SO_4, typical of municipal wastewater.
- A technology for dissolved methane removal and recovery needs to be demonstrated at full-scale to determine the energy balance of the system.
- A life-cycle assessment and business case could be developed from the operation of AnMBR at demonstration-scale to compare the system with conventional secondary aerobic processes and determine its commercial viability.

Appendices

Appendix A Ethical approval letter

8 October 2019

Dear Ms Paissoni ,

Reference: CURES/9359/2019
Title: Demonstration of anaerobic membrane bioreactors for resource recovery in wastewater treatment applications

Thank you for your application to the Cranfield University Research Ethics System (CURES).

We are pleased to inform you your CURES application, reference CURES/9359/2019 **has been reviewed. You may now proceed with the research activities you have sought approval for.**

If you have any queries, please contact CURES Support.

We wish you every success with your project.

Regards,

CURES Team

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Figure A-1 Ethical approval letter for the thesis.

Appendix B Understanding the impact of the type of seed sludge on municipal anaerobic wastewater treatment in temperate climates

Figure B-1 Pilot-scale upflow anaerobic sludge blanket reactors.

Table B-1 Characterisation of influent and UASB effluents wastewater during the first 100 days of operation at pilot-scale.

	Influent	***Effluent UASB-R1***	***Effluent UASB-R2***
	days 1-100	***days 1-100***	***days 1-100***
Temperature (°C)	18.7±1.8	17.6±2.3	17.7±2.4
pH	8.02±0.15	7.68±0.18	7.88±0.11
COD (mg/L)	154±70	61±34	72±39
sCOD (mg/L)	36±5	31±5	30±3
BOD (mg/L)	67.6±11	37±5	43±8
SO_4 (mg/L)	61±3	42±14	51±9
TSS (mg/L)	110±35	38±14	47±12
VSS (mg/L)	107±33	33±14	40±10
Alkalinity (mg_{CaCO3}/L)	302±62	246±34	235±33
NH_4-N (mg/L)	15.0±3.2	19.8±3.0	17.6±2.2
PO_4-P (mg/L)	3.4±0.6	3.8±0.7	3.5±0.6

Appendix C Impact of influent characteristics on demonstration-scale anaerobic membrane bioreactor for municipal wastewater treatment in temperate climates

Figure C-1 Demonstration-scale UASB reactor (in black).

Figure C-2 C-MEM membrane cartridges used in the demonstration-scale AnMBR. Picture reported with permission from manufacturer.

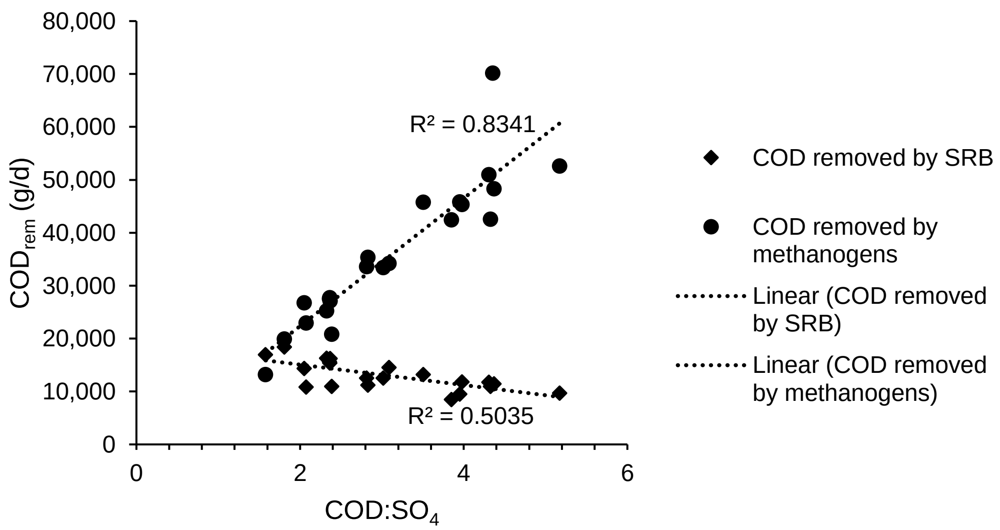

Figure C-3 Correlations between COD removed by suphate-reducing bacteria (and COD removed by methanogens) and COD:SO_4 ratio during Periods III, IV and V.

