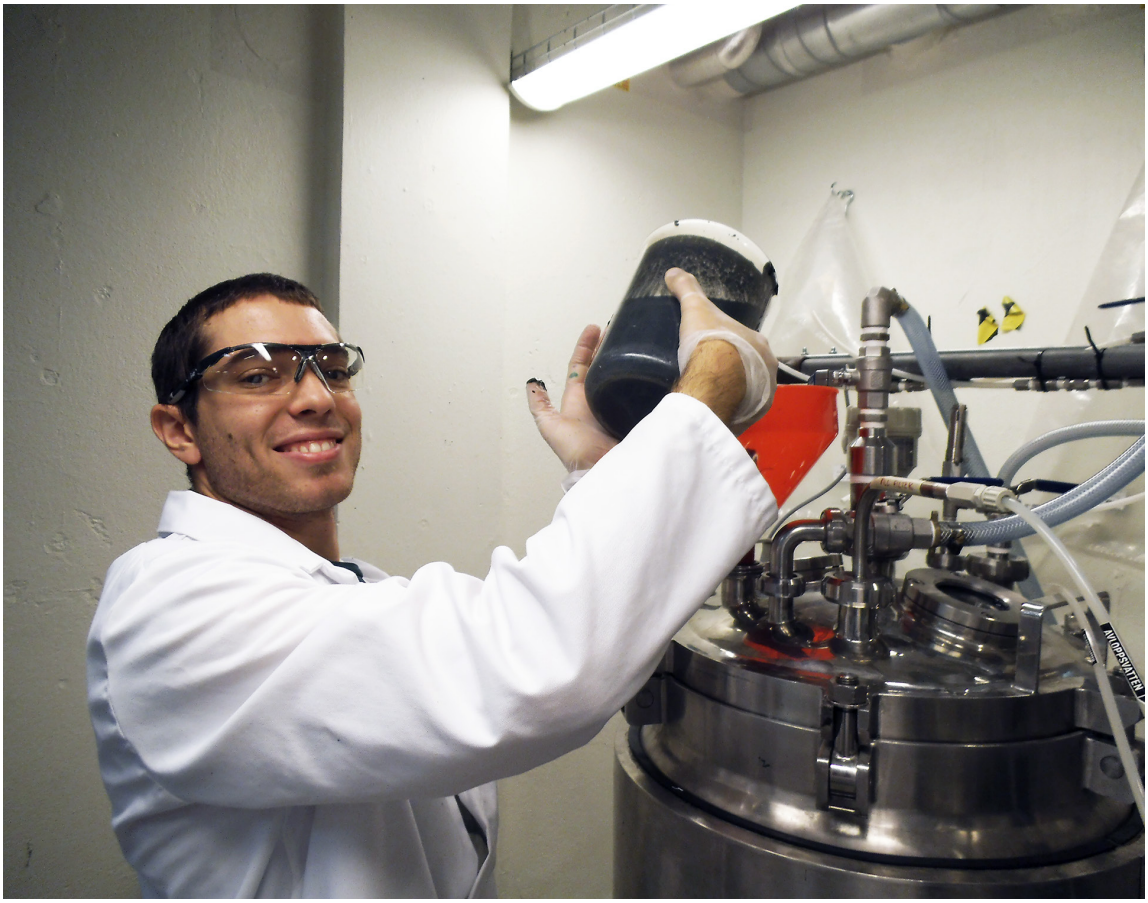
Startup and stabilization of anaerobic membrane bioreactors at ambient temperature



Carlos Benito

Water and Environmental Engineering
Department of Chemical Engineering
Master Thesis 2015

LUNDS UNIVERSITET

Startup and stabilization of anaerobic membrane bioreactors at ambient temperature

by

Carlos Benito

Master Thesis number: 2015-09

Water and Environmental Engineering
Department of Chemical Engineering
Lund University

May 2015

Supervisor: **Hamse Kjerstadius**
Co-supervisor: **Professor Jes La Cour Jansen**
Examiner: **Research associate Åsa Davidsson**

Picture on front page: “Pouring inoculum to reactor 1”. Photo by Hamse Kjerstadius.

Postal address
P.O. Box 124
SE-221 00 Lund, Sweden
Web address
www.vateknik.lth.se

Visiting address
Getingevägen 60

Telephone
+46 46-222 82 85
+46 46-222 00 00
Telefax
+46 46-222 45 26

Acknowledgements

This thesis work could have not been possible without the continuous assessment, contribution and support of *Hamse Kjerstadius*, with his constant effort and both experience and ability to overcome challenges that inspired and helped me to achieve all goals. I am grateful for the opportunity that he gave me to work hand in hand, relying on me during my stay in Lund University. *Hamse* has been my teacher, my colleague and my supervisor. I learned all the secrets of anaerobic technology and most laboratory procedures thanks to his selflessly assistance.

Thanks to *Alfa Laval* for the support and guidance, and for being the main provider of all the equipment in this work.

All the correct equipment operation is the merit of *Leif*, who assisted me and *Hamse* giving his experience as a technician, and resolving all operational problems together with us. Without him it would have taken endless effort to achieve functioning.

I wish to thank all the team of the Water and Environmental Engineering group at Chemical Engineering Department, for helping me feel like home. They were always open to help and provided a very comfortable ambient. Specially thanks to *Maria Messer* and *Maja Ekblad* for all their help in the office and laboratory, and also showing me the most common Swedish traditions during fall and winter, inviting me to *fyka*. I am grateful for being accepted as part of the team and participate in all common activities and leisure.

I want to mention my special gratitude to *Gertrud Persson* for being my laboratory supervisor, appreciating all the patience that she had with me. I want to thank all her time dedicated to show me unknown laboratory analysis methods and to continuous analysis of VFA samples.

Christoph Wenzel and *Marco Fezzi* have been my dearest workmates during this work. I am grateful to them for making much more pleasant all the tough times.

I also thank *Åsa Davidsson* and *Jes La Cour Jansen* for their crucial contribution in helping me as an opponent and being my examiners.

I would like to mention my family, who knew when to listen and when to give me help. Especially to my father, *Enrique*, by which his wisdom helped me finding clear pathways between the dark.

Last but not least, I would like to thank my girlfriend *Anna*, for giving me all her time needed when problems arose. She enlightened my way and was my main moral support, being able to put things straight and helping me focus on what is really important.

Summary

There has been an increasing interest in wastewater treatment in last decades to reduce human footprint. Primarily, anaerobic technology focused on treatment and stabilization of sludge, but now the tendency is to give it a major role in low cost treatment of high/low strength wastewaters, since anaerobic digestion offers energy generation through gas production.

Anaerobic membrane bioreactors (AnMBR) combine anaerobic digestion with membrane filtration. They are becoming a feasible option for treating previous unsuitable low-strength wastewaters, decoupling hydraulic and solid retention times, and providing successful treatment with the benefits of biogas production.

However, the digestion process is optimal at mesophilic or termophilic (35-37 ºC), requiring heating of reactors. The more inexpensive option to treat the wastewater at its ambient temperature is feasible using AnMBR since this type of reactor can offer long sludge retention times. On the other hand, the digestion equilibrium turns out more sensible and delicate, and performing a proper and robust start-up of AnMBR in ambient temperatures is still challenging.

The aim of the thesis was the successful startup and stabilization of AnMBR systems at ambient temperature (25 ºC) and low organic loading rate (OLR). Reactor operation was monitored, and the most relevant process parameters were considered for the aim.

Two pilot-scale AnMBR's (120L) were used with an external membrane configuration. The experiment was carried out in 100 days. Substrate feeding consisted of synthetic dairy wastewater with added nutrients solution. The inoculum was provided from a full-scale anaerobic plant at a digester of BV dairy (UK) treating dairy wastewater at 30ºC.

Main operation parameters were monitored every day, along with gas production and methane yield. Laboratory tests were performed twice a week with samples of the reactors and effluent. A number of parameters were analyzed, the most important of which were total solids content (TSS), alkalinity, fatty acids, biogas content and chemical oxygen demand (COD).

The startup of the two AnMBR's differed greatly. In System 1, stable conditions were acquired in one month of operation. System 2 failed after 20 days of function, and did not achieve successful startup. It was not possible to fully recover it during the days of study due to dramatically slow growth of microorganisms and low stability of the process.

Thus, satisfactory system performance could be achieved but the ambient anaerobic process was vulnerable to inhibitory conditions. Both systems showed that the delicate process operation required fast corrective measures to prevent digestion failure. Causes of instability and failure were: washout of biomass, high content of VFA, low buffering capacity and poor performance of technical equipment and low pH. However, the digestion could stand a lower pH range than found in literature.

In conclusion, the best parameters to control the startup were pH, alkalinity, methane content, biomass content and organic removal. In this sense, low buffering capacity of a reactor makes it vulnerable to inhibition by sudden pH changes, easily solved by systematic addition of a buffering compound. Finally, the use of simple and fast alkalimetric methodologies can give satisfactory process overview compared to complex and more precise techniques for alkalinity measurement.

Table of abbreviations

AD	Anaerobic Digestion
AnMBR	Anaerobic Membrane Bioreactor
CSTR	Continuous Stirred Tank Reactor
OLR	Organic Loading Rate
SRT	Solid Retention Time
HRT	Hydraulic Retention Time
COD	Chemical Oxygen Demand
TSS / VSS	Total / Volatile Suspended Solids
VFA	Volatile Fatty Acids
TNb	Total Nitrogen bound
LMH	$l \cdot m^{-2} \cdot h^{-1}$
NL	Normal Liters (volume at 0 ºC and 1 atm of pressure)
SRB	Sulfate Reducing Bacteria
BAT	Best Available Technique

Table Of Contents

1 Introduction

1.1 Background

One of the biggest concerns in recent decades is how the global human can live in a sustainable society, when energy and resource scarcity arise gradually each year (British Petroleum, 2014; Fritzmann et al., 2007). Thus, contamination of natural environment due to human impact presents a public health risk, polluting rivers and ground water (Obasohan et al., 2010), especially for the industry (Sánchez et al., 2005).

Together with medical advances, wastewater treatment is directly related to the health benefits of different human civilizations. With the invention of sewage systems of the Roman Empire, wastewater was drained from public baths and latrines to sewers outside the cities (Henze et al., 2008). But it was not until the 17th century, it was discovered that decaying organic matter produced flammable gases (Ostrem, 2004). From the first full-scale anaerobic digester in 1895, it was observed that it could be useful not only as a biogas producer, but also for wastewater treatment (Khanal, 2008).

However, lack of knowledge in anaerobic digestion (AD) restricted its use to domestic or isolated farming, far from optimal conditions for biogas production (Ostrem, 2004). This situation started to change from 1950 and especially after the energy crisis of 1973 and when environmental restrictions became a reality (United Nations, 2012), with a substantial increase in wastewater treatment plants. Figure 1-1 shows the main role of AD in the last decades, which consisted of sludge reduction and stabilization. The major treatment, though, was carried out in aerobic digestion, which offered more reliable and mature process compared to AD (Dupla et al., 2004) and capability to treat low organic loading rates (OLR) with high microorganisms sludge production (Ho et al., 2007). Focusing on aerobic technology led to high investments in plants that eluded, at first, possibilities for other technologies that could be more attractive (Kleerebezem and Macarie, 2003).

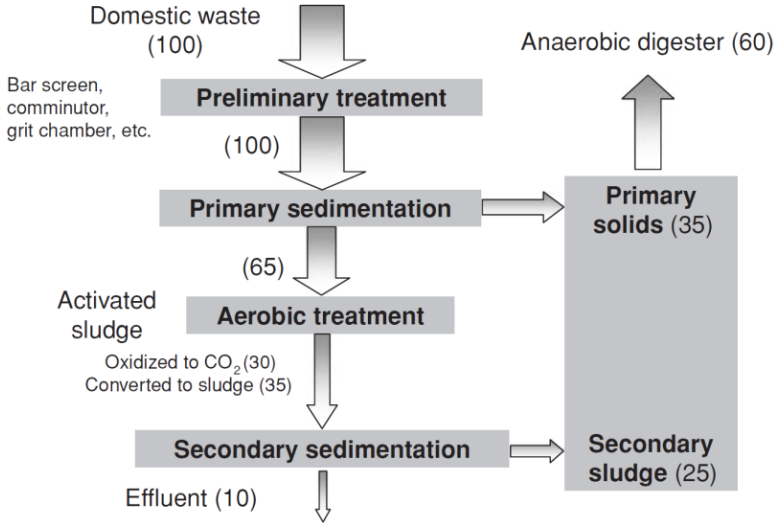


Figure 1-1. Role of anaerobic biotechnology in overall waste treatment. Figure from Khanal, (2008) reproduced with permission from IWA publishing.