Appendix D Investigating the impact of horizontally orientated hollow fibres in pilot- and demonstration-scale anaerobic membrane bioreactors

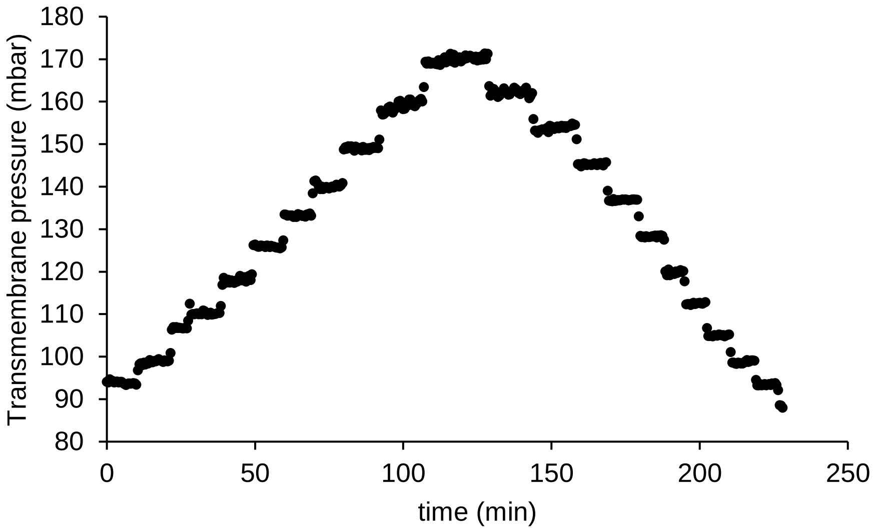

Figure D-1 Critical flux test for AnMBR-HZ. Flux step of 3 LMH.

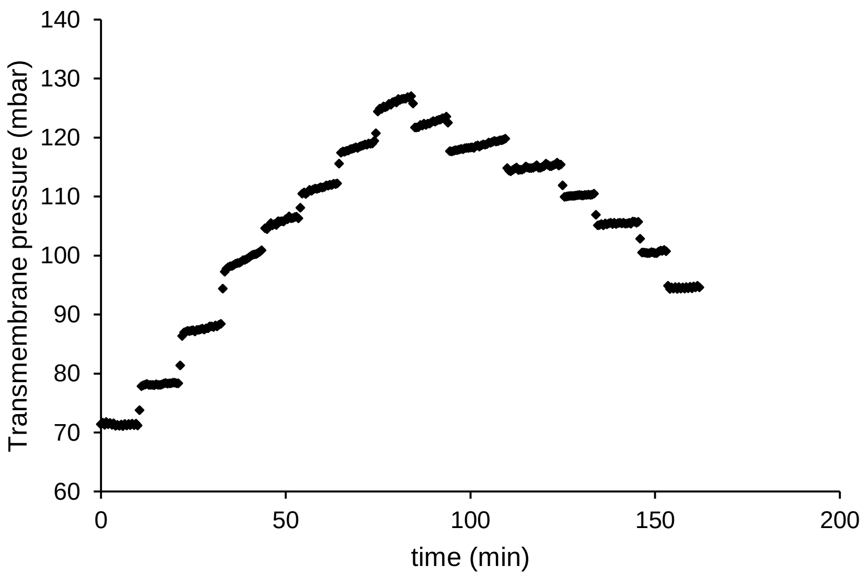

Figure D-2 Critical flux test for AnMBR-VERT. Flux step of 3 LMH.

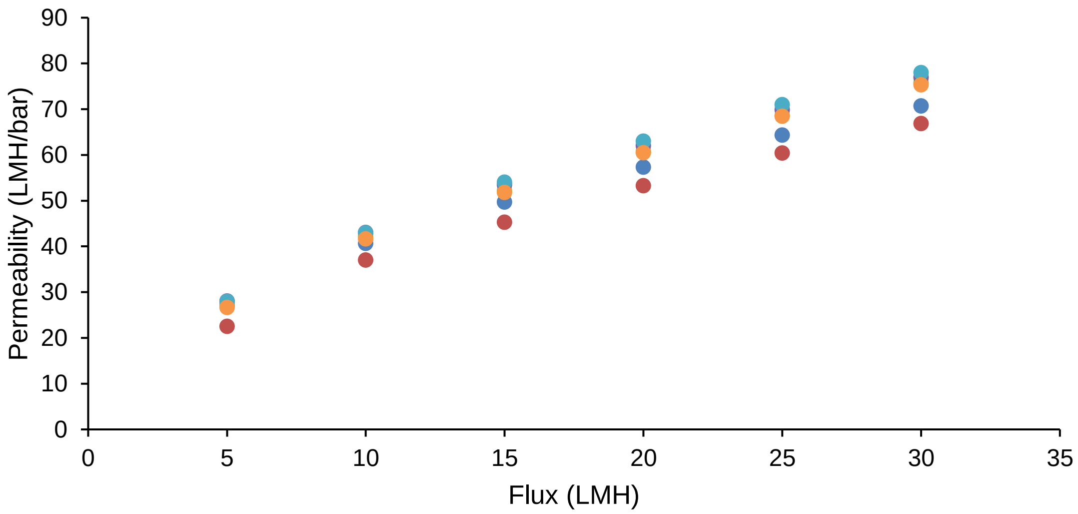

Figure D-3 Variation of clean water permeability in AnMBR-HZ during short-term tests.