Nevertheless, AD appeared to be a very promising technology combining biogas production with wastewater treatment, and it was gradually implemented in the industrial sector, focused

on treating high-strength waste (Khanal, 2008). With multiple advantages over conventional treatments. AD was very attractive in terms of low energy systems, reduced sludge production, destruction of dangerous organisms, minimal odor emissions, little use of chemicals, long-term sustainability and generation of biogas (Ho et al., 2007; Liao et al., 2006; Sánchez et al., 2005; Smith et al., 2012; Van Lier, 2008). Years of research and development in AD have been enabling more extended application, where only in Europe the number of plants increased from 15 in 1995 to 200 in 2010 (De Baere and Mattheeuws, 2011).

Also, another line of work relies on the use of membranes in conventional aerobic bioreactors, which has a background of research of more than 30 years. Membranes offer an important improvement for effluent quality, with potential to remove suspended solids, chemical flocks and other compounds (Hammer Sr. and Hammer Jr., 2011). During the last decade, it has been an exponential research for developing this technology, thanks to decreasing costs and materials optimization for the membranes (Yang et al., 2006).

Anaerobic technology combined with membrane filtration represents a cost-effective wastewater treatment with biogas production (Ghyoot and Verstraete, 1997; Visvanathan and Abeynayaka, 2012). The system can be potentially used as a way of substitution for primary and first waste stream treatment. Hence, it has been quite an important topic for research during the last two decades (Lin et al., 2013).

The implementation of Anaerobic Membrane Bioreactors (AnMBR) gives a new perspective of possibilities for any kind of wastewater, improving the effluent quality by offering total biomass retention thus great removal of suspended solids, compared to other systems (Lin et al., 2013; Visvanathan and Abeynayaka, 2012). It also permits a long solid retention time (SRT) with low hydraulic retention time (HRT), which helps microbial growth in smaller reactors without being washed out (Liao et al., 2006; Visvanathan and Abeynayaka, 2012).

Despite these advantages, AnMBR's still carry some bottlenecks inherent to each AD and membrane technology. There are crucial aspects regarding system performance, operational parameters and membrane fouling issues that still need development (He et al., 2005). The poor stability of the reactions and multiple inhibitory substances can affect all the process, if it is not monitored thoroughly (Vannecke et al., 2014).

Also, AD usually takes place at mesophilic (37ºC) or termophilic (55ºC) temperatures, because it is optimal for growth of microorganisms. However, there is still research needed to evaluate the feasibility of the digestion in unheated wastewaters, at ambient temperature. There are two main groups of microorganisms especially sensitive to temperature in AD, methanogenesis and hydrolysis (Smith et al., 2012). These groups need special attention when operating below their optimal growth temperature because reactions are slowed down. Hence, a period of acclimation is required to adapt the microorganisms to the new temperature.

There have been successful demonstrations of methanogen microbes development below mesophilic temperature, achieving successful acclimation with notable biogas production (Bialek et al., 2012; McKeown et al., 2009). However, most of them are performed at laboratory scale, and only evaluate AD without considering membrane technology (O'Reilly et al., 2009). Furthermore, they focus on the feasibility of the reaction, but omit the importance of the startup and acclimation period of the process.

Long and delicate startup times are frequently underestimated, provoking system failure if not monitored in detail, and making almost impossible to recover (Cao et al., 2011; Labatut and Gooch, 2012; Lahav and Morgan, 2004; Smith et al., 2012). Although startup could be achievable relatively fast in mesophilic conditions (Griffin et al., 1998), periods of 2 to 4 months are quite common (Khanal, 2008). If started at low temperature, conditions are even worse, reducing biomass growth and requiring longer SRT to stabilize (Smith et al., 2012).

Low temperature anaerobic digestion has proven its feasibility using membranes in laboratory and pilot scale (Ho et al., 2007; Smith et al., 2013), enabling a viable digestion with minimum loss of methanogenic microorganisms to produce sufficient amount of biogas. However, little is known about start up periods at ambient temperatures in pilot scale experiments, and evaluation of its capacity to develop a competitive alternative to conventional systems (Skouteris et al., 2012). There are multiple options in reactor configurations and design that require further experiments to assess the best available technique for developing AnMBR (Smith et al., 2012).

Strategies to develop a fast and successful startup of AnMBR's at low temperatures are yet to be studied, in order to assure that startup of these processes at industries will not fail.

1.2 Aim

The aim of this thesis was to accomplish a successful startup of an AnMBR at room temperature (25ºC) and low OLR while keeping stable process. Additionally, to evaluate the operation parameters to control the state of the process. This led to the following research questions:

- What is the best operation strategy to perform a stable and successful startup of AnMBR?
- Does ambient temperature and low OLR affect digestion performance in any way?
- Which are the most important process parameters to monitor the startup period and reveal digestion upsets in the AnMBR?
- When an AnMBR is unstable, are corrective measures effective to avoid digester failure?

1.3 General method

The stability of the process was evaluated using biogas production, biomass content and organic removal as selected process parameters related to microbial activity. These were in turn indicators for startup monitoring along with other operational parameters also used.

2 Literature study

2.1 Anaerobic digestion

The word anaerobic comes from the Greek word *αναερόβιος* which literally means *life without air*. Indeed, anaerobic digestion (AD) is defined as a biological treatment process where microorganisms break down organic matter in the absence of oxygen. This phenomenon occurs naturally in places where organic material is available under anaerobic conditions, such as stomachs of ruminants, sediments of lakes and ditches, sewage or municipal landfills (Henze et al., 2008).

AD is very effective processing all kinds of feedstock containing organic digestible material, such as industry, agricultural, sewage and solid waste, with varying degrees of degradation (Ostrem, 2004), and leaving mineralized compounds like ammonia, phosphate or sulfate in the solution. It can be applied at any scale and place, producing very small amounts of sludge and valuable biogas generation in terms of methane and carbon dioxide (Henze et al., 2008).

The full process comprises very complex and multi-step stages that include physicochemical and biochemical reactions, as illustrated in Figure 2-1. These mechanisms can take place stepwise and in parallel, and each of them is linked to the rest of intermediate processes. In general, there are four distinctive parts of the process: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Each of them have a physiologically unique microorganism population that requires disparate environmental conditions (Ostrem, 2004). The feedstock has to undergo all of these stages to succeed a full digestion.

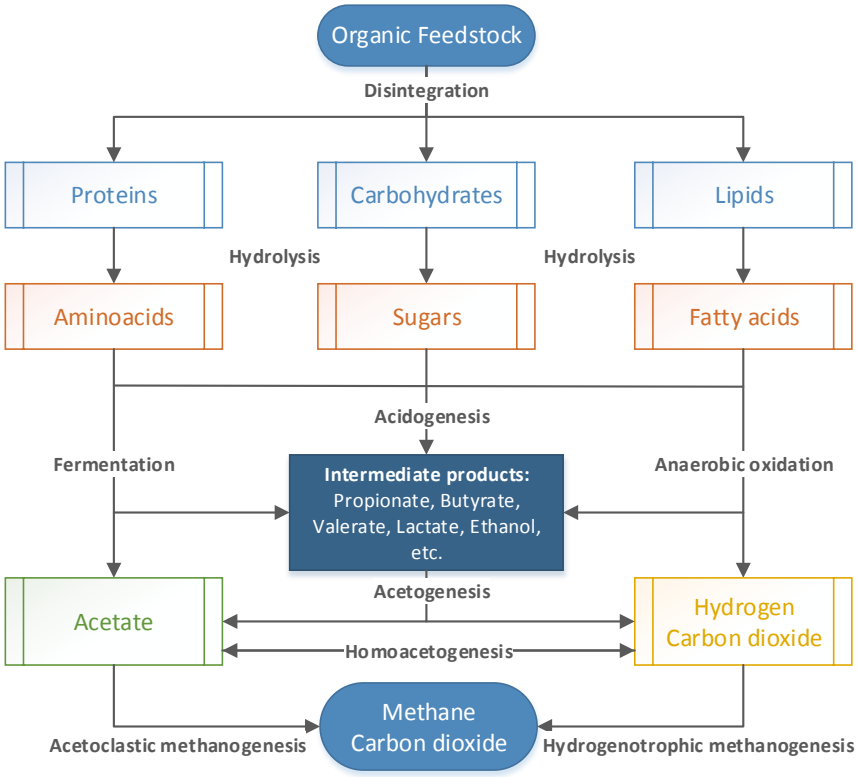


Figure 2-1. Reaction pathways of AD process. Adapted from Henze et al. (2008); Khanal (2008) with permission from IWA publishing.

In the hydrolysis stage, complex molecules are broken down to constituent monomers by enzymes excreted by fermentative microorganisms. Acidogenesis is the next stage of the process, in which short chain fatty acids and alcohols are formed, as well as hydrogen and carbon dioxide. It can also be considered as the core of the digestion, including anaerobic fermentation and oxidation (or respiration as Khanal (2008) refers).

Acetogenesis refers to the process before methane production, when acetate, H_2 and CO_2 are produced through carbohydrate fermentation. Methanogenesis is the last stage, in which acetate or carbon dioxide and hydrogen gas are converted into methane. Approximately 2/3 of the production is derived from acetate conversion, and the rest corresponds to hydrogen conversion (Henze et al., 2008).

The rate-limiting steps are generally hydrolysis and/or methanogenesis, depending on the type of substrate and operational conditions (Smith et al., 2012). Hydrolysis can be difficult to develop when the feedstock is very complex organic material, for instance in municipal solid waste. Methanogenesis can also be limiting because the microbial population is notably more sensitive to environmental conditions compared to the rest of the global microorganism consortia. Therefore, AD is very susceptible to any abnormal situation like the presence of inhibitory substances, abrupt pH changes or unstable organic loading rates (De Vrieze et al., 2014).

The overall balance of the reactions depends on the equilibrium of all reactants and products that conform the digestion (Labatut and Gooch, 2012). The most important intermediates of the digestion are volatile fatty acids (VFA, mainly acetate) and hydrogen, because they represent the direct precursors to biogas generation through methanogenesis.

Under stable operation, degradation of VFA is developed partly by hydrogen-producing microorganisms, which are in close relation to hydrogen-oxidizing methanogens (Labatut and Gooch, 2012). Acetic acid and H_2 are immediately utilized by the methanogens and converted to methane (Lahav and Morgan, 2004).

Each group of microorganism community also adapts with certain flexibility to the process conditions. Some archaea microorganisms (methanosarcinaceae) can withstand high concentrations of ammonium and VFA, while others (methanosaetaceae) are present when VFA is residual (De Vrieze et al., 2014).

2.1.1 Digestion below mesophilic temperature

AD can take place at a wide range of temperatures, but most of the reactors operate generally at mesophilic (~37ºC) or termophilic (~55ºC) temperatures, each one with its own group of characteristic microorganisms. Although psychrophilic (<10ºC) digestion has proved to exist (Smith et al., 2012), the other two temperature regions are most common because they have optimal growth conditions in AD (Khanal, 2008). However, the energy supplied to maintain such temperatures makes it unsuitable for a lot of different applications that require low energy intake (Lin et al., 2013).

In this way, operating without external heating (i.e. at influent temperatures) is an attractive proposal for broadening AD applications, now limited to warm climate locations (Liao et al., 2006; Seghezzo et al., 1998) or that require heating.

However, the feasibility of AD at lower temperature depends on the retention of psychroactive biomass (Bialek et al., 2012). When temperature decreases, washout of biomass is inten-

sified, hydrolysis of solids slows down significantly and viscosity of the liquid increments (He et al., 2005; Liao et al., 2006; Smith et al., 2012).

In lower temperatures, hydrogen-related AD pathways are favored, due to the higher solubility of H_2. Hence, hydrogenotrophic methanogenesis and homoacetogenesis take protagonism over aceticlastic methanogenesis (Smith et al., 2012). However, the general growth rate of microbes slows down (Sánchez et al., 2005). Rate-limiting steps in the digestion can be either hydrolysis or methanogenesis, depending on substrate and operating conditions.

An increase up to twice as longer solids retention time (SRT) is therefore required to avoid washout. It is resolved using different techniques, such as type of reactor (Bialek (2012) used a special reactor at 10ºC) or coupling a membrane to the reactor which separates the HRT from the SRT by the use of the membrane as a physical barrier (Sánchez et al., 2005; Smith et al., 2012).

The latter option has been of more interest due to the potential efficiency and cost-effective system compared to special and costly reactor designs. Membrane reactors effectively retain biomass when integrated into anaerobic digesters (Khanal, 2008).

2.2 Membrane technology

Membranes are selective barriers characterized by a porous material with orifices in their structure of a determined size. These orifices allow the passage of particles below a certain size while bigger particles are retained in a solution. From the perspective of this thesis, membranes are systems designed for microbial biomass immobilization. They can be characterized by different aspects such as pore size, material, building construction and configuration (Smith et al., 2012).

Pore size is generally classified as microfiltration (>0.05µm; MF), ultrafiltration (>0.002 µm; UF) or nanofiltration (below 0.002 µm). The type of suitable filtration depends on the desired quality of the effluent (sewage restrictions, agricultural irrigation or industrial reuse). Pore size should be as big as possible in order to use a reasonable and cost-effective operation of the membrane (Visvanathan and Abeynayaka, 2012). Selecting a membrane with a too small pore size will be more expensive than necessary, and cause more clogging issues. Therefore, most of membranes used in AnMBRs are MF or UF (Smith et al., 2012).

Membrane materials are generally ceramic, metallic or polymeric. Although the first two have a better performance than polymeric, the latter is much more attractive due to the cost of material source and building, and is the most commonly used (Lin et al., 2013).

The building construction includes flat sheet, hollow fiber or tubular construction; being the first two the most used and experimented with (Skouteris et al., 2012). The key elements that define a membrane performance are the driving force per unit membrane area (i.e. transmembrane pressure or TMP), the membrane permeability (in terms of permeate and retentate flux) and the fouling of membrane.

2.2.1 Membrane Fouling

Fouling consists of the accumulation of inorganic and organic foulants in the internal parts of the membrane pores and also at the membrane surface (i.e. cake layer). This phenomena is the result from very complex processes that reduce performance of the filtration process. It reduces flux, increases TMP and provokes less quality and quantity of effluent.

Membrane fouling is considered as one of the major contributors to the operating cost and maintenance of membranes (Smith et al., 2012). Therefore, it is a major drawback when considering AnMBR's installation, hence very important to consider (Dereli et al., 2014).

The potential foulants can be main biomass solids as well as supernatant substances. They can be classified as organic or inorganic. Colloidal solids, soluble microbial products (SMP), extracellular polymeric substances (EPS) and cell debris are examples of organic foulants (Skouteris et al., 2012). Inorganic foulants include precipitates like struvite, but are less common than organic ones and only appear in specific wastewaters (Smith et al., 2012).

The process of fouling can reduce or block the pores (internal) or form a biological cake layer (external) on the membrane surface. Internal fouling is mainly caused by cell debris and colloidal particles. They accumulate in pores and reduce surface area for filtration (Liao et al., 2006). On the other hand, the cake layer is a very heterogeneous structure, and it is formed by different foulants (Skouteris et al., 2012).

The problem of fouling depends on two aspects. The first is related to operational parameters such as SRT, loading strengths, fluxes and membrane operation. The second is intrinsically of presence of foulants in sludge and influent (biomass concentration, presence of EPS or SMP, and particle size distribution). For instance, Dereli et al. (2014) concluded that suspended solids have a direct relation with membrane fouling in concentrations above 20 g/l. However, fouling issues did not differ significantly when operating at SRT of 30 and 50 days.

Due to the complex nature of biofouling as well as reactor types and sources of feed, little is known about which parameters of operation contribute to fouling (Dereli et al., 2014). There has been, though, some indicators that may help to enhance knowledge on this issue. For instance, Liao et al. (2006) mentioned that cake deposition is typical in continuous stirred tank reactors (CSTR). It is also well known that membrane materials lead to different fouling mechanisms (He et al., 2005; Liao et al., 2006; Skouteris et al., 2012; Smith et al., 2012).

The procedures to revert fouling issues focus on reducing fouling rate and chemical cleaning procedures. The first procedure includes membrane operation below a certain flux and keeping a high shear across membrane surface, using biogas sparging, backwashing or velocity gradients (Liao et al., 2006). All these techniques focus on external fouling, because internal is usually irreversible (Skouteris et al., 2012).

Although more research needs to be performed in order to follow the best procedure, it is presumable that a combination of fouling control measures is more effective than one method alone (Smith et al., 2013). When the fouling is critical, it requires a full replacement of the membrane.

2.3 AnMBR design and control

An AnMBR is basically an anaerobic bioreactor coupled with a filtration membrane. A good system design and control is basic for a robust and stable operation, preventing process upsets. Its design and control measures should be based on influent type and strength, which determine the type of reactor and membrane to use. The system has to perform at short hydraulic retention times (HRT) and high SRT to reduce reactor volume while enabling low temperature digestion. Last, it should be focused on maximum production of methane and sufficient chemical oxygen demand (COD) removal. (Smith et al., 2012; Ward et al., 2008).

2.3.1 Membrane configuration

There are three available configurations of membranes, which can be classified as their location in the system (inside or outside the reactor), or the operation according to the driving force of membrane. Figure 2-2 shows a schematic view of the three designs.

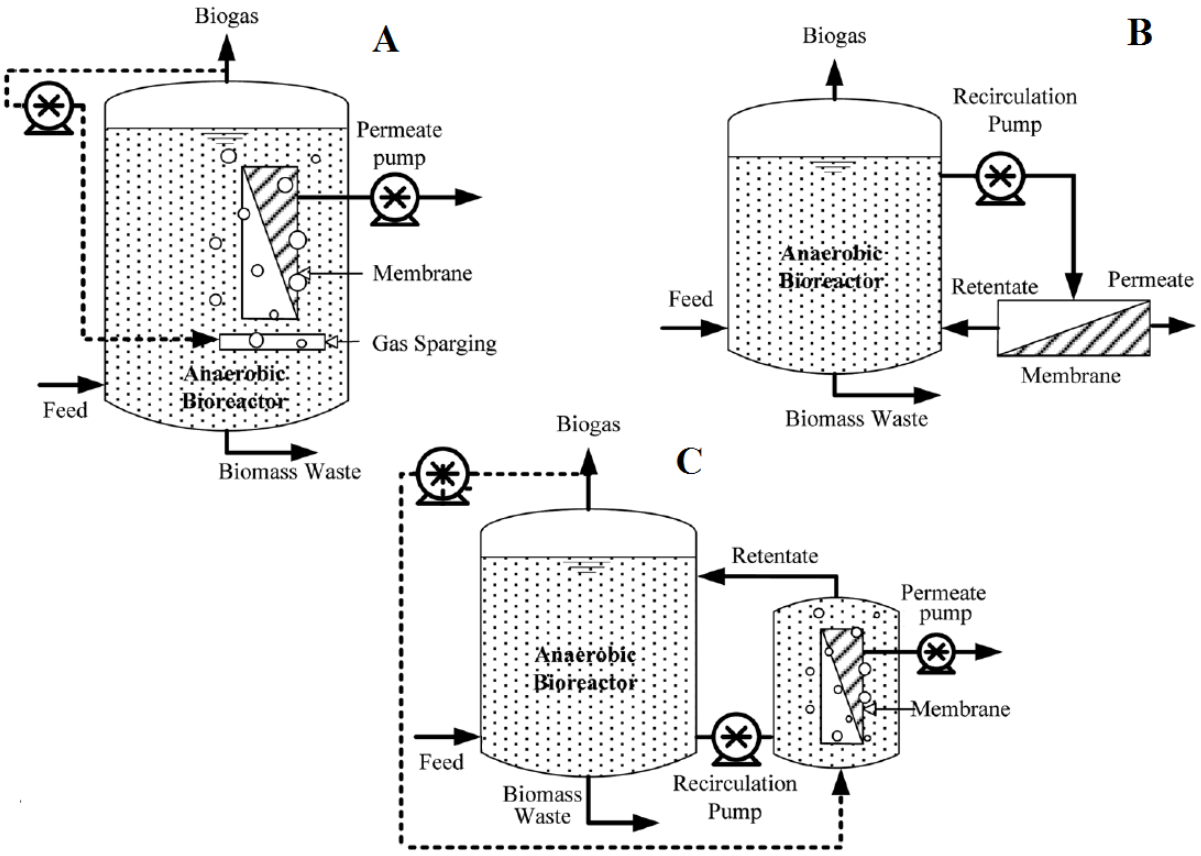


Figure 2-2. Membrane configurations A) Internal Submerged. B) External cross-flow. C) External Submerged. Adapted from Visvanathan and Abeynayaka (2012) with permission from Techno Press.

Cross-flow configuration uses membranes under pressure to produce permeate liquid. The other two are operated under vacuum driven force or gravity to pull the permeate through the membrane. Although immersed version has greater performance (Martin-Garcia et al., 2011), external membranes are most common because they allow maintenance intervention (cleaning, replacement or inspection) without affecting the anaerobic reactor (Liao et al., 2006). Furthermore, vacuum driven configurations is preferred over cross-flow due to less amount of water required. However, it requires addition of pumping to recirculate the retentate to the reactor (Smith et al., 2012).

2.3.2 Reactor types

Among all the reactors available, the most common AnMBR is the CSTR coupled with a membrane, because it has a simple construction and use. However, there are other reactors available such as the upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB) or fluidized bed reactors (Martin-Garcia et al., 2011). These reactors usually have greater performance in terms of membrane fouling and biomass yield, but they are also more complex and expensive in all aspects (Liao et al., 2006).

Without considering the attached membrane, the biomass retention is the characteristic for differencing the type of reactor. For instance, an UASB reactor is less likely to suffer from fouling because its design allows better biomass retention, although it is not totally discarded (Smith et al., 2012). CSTR reactors do not have any biomass retention by themselves, hence SRT can only be decoupled from HRT with addition of a membrane (Liao et al., 2006).

Reactor location and shape will also determine the temperature of digestion and ensure complete homogenization. All of the designs are cylinder shaped to perform optimal mixing, and temperature should be orientated to be as stable as possible. Surface coatings, underground reactors or homogenizing feeding tanks can minimize the energy intake and digester upsets (Ward et al., 2008).

Depending on the substrate, there are also different configurations more suitable for every type of wastewater. For instance, domestic wastewater was proved to be more effective using UASB reactors with either external or immersed membrane configuration (Martin-Garcia et al., 2011).

2.3.3 Monitoring parameters

Proper system operation relies on a careful process control and monitoring to ensure organic removal and stable biogas production. But it also prevents the potential instabilities that may occur. Automation of control devices and on-line measurements can be a very helpful monitoring strategy to succeed in operating an AnMBR, especially in short term periods like startup times or drastic influent changes (Ward et al., 2008).

A poor monitored AnMBR will not operate at full performance, and will have a higher risk of instability, as it was stated by Labatut and Gooch (2012). These reactors should have all the appropriate sensors, and also keep a constant revision and checking of the equipment for a correct measuring. Even the simplest devices can decalibrate and provoke wrong corrections if not taken into account.

There are different classifications of the parameters to be monitored, depending on the phase (liquid, gas), complexity, ease of use and cost. Critical parameters such as temperature, pH and alkalinity (including VFA estimation) can be easily monitored at very low cost; Gas production and TMP sensors are more expensive but still very important to check.

Other minor monitoring aspects are related to off-line measurements using laboratory equipment. They cannot be measured constantly or do not require constant measurement, like chemical oxygen demand (COD) concentration or solids content. However, they offer valuable information of how is the development of the anaerobic digestion, and should be performed frequently.

Other periodic measurements in laboratory are nitrogen, sulfur and phosphorous content in reactor and influent. The use of determined substrates may contain elevated concentrations of certain substances like sulfate rich or nitrogen rich wastewaters, and they should be monitored in order to prevent digester failure.

2.4 Start-up of anaerobic membrane bioreactors

As mentioned in previous chapters, due to the slow growth rate of microbial population in anaerobic digestion, especially for methanogen archaea, a startup period requires significant importance. This period extends until a steady state is reached, where nominal parameters are

achieved with continuous operation. The key elements to understand the startup process are inoculum characteristics and reactor conditions during this time.

The inoculum is the source biomass that is used as a seed for starting the anaerobic digestion. It characterizes the composition and organization of the microbial community that will grow inside the reactor during the first weeks of operation (startup period). Therefore, it is crucial to characterize which type of substrate influent will feed the digester in order to select the appropriate microorganisms (De Vrieze et al., 2014).

According to Khanal (2008), aerobic systems have a short startup time (1 or 2 weeks) compared to anaerobic, which can take several months and even longer if the reactor operates below mesophilic conditions. This time can be easily reduced if the inoculum amount is increased, which will contain more microorganism population and then require less time to reach steady state.

Another strategy consists of the selection of diverse microbes that will constitute the inoculum. The richness in different microorganism population will play an important role when assimilating drastic changes in operating conditions. It will also support high concentration of toxic substances, and will ensure reactor stability even at abnormal conditions (De Vrieze et al., 2014).