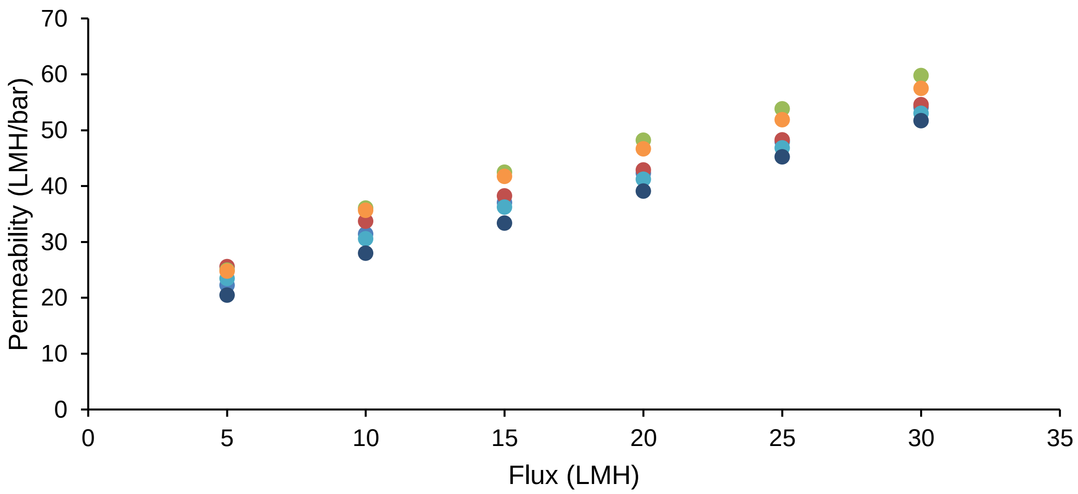

Figure D-4 Variation of clean water permeability in AnMBR-VERT during short-term tests.

Appendix E Overall discussion

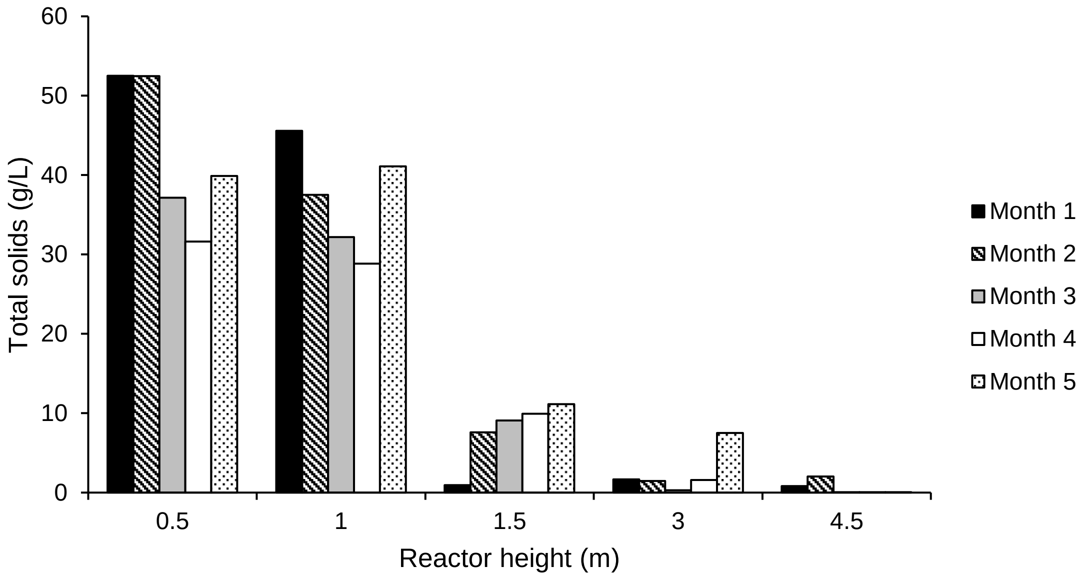

Figure E-1 Evolution in time of the total solids concentration in the sludge bed along the height of the demonstration-scale UASB reactor.

Table E-1 Statistics of elemental composition of the precipitate found in the degassing pipes obtained with scanning electron microscopy.

Statistics	***C***	***N***	***O***	***Na***	***Al***	***Si***	***P***	***S***	***Cl***	***K***
Max	56.49	6.05	6.16	0.16	0.07	0.06	0.21	83.20	0.22	0.11
Min	14.82	6.05	1.98	0.16	0.03	0.03	0.21	30.74	0.22	0.11
Average (%)	27.27		3.69					67.88		
Standard Deviation	15.32		1.49					19.29		

Figure E-2 Demonstration-scale degassing system for the removal of dissolved methane.