Low temperatures also may impact the choice of an appropriate inoculum for seeding AnMBR. In this way, Smith et al. (2013) observed that mesophilic inoculum was suitable for seeding psychrophilic AnMBR treating low strength wastewater. He concluded that best procedure is to mix multiple sources of mesophilic and psychrophilic inoculum, especially if the wastewater will not be heated and temperature will differ seasonally.

In addition, McKeown et al. (2012) suggested the use of pre-acclimated inoculum biomass to reduce start-up times, because the biomass will already be adapted to the new temperature conditions.

Furthermore, other reactor conditions can set the proper ambient of microbes development, and they also affect start-up times. The most important is to know what are these optimal conditions (mainly pH, loading rates or wastewater strength), and control them in order to facilitate the microbial comfort. After a steady state is reached, long-term reactor conditions can be set and a new period of transition will happen to acclimatize the microbial population (Smith et al., 2012).

For instance, Cao et al. (2011) observed a fast start-up in UASB reactors using alkalinity concentrations of 1500 mg/l. After the startup, it could operate stable with less concentration (500mg/l). Also, an OLR start of 2 $kg \cdot m^{-3}\ d^{-1}$ reduced startup times compared to starting at 0.25 $kg \cdot m^{-3}\ d^{-1}$, with a COD influent of 2000 mg/l.

Last, it is crucial that influent fluctuations remain constant during startup times. After the inoculation, fluctuations should be avoided until a steady state is reached because biomass may not adapt itself to it (Skouteris et al., 2012).

2.5 AnMBR operation and inhibition factors

Given the complexity stated in previous chapters, a proper knowledge and expertise in the maintenance of AnMBR is required to succeed in a correct functioning and avoid failures.

Labatut and Gooch (2012) studied the operation of some anaerobic digesters, and found that inadequate operation of the systems lead to low efficiency in the digestion and half of the potential power capacity was obtained.

Perturbations in the system are expressed by a loss of equilibrium in anaerobic reactions. They may occur by the alteration of substrate characteristics, or by a change in environmental conditions. Therefore, concentration of main intermediates of AD are one of the most relevant parameters when detecting process upsets. The core principle when operating an AnMBR is to keep the parameters to constant values.

There are also other key indicators that reveal system stability; some of them are related to reactor conditions (temperature, pH, flux and TMP). Others imply substance concentrations, either from biomass (suspended solids, biogas content or OLR) or inhibitory compounds (ammonia, sulfate, phosphate or VFA).

Toxicity is generally discussed in terms of concentration rather than specific compounds, because the presence of any compound in a considered high concentration can cause inhibition (Skouteris et al., 2012).

2.5.1 Temperature of the reactor

The temperature of operation is one of the most delicate parameters that can negatively affect the efficiency of AnMBR. Microbial population require relative long periods of acclimation in order to develop under a certain temperature. Also, if the temperature is changed, it needs to be gradually adapted to provide good acclimation, which can take several days. If the process is not monitored correctly, or sudden temperatures changes occur, it can provoke digester failure.

There are system designs such as surface coatings and underground locations that minimize temperature changes. However, if no heating is added to the substrate, a good quality inoculum is required to allow fast adaptation of microbes to new temperatures.

Skouteris et al. (2012) observed that the temperature of operation affected the COD removal efficiencies. A reduction from 95% to 85% COD removal was observed reducing temperature from 25ºC to 15ºC.

2.5.2 pH and alkalinity

Although the tolerance of global AD microbes is very big, the optimal pH for methanogens is quite narrow, between 6.8 and 7.2, although it is possible to operate the anaerobic digester with a wider range of 6.5 – 7.6 (Khanal, 2008). However, under pH 6.4 the methanogenesis is totally inhibited (Ward et al., 2008). Because not all of the microorganisms have the same optimal pH range, it is important to determine the limiting reactions in the anaerobic process, according to the influent characteristics and reactor conditions.

The pH is not only important by its own inhibition, but also because of presence of other inhibitory compounds. In general, only the non-ionized forms of possible toxic compounds are responsible for inhibition. The proportion of ionized and non-ionized is determined by the pH of the system. Therefore, the pH can affect indirectly the potential toxicity of other substances (Ward et al., 2008). For instance, VFA can affect the methanogens at pH below 7 but, on the other hand, ammonia is toxic above pH 7 (Dupla et al., 2004).

In terms of routine monitoring, pH measurement cannot form the sole indication of imminent failure, because in medium or well-buffered waters high VFA concentration would have to form in order to cause a detectable drop in pH (Lahav and Morgan, 2004). Another related parameter is needed for the evaluation: alkalinity.

The alkalinity (also known as buffering capacity) represents the equilibrium of ions present in a solution that may interfere to changes in pH. It shows the pH response when the system is affected by other low or high pH substances. Hence, alkalinity is an extension of the pH measure, because it informs of the presence of other substances. For instance, the accumulation of fatty acids can reduce the alkalinity before the pH is affected. Therefore, alkalinity plays an important role in the evaluation of system stability and strength.

The buffering capacity can be controlled using different techniques. Addition of buffering solutions like bicarbonate, carbonate salts or strong bases affect direct or indirectly to the carbonate equilibrium of the system. Also, reducing organic loading rates can increase the alkalinity by reducing concentrations of acid intermediates (Ward et al., 2008).

In normal AD systems with neutral pH, the main source of buffering capacity is bicarbonate ion (HCO_3^-), and its concentration is related to the percent of CO_2 in the gas phase (Labatut and Gooch, 2012). However, if the concentration of other buffering compounds is high, they gain importance over the carbonate ions. Examples of buffering compounds are fatty acids, phosphates, sulfates and ammonium.

By taking into account these factors, the pH of the system can be adjusted to balance the speed of all the reactions, promoting the limiting step above the rest. For instance, it is best to keep the pH around 6.5 if the hydrolysis is the limiting factor in the process, because the optimal reactions of it take place between 5.5 and 6.5 (Ward et al., 2008).

The recommended levels of alkalinity have to be able to withstand moderate shock loads of VFA, as it is one of the first compounds that accumulate when methanogens stop producing methane. A good indicator is the ratio alkalinity/VFA, which represents reactor sensitivity to pH change. Literature studied proposed alkalinity levels around 1000 mg/l as $CaCO_3$ (Dupla et al., 2004; Sánchez et al., 2005). The addition of buffering substances such as sodium bicarbonate can help maintain a proper alkalinity.

2.5.3 Alkalinity tests

Despite its accepted and wide use, alkalinity still remains uncertain as for what is the best available technique (BAT) for how to perform the analysis. The APHA (APHA et al., 1999) standard methods is one of the major references for water and wastewater analysis, and its standardized methodology is widespread around all the research and industry sector. However, the standardized procedure of alkalinity applied to anaerobic digesters has been proved the least precise compared to other methods (Lahav and Morgan, 2004).

There are different conditions to use the BAT for each case. For instance, fast and cost-effective methods could be applied when regular monitoring is done, focusing on inexpensive techniques rather than precise. On the other hand, more attention should be paid in delicate periods such as startup times or when changing operational conditions in order to obtain accurate analysis.

Moreover, there could be situations in which no BAT is possible due to lack of laboratory resources or other reasons. In those cases, information about how reliable is the method selected should be taken into account to correctly operate an anaerobic reactor.

Among all the different techniques, titration methodologies have been established as the more suitable for a wide range of circumstances. The debate lies on which titration methods should be used in order to recognize total, carbonate and/or VFA types of alkalinity.

2.5.4 Long and short chain fatty acids (volatile fatty acids)

Fatty acids are key intermediates in the anaerobic digestion, because they represent the direct source of substrate for methanogen microbes. Roughly 2/3 of the biogas production comes from this reactants, while the rest corresponds to hydrogen (Labatut and Gooch, 2012). However, the methanogenesis can be inhibited by means of concentration of VFA's or indirect pH alteration, as stated before.

High concentrations of VFA (above 4g/l) or long chain fatty acids (LCFA) can totally inhibit methanogens (Ward et al., 2008). Lipids are an inhibition factor according to Demirel et al., (2005) and Kushwaha et al. (2011) in terms of long chain fatty acids. Dereli et al. (2014) suffered from LCFA inhibition due to high lipid content of wastewater, obliging to reduce OLR.

The type of substrate that is going to feed the reactor plays a role in revealing the amount of fatty acids that the reactor will have to digest. The previous stages of methanogenesis could reveal the concentration of intermediates, according to the fast or poor degradation of the feedstock during the first stages of the anaerobic digestion, such as hydrolysis.

VFA concentration is more sensitive compared to other indicators (Labatut and Gooch, 2012) and it encompasses a group of 6 acids (acetic, propionic, butyric, valeric, caproic and enanthic). The main fatty acid present in the digestion is usually acetic acid, but propionic and butyric acid are inhibitory to methanogenesis at lower concentrations than acetic acid (Weiland, 2010).

During a correct operation, VFA concentration is lower than 500mg/l, and between 1500 and 2000 the biogas production is reduced (Labatut and Gooch, 2012). However, what is more important is the sudden change in VFA concentration that reveals digestion stress.

2.5.5 Ammonia and sulfide

Ammonia is produced in the degradation of nitrogenous, protein-rich substrates. Ammonia can inhibit the digestion process and decrease its overall performance, mainly affecting methanogenesis. The ammonia diffuses through the cell membrane of the microorganisms and interferes with proton transport across the membrane and/or causes potassium deficiencies (Chen et al., 2008).

Because the two principal forms of inorganic ammonia in a solution are ammonia and ammonium, the effect is studied as the sum of both (total ammonia nitrogen, TAN), although free ammonia is the main cause of inhibition (Chen et al., 2008). Concentrations over 1500 mg N/l as TAN have been reported to be totally inhibitory at pH above 7.4 (Labatut and Gooch, 2012). However, depending on the reactor conditions and the degree of acclimation of the microbial community, the inhibition can start from 100 mg N/l or more (Chen et al., 2008; De Vrieze et al., 2014; Vidal et al., 2000).

Lower temperatures and presence of other ions such as sodium, magnesium and potassium can reduce the inhibition of ammonia. On the other hand, the presence of ammonia in low concentrations is beneficial to anaerobic process, since nitrogen is an essential nutrient for anaerobes. (Chen et al., 2008)

Sulfide has a relevant presence in industrial wastewaters. In the digester, it is reduced to sulfate by the sulfate reducing bacteria (SRB). The inhibition from sulfide may be present by direct high concentration, or indirectly. SRB bacteria compete with methanogens for common organic and inorganic substrates, and can cause inhibition for lack of available feed sources (Chen et al., 2008).

Sulfide inhibition concentration was reported to have an effect above pH 7 in the unionized form, at levels of 90-250 mg S/l as H_2S. The optimal levels of sulfide vary from 1 to 25 mg S/l (Chen et al., 2008; Lens et al., 1998)

2.5.6 Suspended and volatile solids

The content of solids is a representation of the organic matter present in the reactor. They are a good indicator of biomass present in the anaerobic reactor. A well operating AnMBR tends to accumulate biomass solids depending on the SRT.

The accumulation is positive when the reactor is starting up, but should be controlled in stable operation, because suspended solids can contribute to membrane fouling at high concentrations, as stated before (Dereli et al., 2014).

2.5.7 Membrane operation

When considering membrane operation, several aspects should be considered, all of them oriented on minimizing fouling. A good combination of filtration and relaxation modes is essential to produce effluent quality while controlling fouling issues in long-term operation.

Membranes can be operated under constant fluxes or constant TMP. The concept of critical flux is defined as the flux where irreversible fouling appears. Although it is preferable to operate below a determined critical flux, it is likely impossible that no fouling will appear in the long-term periods (He et al., 2005). Therefore, frequent cleaning and mitigation procedures might be adequate to success in keeping a good membrane performance.

However, the best conditions for minimizing fouling do not necessarily coincide with optimal conditions for best sludge filterability. Hence, a compromise between membrane and overall system performance has to be found (Dereli et al., 2014).

2.5.8 Hydraulic and Solids Retention Times

HRT and SRT are important operational parameters that impact treatment performance and affect membrane fouling. HRT does not appear to have much influence on AnMBR operation by itself, hence setting a low HRT will not affect very much the performance even in low temperatures. However, under 10ºC it does may have an impact in COD removal efficiency (Smith et al., 2012).

HRT values have been studied from 2 hours to a few days (Skouteris et al., 2012). The decrease of HRT significantly reduces reactor volume, hence capital cost of a new AnMBR (Liao et al., 2006). On the other hand, a low HRT enhances growth of biomass leading to ac-

cumulation of SMP which accelerates membrane fouling, causing particle deposition and cake formation (Skouteris et al., 2012).

In AnMBRs, HRT can be decoupled of SRT. There has been SRT reported from 20 days to more than a year, and theoretical infinite values. When the microbial growth rate is positive, the higher SRT, the more biomass concentration is in the reactor. Therefore, better stabilization of organic matter takes place with more biogas produced. However, it also accumulates organic and inorganic products and precipitates in the system (Dereli et al., 2014).

It is important to consider a high SRT/HRT ratio, rather than relying on absolute independent values, because it will ensure operational feasibility at low temperatures, reasonable low volume reactors and avoid washout of slow-growing microorganisms (Khanal, 2008; Smith et al., 2012).

Conversely, increasing the ratio may result in greater risk of membrane fouling, because of more suspended biomass (SMP and EPS). Therefore, a good ratio of operation may be studied to properly mitigate membrane fouling without compromising reactor performance (Smith et al., 2012).

2.6 Applications of AnMBR

AnMBR offer a wide range of applications in wastewater treatment, depending on the type of wastewater to be treated and the effluent destination. It has been historically focused on high rate wastewaters, but in the last years there has been more interest in using AnMBR as an alternative strategy for low strength wastewaters at low loading rates and temperatures, such as domestic wastewater (Smith et al., 2012).

Other type of available wastewaters could be from food processing industry, manures, slaughterhouse, chemical and industrial types. Each one of them are characterized for very different organic strengths and require different strategies of operation as well. For instance, waste streams with high solids content are characterized by slow biodegradability of feedstock, hence hydrolysis may likely to be the rate limiting step. This information is very useful when planning an AnMBR installation. (Liao et al., 2006)

With respect to effluent characteristics, AnMBR treatment does not remove nutrients and therefore additional treatment may be required in some cases where there are sewage restrictions. For instance, municipal wastewaters may be adequate using AnMBR as the main treatment with an aerobic reactor for nutrients removal. Conversely, the nutrient richness of the AnMBR effluent may be positive in agricultural irrigation without post-treatment (Smith et al., 2013).

3 Materials and methods

The study assessed the startup of two identical AnMBR's during 100 days of operation. Main reactor parameters were monitored, and laboratory analysis of reactor, effluent and gas samples were performed. Additionally, maintenance tasks such as equipment cleaning and system improvements were carried out frequently. All of these will be described in detail in the next sections.

3.1 Equipment description

The equipment consisted of two independent AnMBR's with external membrane configuration. Figure 3-1 shows the main elements of each system, including the reactor, the membrane, the feeding tank, all the streams (Substrate, sludge, biogas and effluent), inlets and outlets (arrow streams) and all the pumps. The following list includes most important parts:

- CSTR reactor (180L).
- Feeding tank (270L) to prepare daily substrate for the system.
- Flat sheet membrane (PVDF material, 0.2µm pore size, 8 layers with total surface of 0.5168 m^2) submerged in an external filtration chamber (35L).
- Permeate regulator resistance to keep constant outlet flow.
- 3 liquid flow pumps and 1 gas pump.
- Control panel unit.
- Other elements such as connections, valves, hoses and sensors.

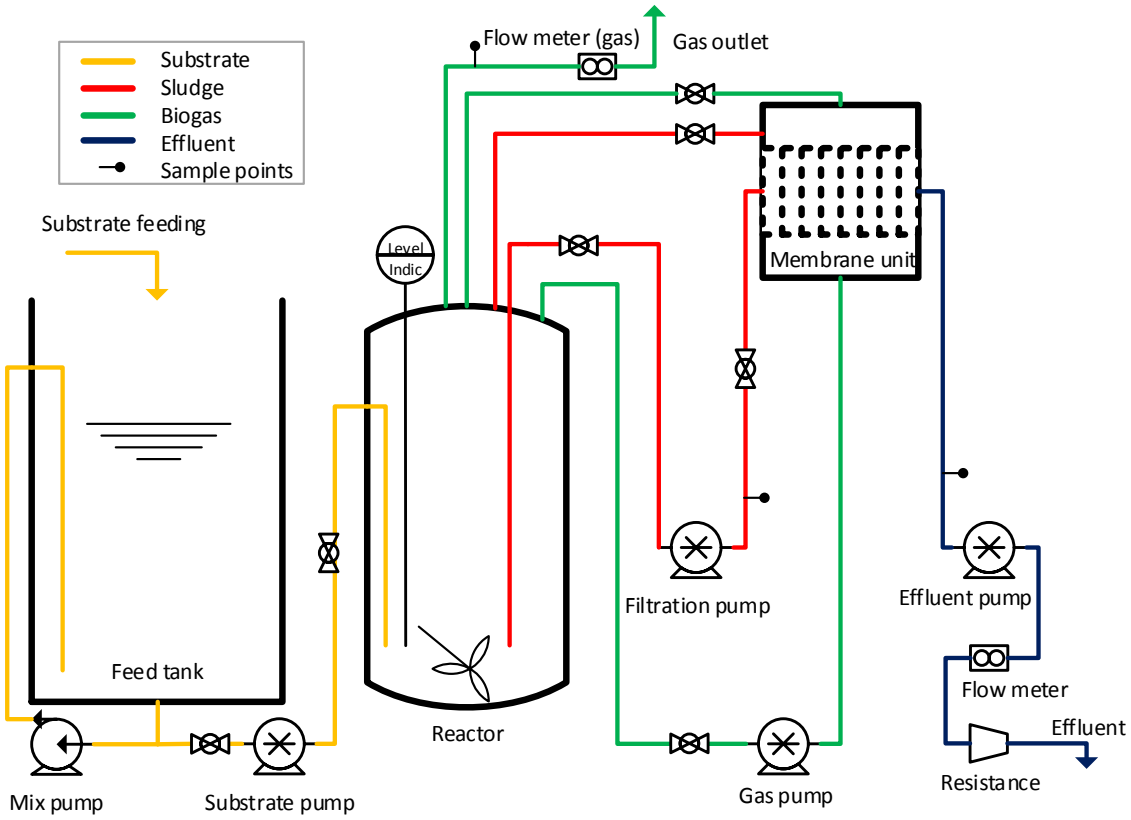


Figure 3-1. Schematic diagram of the system.

Figure 3-2 shows all the system with reactor 1 (left) and reactor 2 (right). Each reactor was equipped with an internal magnetic stirrer (the electric motor can be seen at the bottom of reactor in Figure 3-2). The feeding tank had a centrifuge pump to mix the substrate.

The membrane configuration was external, where the membrane was submerged into the reactor liquid. It had gas, sludge and effluent connections as well as an upper lid for maintenance operation. The gas pump recirculated constantly the biogas inside the system between the membrane chamber and the reactor. The biogas was driven over the membrane for self-cleaning purposes with gas sparging.

The pipe circuit had 3 different sample points (see Figure 3-1), one for each type of sample: reactor, effluent and gas. All the liquid circulation system was carried out with Quattroflow® fluid pumps, allowing wide ranges of speed and ease of operation.

The systems were located at the department of Chemical Engineering (VA-Teknik), Lund University. Conditions of temperature (approx. 23ºC) and humidity were assumed constant.

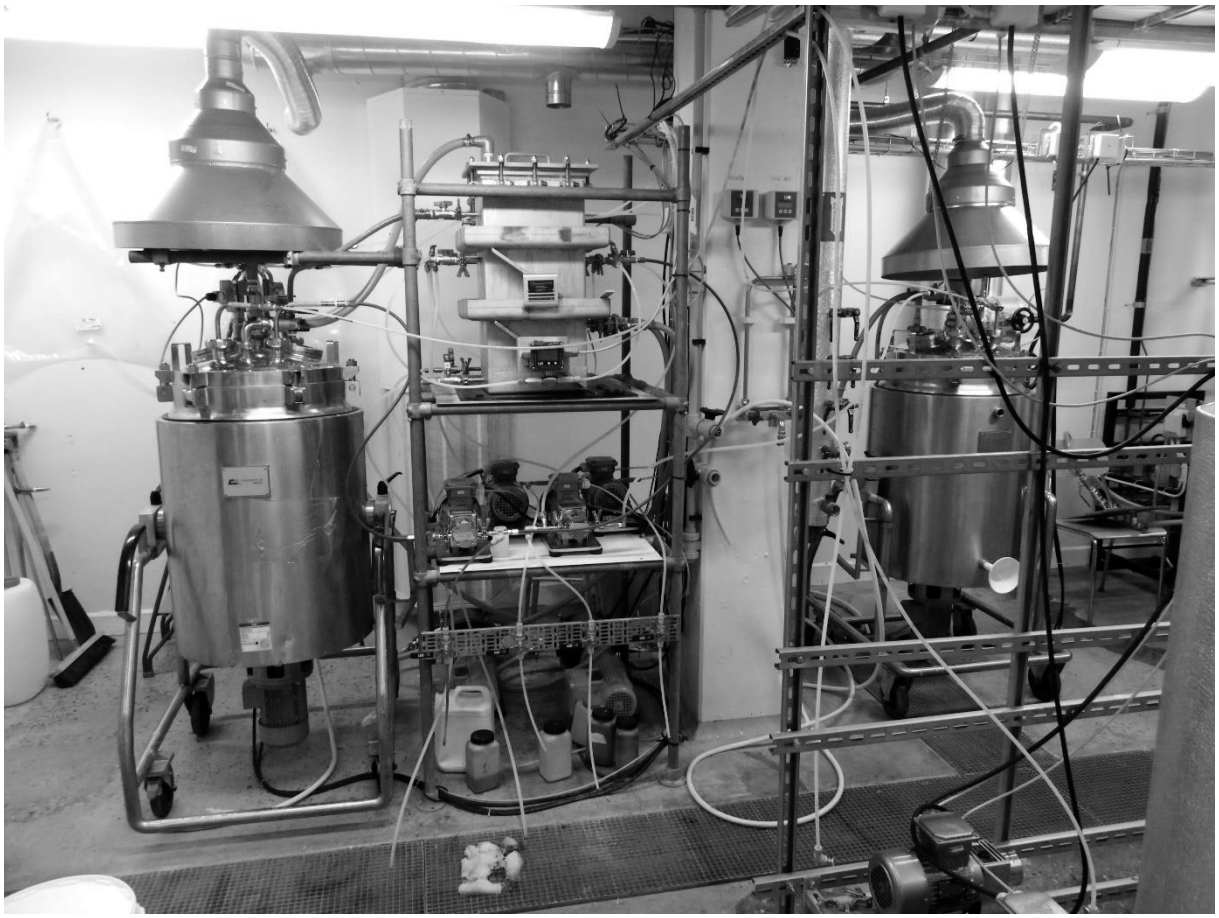


Figure 3-2. Image from all the system except substrate tanks. Reactor 1 (left), 2 (right), membrane filtration unit and filtration and biogas pumps (center).

3.1.1 Equipment improvements

During the days of study, some minor parts of the AnMBR's were adjusted or changed to improve consistency of the experiments and ease of operation. The most remarkable included pump configurations that increased performance, metal pipe connections for better sealing

from outside air, and better substrate mixing. It is assumed that these enhancements did not affect the results of the experiments.

3.2 Programmed tasks

Figure 3-3 presents a schematic view of all the tasks programmed during the experiment. These tasks were set gradually from the beginning of the experiment, because there was little knowledge on how the system would develop during operation.

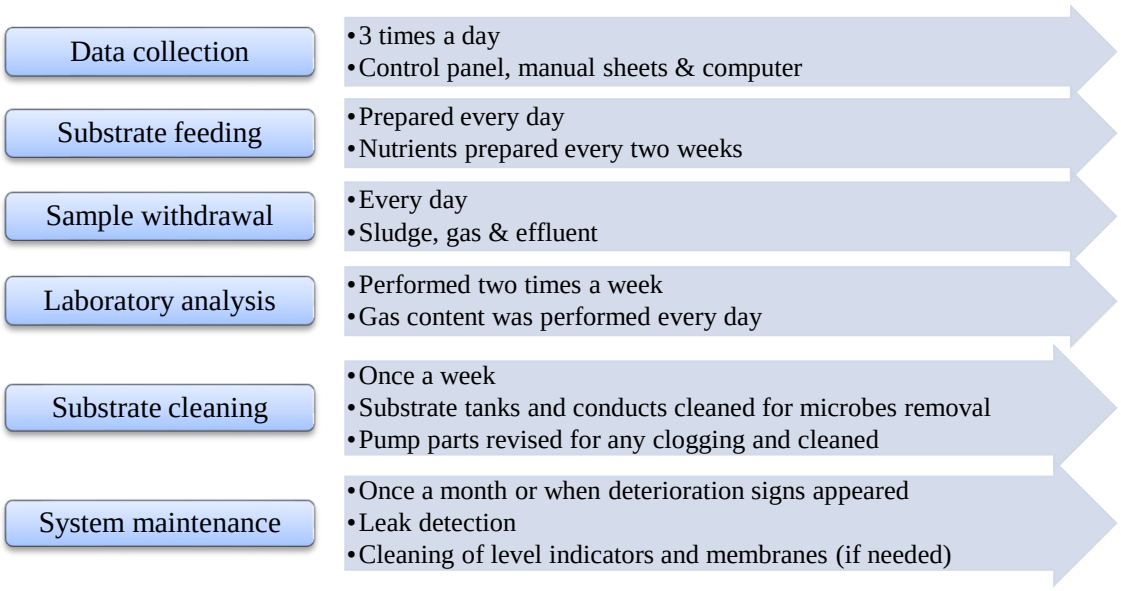


Figure 3-3. Programmed tasks for the experiment

3.3 Inoculum and substrate

The anaerobic microbiological inoculum was taken from a full-scale anaerobic digester of BV dairy (UK) treating dairy wastewater at 30ºC. It was diluted with water to get a concentration of roughly 8000 mg VSS/L.

The feeding substrate was intended to simulate wastewater from the dairy industry. The full substrate included synthetic dairy wastewater, micro and macro nutrients, and a buffer compound for pH correction.

Substrate was prepared every day, and was applied to both reactors following the same schedule.

3.3.1 Substrate preparation

To prepare the synthetic wastewater, an average concentration of 112g of milk powder (Nestlé Nido®) per 100L of tap water was prepared. Additionally, a number of specific nutrients were added to the substrate, according to Shelton and Tiedje (1984). The concentration of nutrients in the substrate was 1 ml of macronutrients stock solution per liter of tap water, and 0.01 ml of micronutrients per liter of tap water. See Table 3-1 for full list of macronutrients and Table 3-2 for micronutrients). The nutrients were used as a mineral source for anaerobic feeding.

Table 3-1. List of macronutrients for substrate preparation.

Macronutrient Compound	Concentration (g/l of stock bottle)
KH_2PO_4	2.7
K_2HPO_4	3.5
NH_4Cl	5.3
$CaCl_2 \cdot 2H_2O$	0.75
$MgCl_2 \cdot 6H_2O$	1
$FeCl_3 \cdot 6H_2O$*	0.28
$NaHCO_3$	1.2

**Note: $FeCl_3$ was used instead of $FeCl_2$ for availability issues.*

Table 3-2. List of micronutrients for substrate preparation.

Micronutrient Compound	Concentration (mg/l of stock bottle)
$MnCl_2$	50
H_3BO_3	5
$ZnCl_2$	5
$CuCl_2$	3
$NaMo_4 \cdot 2H_2O$	1
$CoCl_2 \cdot 6H_2O$	50
$NiCl_2 \cdot 6H_2O$	5
Na_2SeO_3	5

Due to pH degradation for microbial activity in the feeding tank, an extra 30g of Na_2CO_3 per 100L of feeding wastewater were added as a buffer compound to keep substrate pH constant.

In System 2, there were punctual situations where substrate was prepared without adding milk powder, to allow a pH increase in the system. These periods are further mentioned as *recovery modes*. From day 70, half the concentration of milk powder was used in this system for demonstrating better pH stabilization.

3.3.2 Analysis test

Samples of the inoculum and the substrate preparation was taken to perform analysis. Table 3-3 shows the main results. The substrate analysis was performed right after its preparation, using a different concentration of milk powder (75g/100l) compared to the one used for everyday substrate preparation (112g/100l). The temperature of the tap water used was also different (the average temperature in the feeding tanks was 20ºC). The buffer compound was not added to this substrate analysis, which elevated the pH to 10.

Table 3-3. Main parameters for inoculum and substrate samples.

Parameter	Inoculum	Substrate
Temperature (ºC)	29	7.1
pH	7.06	7.91
COD (total / soluble) (mg/l)	91600 / 2460	1190 / 402
Total Carbon / Total Organic Carbon (mg/l)	1159 / 730	337.9 / 333.4
Total Nitrogen (TNb)* (mg N/l)	1060000	28.8
Total Phosphate** (mg P/l)	157000	6.22
Total alkalinity (mg $CaCO_3$/l)	7620	421.9

*Total nitrogen bound: Includes organic, inorganic, ammonium, nitrate and nitrite nitrogen.

** PO_4, PO_3, PO_2 and other phosphate ions.

3.4 Operational conditions

The two reactors were started with the same operational parameters, which are shown in Table 3-4. These values were kept constant for all the study, except for the substrate strength in System 2, which was reduced during some periods of the experiment to equilibrate the system.

Table 3-4. Main operational parameters used in AnMBR's

Parameter	Units	Value
Flux	l/m^2*h	8
Hydraulic Retention Time (HRT)	h	53
COD concentration substrate	mg O_2/l	1700
Effluent (permeate) flow	l/h	4.11
Gas sparging (scouring rate)	m^3/m^2*h	0.5
Total active volume (reactor and filtration unit)	l	150
Influent flow	l/day	71
Organic Loading Rate	Kg COD/m^3/day	0.72

The operation time was in continuous mode, with cycles of 14 minutes: 10 minutes of filtration mode and 4 minutes of relaxation. During relaxation mode, the feeding and permeate pumps were put in standby, and there was no membrane filtration to allow transmembrane pressure recovery. The feeding section was completely stopped every day at a certain time during half an hour for new substrate preparation and tank filling.

3.5 Maintenance

The AnMBR's required periodical checking and adjustments to ensure desired performance at the operational conditions above mentioned. Maintenance was adapted according to the evolution of the response in performance of the systems. This was applied specifically on pipes, pumps, level indicators, substrate tanks and, most important, membranes.

3.5.1 Pumps and substrate tank

Pumping system required practical checking of performance frequently, as it was considered a critical point in the system to ensure full operation. Wearing parts were replaced in some of the pumps, and other ones were thoroughly cleaned at least once.

Substrate tanks were cleaned every week with generic dishwasher detergent to wash out any undesired aerobic microbes that could grow with air (oxygen) available, and alter the substrate conditions such as pH and temperature.

3.5.2 Membrane cleaning

The membranes used in the experiments were prototypes provided by Alfa Laval. They required a pre-cleaning before installation in the filtration unit, in order to remove the glycerin used as a protective coat during fabrication and transport. The pre-cleaning consisted in submerging the membranes in a basic solution (at pH=10 with NaOH) at 30ºC for 1 hour.

During the study, chemical cleanings of the membranes were also performed, in order to remove sludge particles that could block the pores of the membrane (i.e. membrane fouling). The transmembrane pressure (TMP) was used as a signal for this phenomenon, and membranes were cleaned when the TMP was above 100 mbar.

To start the cleaning process, the system was stopped, and two chemical solutions were prepared. The first was with hydrochloric acid solution (at pH=1.5) at 30 ºC; the acid solution removed any scales, metal dioxides or salts like $CaCO_3$ present in the fouling. The second was a basic solution of pH=11 at 30 ºC, with Ultrasil® 10 detergent, acting as a surfactant ideal for milk waste proteins and fat. Each solution was flushed one hour in backwash, with an average water column pressure of 30 cm.

3.6 System monitoring

The AnMBR's were monitored and controlled in three main ways. The first was the Control Panel Unit, which displayed the data of level sensors, TMP sensors, flow meter (permeate) and pumps speed. The same panel was used for adjusting pumps speed (including the gas pump). Figure 3-4 shows the control panel for each AnMBR displaying all mentioned parameters in a schematic view of the system, the screens were touch sensitive to edit values.

Figure 3-4. Control panels of both systems. Note that the left panel has red buttons and the right has green ones. These colors were alternated informing of filtration or relaxation mode.

The second consisted of a thermal mass flow meter from Vögtlin for measuring gas flow. It was calibrated for a gas composition of 60% CH_4 and 40% CO_2 and a maximum flow of 600ml/min. It was connected to a computer with a dedicated software (Red-y) that informed about the flow and temperature of the gas output in normal liters (NL).

The third type was a manual procedure. The pH and temperature (pH-T) were measured daily in place, using the sample points and a portable pH meter in the room. The pH meter was also used to measure pH-T of the feeding tanks. According to the tanks dimensions, a roller meter was used to measure the quantity of substrate added to the systems.

3.7 Sample analysis

Samples were collected from the sampling points indicated in Figure 3-3. Every type of sample had its own parameters to analyze, as well as the frequency of it. For everyday analysis, pH and temperature of the reactors and substrate were measured using a WTW Sentix ® portable digital pH meter (model 3110 with electrode num. 41). The pH meter was calibrated once every two weeks based on 2-point calibration (pH=4 & pH=7).

The other samples were taken to the laboratories next to AnMBR's location, in the same department (chemical engineering) for further analysis. Reactor, effluent and gas samples were analyzed. A description of each one is presented as follows.

3.7.1 Reactor samples

Samples were collected manually using closed recipients. They were immediately analyzed for minimum alteration of the values. Table 3-5 shows the full list of parameters analyzed and the method used.

The majority of them were tested with Hach Lange Cuvettes®. The prepared cuvettes were analyzed in a Hach Lange spectrophotometer (model DR 2800). Not all the methods were used at first, due to availability issues. Also, some dilutions were made to ensure range of detection, using precision tools. For some analysis, samples were centrifuged at 10.000G for 10 minutes. See Appendix I & II for the laboratory procedures and calculations.

VFA was measured with a Gas Chromatograph Agilent 6850 Series equipped with a flame ionization detector (FID) and a column with dimensions: 25 m x 0.32 µm x 0.5 µm. For the TSS & VSS, *Munktell* filter papers of 1.6 µm were used along with a 105ºC and 550ºC oven.

Table 3-5. List of analysis in the laboratory for reactor samples

Parameter	Units	Centrifugation	Dilution	Method
Total COD	mg O_2/l		✓	Hach Lange
Soluble COD	mg O_2/l	✓		Hach Lange
Conductivity	µS/cm			WTW conductivity meter
Alkalinity	mg $CaCO_3$/l			Various method (see 3.7.4)
Volatile Fatty Acids (VFA)	mg COD/l	✓		GC – FID
Total Carbon / Total Organic Carbon	mg C/l		✓	Shimadzu TOC analyzer
Total Nitrogen (TNb*)	mg N/l		✓	Hach Lange
Total Phosphate**	mg P/l		✓	Hach Lange
Ammonium	mg N/l	✓		Hach Lange
Phosphate (PO_4)	mg P/l	✓		Hach Lange
Sulfate	mg SO_4/l	✓	✓	Hach Lange
Sulfide	mg S/l	✓		Hach Lange
Total Suspended Solids (TSS) & Volatile Suspended Solids (VSS)	mg/l			Standard methods (SS-EN12879 & SS-EN12880)

*Total nitrogen bound: Includes organic, inorganic, ammonium, nitrate and nitrite nitrogen.

** PO_4, PO_3, PO_2 and other phosphate ions.

3.7.2 Effluent samples

Along with reactor samples, the effluent was also sampled with 50 ml bottles. The analysis performed for the effluent was turbidity, VFA, COD (in HL LCK-614) and conductivity.

3.7.3 Biogas samples

For measuring gas content, samples were taken through sample point with a rubber septum in the output line. A standard micro syringe of 500 µl (500 mm Bevelled 1) was used to take the gas samples to the Varian 3800 gas chromatograph, equipped with a thermal conductivity detector (TCD) and a column with dimensions: 2.0 m x ⅛ inch x 2.0 mm.

A reference signal of 100% CH_4 was used to be compared with the samples. The program was set to measure CH_4, CO_2 and O_2.

3.7.4 Alkalinity tests

Different tests were performed as a way to evaluate best available technique (BAT) to measure alkalinity (including total alkalinity, VFA alkalinity or carbonate alkalinity when available). Total alkalinity from Standard Methods (APHA), Jenkins et al. (1983), Ripley et al. (1986), and finally Moosbrugger et al. (1993) were assessed to find strong and weak points for a practical and reliable analysis of AnMBR's alkalinity.

All methods include only titration procedures, which were performed with a BRAND digital burette III, using hydrochloric acid at 0.05 N and WTW Sentix ® pH meter. The down points for annotation were collected from all methods to be done at once, and they were 6.7, 5.9, 5.75, 5.2, 4.5 & 4.3.

For the calculations, see Appendix II. Moosbrugger et al. (1993) utilized a dedicated MS-2 program. However, Chris Brouckaert developed an MS Excel version of the program (Vannecke et al., 2014). This excel version was used in the experiment for calculating alkalinity.

4 Results and discussion

The start-up of two pilot-scale AnMBR reactors in equal conditions was evaluated performing laboratory analysis and monitoring operational parameters. Despite the identic start point from both systems, they followed substantially different performance. System one achieved a successful operation, while the second did not develop a satisfactory functioning.

The key factors of this contrast were compared in the next sections to draw clear indicators of what went wrong, and the necessary steps to fulfill a successful startup. Additionally, results from other similar experiments were compared with this study to give a better scope on the aim of this work.

4.1 General performance of AnMBR

The summary of the performance of both reactors can be seen in Table 4-1. COD removal refers to the ratio between COD in effluent compared to the inlet. Methane yield and content define the real performance of an AnMBR. Flux and OLR are characteristic for its importance in membrane operation and organic strength of the feed, respectively. Volatile suspended solids (VSS) represent the average biomass content in the reactor.

Comparing each system, removal rate of COD was excellent in System 1, while the second did not achieve an average above 80%. AnMBR tend to perform at reasonably high COD removals; hence, values below 80% may indicate a sign of operational problems, according to Lier et al. (2008).

Table 4-1. Summary of AnMBR's performance. Values are the mean ± StDev

Parameter	System 1	System 2
COD removal (%)	95.6 (±3.5)	76.4 (±5.5)
Flux (LMH)	7.66 (±0.82)	7.58 (±0.88)
Methane yield (m^3 CH_4/kg $COD_{removed}$)	0.26 (±0.16)	0.04 (±0,17)
Methane content (%)	62.8 (±12.1)	9.7 (±14.0)
OLR (kg COD/m^3·day)*	0.67 (±0.22)	0.51 (±0.35) / 0.29 (±0.07)
VSS (mg/l)	8729 (±1626)	7939 (±2100)

*The OLR of System 2 was changed once.

In terms of equipment stability, flux was close to the operational setting (8 LMH), as well as OLR (0.72 kg COD/m^3·day). Note that System 2 had recovery periods (i.e. periods when only feeding the system with water and nutrient mixture), which explains the high deviation. Also in System 2, a new lower OLR was set at the last weeks of experiment (0.36 kg COD/m^3·day). This new OLR had much less deviation compared to the first OLR setting, meaning a better influent stability.

As for biogas yield, System 1 developed a good operation, although being under the theoretical maximum yield (0,350 m^3 CH_4/kg COD removed). In System 2, there was almost imperceptible gas production (deviation is much higher than the average low value).

The biomass content (VSS) of both systems were in average similar to the starting point of the reactors (8000 mg/l). There is substantial deviation in the results (higher in System 2 than in 1). In general, higher concentration was expected than the obtained at the end of the experiments. The low growth rate of microbial population could be the reason for this small VSS increase. Concretely, System 2 suffered from biomass leakage and lost an important part of VSS during the experiment.

It is important to state that operational performance did not develop with clear stability, showing more changes during the first weeks of the study. This applies especially to VSS content, COD removal rate and gas yield. Therefore, more attention to these parameters in this period might have helped to apply corrective measures.

Biogas production in terms of its methane generation is presented in Figure 4-1. It shows the cumulative methane production of System 1, System 2, and an expected production of 32 NL/day. The expected series was calculated based on operational values (OLR setting and reactor volume), and an estimated 80% degradation of the theoretical production of 350 NL/kg COD according to Speece (1996).

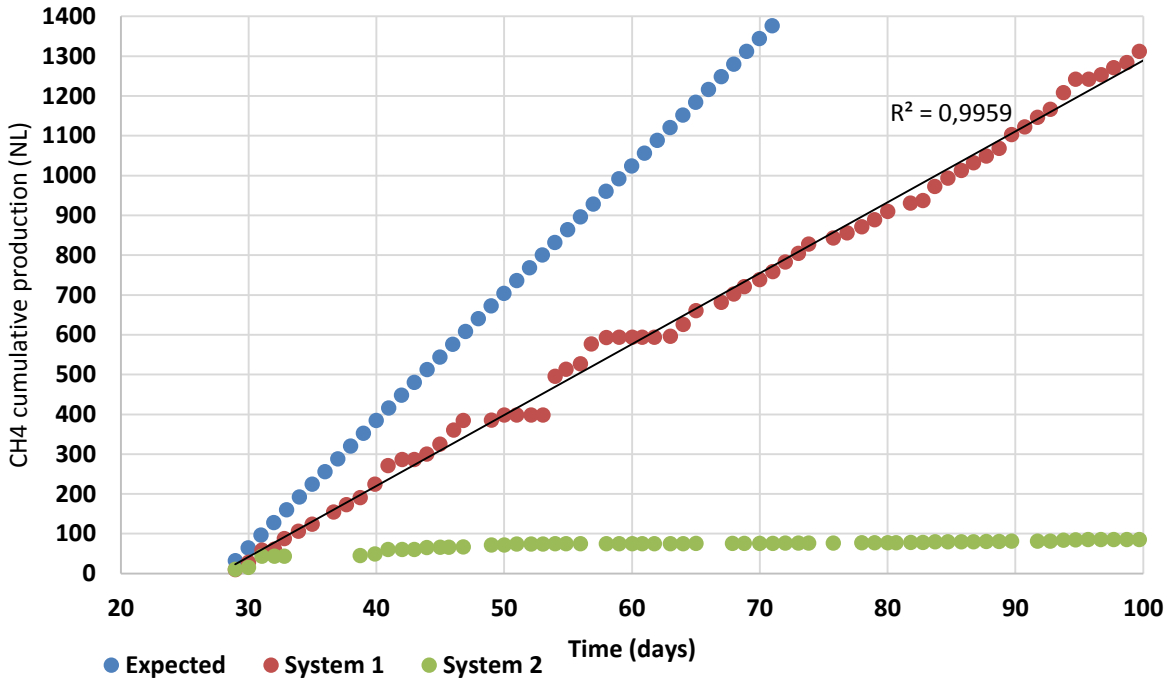


Figure 4-1. Comparison between expected and each system on CH_4 cumulative production.

Data was disregarded from the first days due to incomplete biogas content analysis. According to the setting parameters, System 1 produced roughly 60% of the daily 32 NL CH_4 that was expected. Although being far from expected, methane yield in System 1 follows a steady linear trend (0.99 R^2 regression). Causes for the lower production were related to an actual less COD degradation than expected, less biomass yield due to ambient temperature, or dissolved methane in reactor. Dissolved methane corresponded up to 10% of methane production. Smith et al. (2013) experienced dissolved methane as a major problem operating a reactor at 15ºC. This issue was more pronounced when decreasing temperature below mesophilic.

In System 2 there was hardly any amount of methane generation, being incapable of producing almost any from day 40. This demonstrates the failure of System 2 in one of the major aspects of AnMBR, which is biogas production from wastewater. In this regard, it is important to remark that System 2 did apparently work at least the first days. The very first data available is similar to System 1, but short after it stopped producing methane. Therefore, it is presumable that the two reactors did not have the same operational conditions, and that system two suffered from digestion upsets. A thorough analysis of other indicators is presented below to enlighten any issues of System 2.

One of the more sensitive parameters that was affected by these issues was the organic loading rate (OLR). As stated before, while OLR setting in System 1 was kept constant, in System 2 there were adjustments to enhance reactor performance. Some recovery periods (periods without milk powder feeding, i.e. without organic loading rate) were applied in order to balance the buffering capacity of the reactor. This phenomena is described in more depth in the stability section.

Figure 4-2 shows daily OLR for each system, including recovery modes, the two OLR settings and the date when new OLR setting (half the amount of original OLR) was applied in System 2. Recovery data points were also marked in the figure.

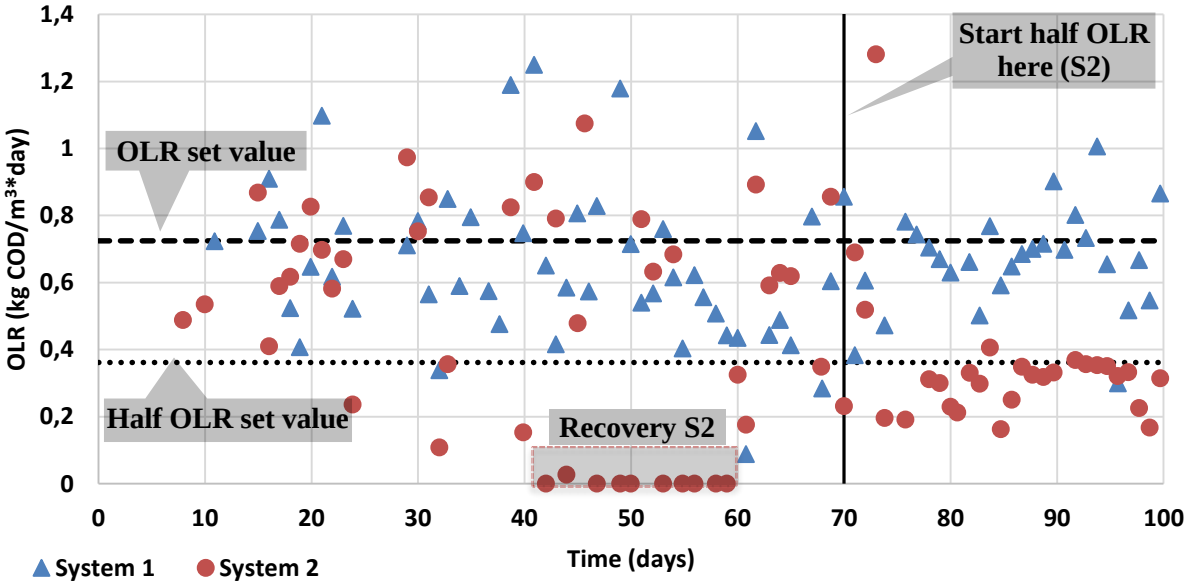


Figure 4-2. Changes in the organic loading rate during the study.

The OLR set lines correspond to the desired values that were intended to keep. Using these set lines as reference, it can be seen how many loading shocks affected each system. As mentioned before, recovery modes were used in System 2 as well as half loading rate from day 70 to recover pH stability. All of these phenomena were marked in Figure 4-2.

Until day 40, System 1 was more close to the set line compared to System 2, where values are more dispersed. This means that it suffered from unstable loading rates during these days. The high variability of OLR was caused by the poor performance of the pumping system. The design of the pumps was vulnerable to solids present in the influent. These solids could block the pumps and affect their performance. The OLR was sometimes increased in order to keep a constant daily flow, but detrimental as organic shock loads were applied.

After day 70, there is significant difference between the values from the first days, showing a more stable OLR in both systems. This is coincidental with a better maintenance of the equipment (cleaning schedules and less presence of solids), which allowed better stability in the digestion.

Daily biogas production is another clear indicator of reactor balance. High variance in the values may reflect alteration of reactor volume (loss or overload). It also can reflect loading shocks in second place (excessive development of different microorganisms). If there is an overload in the reactor, the liquid gas equilibrium breaks and causes an abnormal overproduction of biogas. Conversely, when reactor volume goes down, there is no gas outlet due to the slight suction effect of an emptying reactor.

Alteration of the reactor volume could have caused more stress in the active biomass, as well as an increasing risk of air intrusion in the systems. When reactor volume is reduced, a negative pressure is generated (below atmospheric pressure) and it may facilitate the intrusion of oxygen inside the reactor and break the anaerobic conditions, therefore causing inhibition of the methanogens.

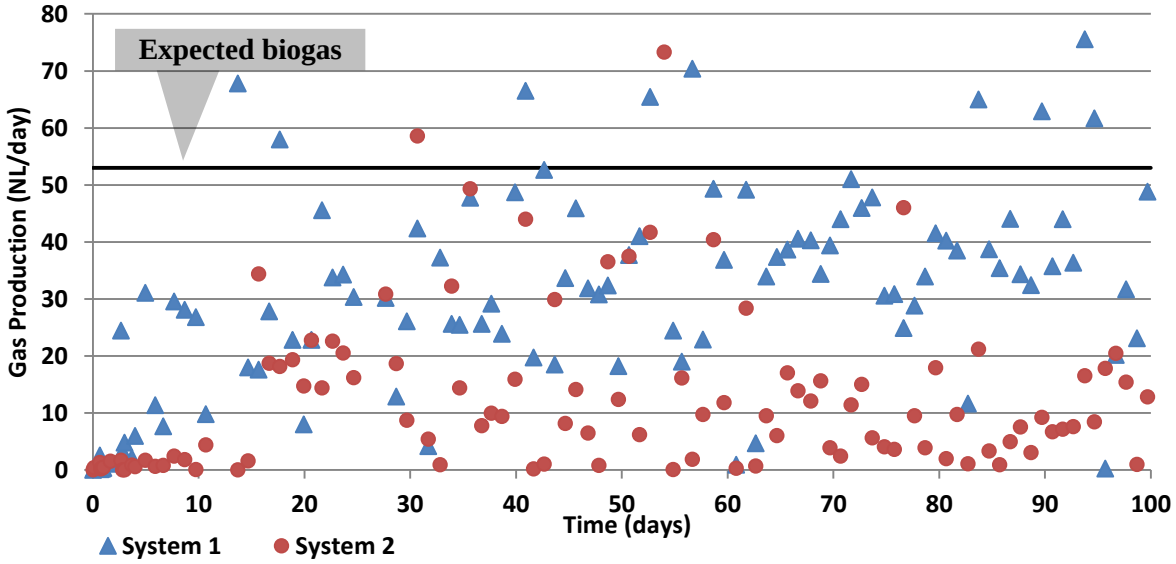


Figure 4-3. Daily gas production in each system (Normal Liters).

The gas production is shown in Figure 4-3. Both of reactors had very disperse values during the first half of the experiment time. More concretely, there was no biogas production in System 2 during the first days. The last days of experiment show a more stable biogas production in both cases, with clear advantage of System 1 over System 2.

The combination of organic loading rates and biogas production reveals that there were unfavorable conditions to develop a satisfactory startup, especially in System 2. Although System 1 could overtake this conditions, frequent maladjustments might have affected a successful startup of System 2, such as sudden loss of reactor volume (up to 75%) and organic shock loads, as mentioned before.

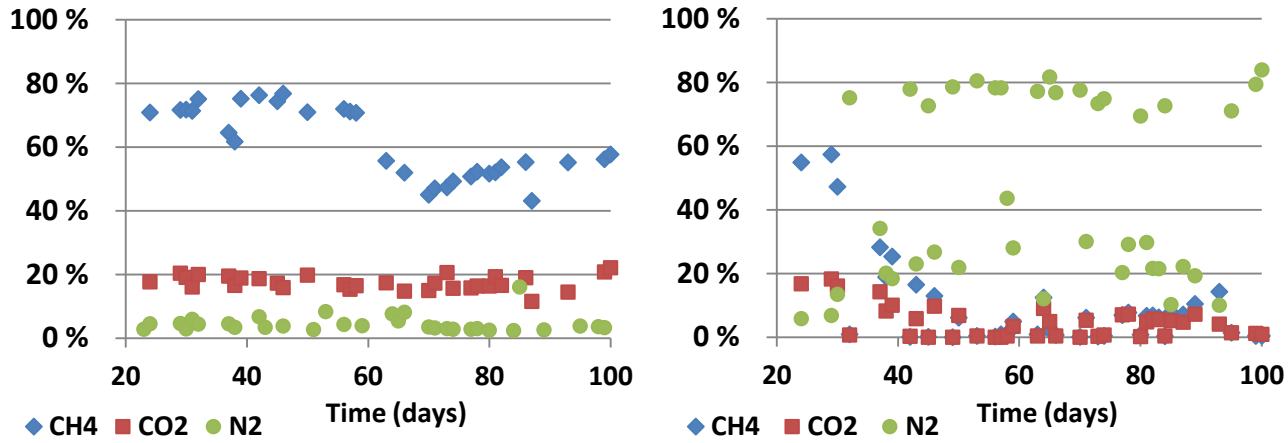


Figure 4-4. Gas composition of System 1 (left) and System 2 (right).

Biogas content is shown in Figure 4-4 for every reactor. As mentioned before, each system followed totally different evolution during the study.

The methane content in System 1 was stable until day 60, with around 70% CH_4 and 20% of CO_2 and almost no air (shown as nitrogen gas). From day 60, methane proportion dropped to 50% and recovered a 10% in 30 days to a new stable state at 60% with same content of CO_2. This showed a considerable slow recovery compared to other literature (Dupla et al., 2004), especially considering that operational parameters did not change. The methane content drop happened at the same time as a biomass loss, which could have washed out methanogens. This effect was reflected in a higher effluent COD and decrease of VSS in System 1.

In System 2, a stable period can be deduced from the first values (day 0 to 20). However, from day 30 methane content was totally lost until the end of the experiment. Only in the very last days it started to rise timidly again, as a consequence for a regained process after recovery periods and low stable OLR.

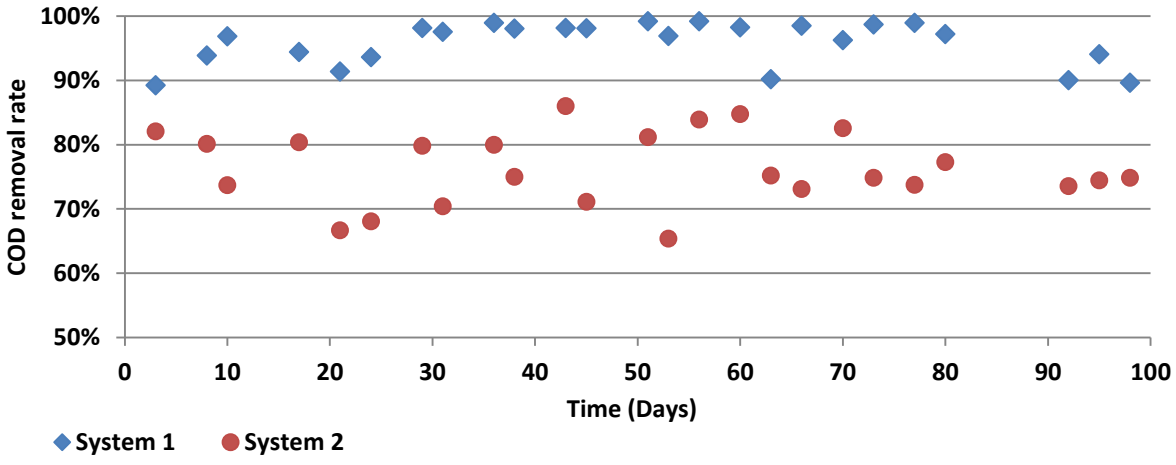


Figure 4-5. COD removal rates (effluent COD vs influent COD) in each system.

Figure 4-5 shows the COD removal rates of the systems. The COD removal is a ratio that compares how much COD is left in the effluent compared to the influent. It presents the relative efficacy of the AnMBR in removing organic matter from the liquid. This rate was significantly stable mostly at high values for System 1, whereas System 2 varied from 70% to 85% removal capacity. It also performed with abrupt variations during the intermediate periods

(days 20 to 70), mostly because of digestion upsets and membrane performance. Also, the recovery periods and lower OLR could have affected the ratio. Overall, the removal rate was a factor dependent on the stability of the digestion process. And its results explained the success of anaerobic digestion.

In Figure 4-6, a representation of the effluent quality is presented (COD and VFA in effluent). It also showed significant difference between systems. While System 1 kept most of the days an effluent COD below 100 mg/l, System 2 had concentrations of 200 – 500 mg/l.

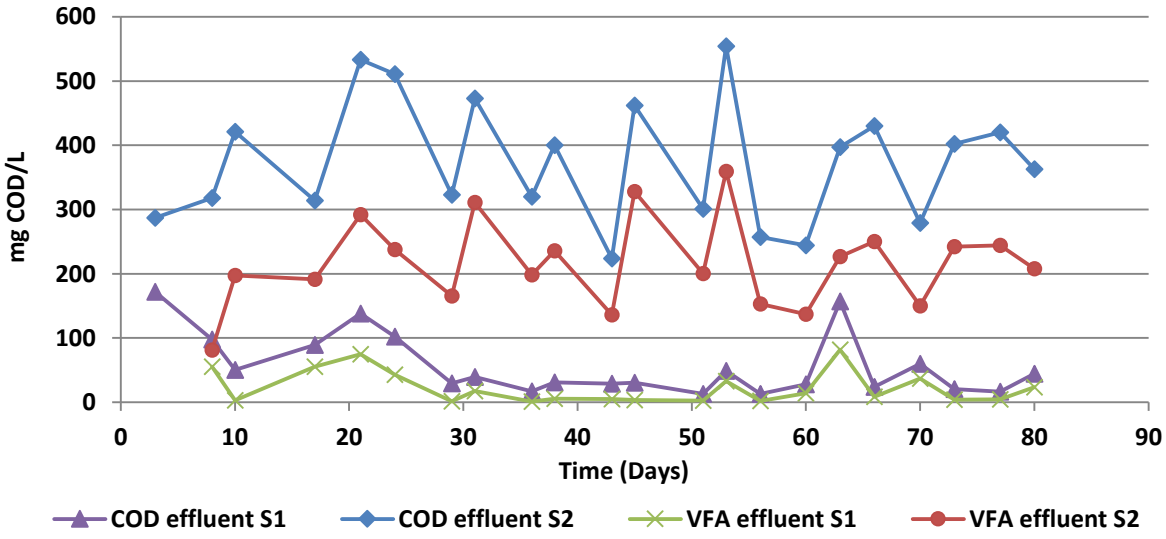


Figure 4-6. Effluent COD & effluent VFA

Note that after day 60 there is a temporary increase of effluent COD in System 1, which coincides with the loss of methane proportion in Figure 4-4 (left), and therefore supporting the hypothesis of biomass washout through effluent.

It is worth mentioning that in both systems, COD and VFA in effluent followed the same variation, as Figure 4-6 shows. This is because an important portion of the effluent COD corresponded to VFA, between 40 to 70%. Sánchez et al. (2005) also experienced this relation.

The effluent levels in System 1 were considered acceptable for the reactor characteristics, with similar concentrations of 30 – 240 mg COD/l in literature (Ho et al., 2007; Liao et al., 2006; Lin et al., 2013; Skouteris et al., 2012; Visvanathan and Abeynayaka, 2012). However, the concentrations in System 2 were higher than literature (300 – 400 mg COD/l).

4.2 Stability parameters of reaction process

The following sections describe the effect of pH, VFA, alkalinity and suspended solids (TSS & VSS) as the main indicators for evaluating a stable operation of the AnMBR. The monitoring of all these parameters provides good information of the process stability and performance.

4.2.1 Effect of VFA and alkalinity on pH

One of the important parameters that enables reaction stability is pH of the reactor. While the majority of anaerobic microbes can live in a relative wide range of pH, methanogens develop

best in a range between 6.8 and 7.4. Under values of 6.4 the methanogens are mostly inhibited (Khanal, 2008). Therefore, aiming to a neutral pH operation is desirable in normal AnMBR.

From the very first days of operation, pH never reached neutral values in any of the systems, and it was mainly around pH of 6.5. Figure 4-7 and Figure 4-8 show the evolution of pH and temperature during the experiment. Again, each system followed a different pattern, as well as different operational modes. However, both systems had a constant temperature of 25±1 ºC.

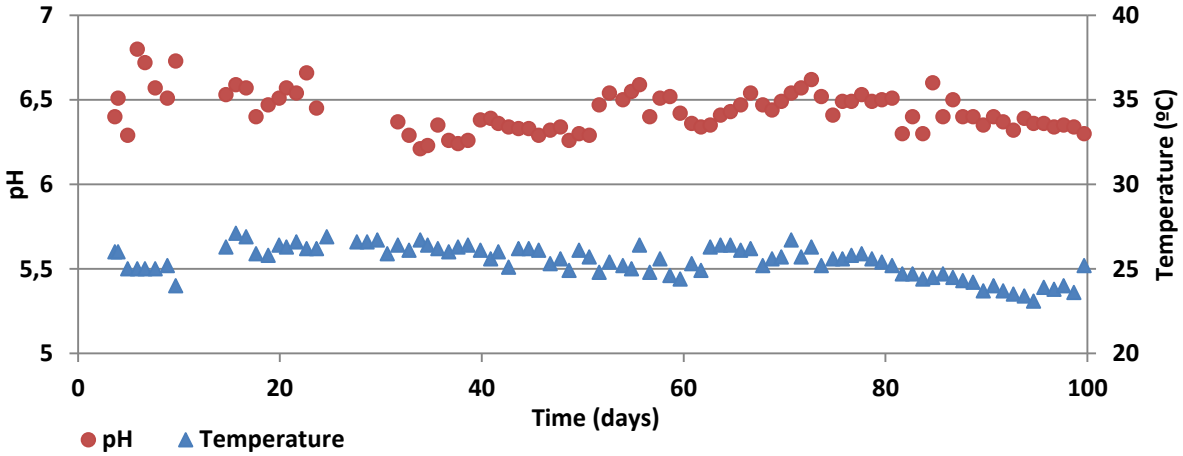


Figure 4-7. pH and temperature evolution during the experiment in System 1.

Although being under pH 6.4 in some periods, System 1 maintained a relatively constant pH of 6.5 without any special intervention. These data reveal that no relation can be found between the pH of System 1 and an inhibition of methanogenesis.

The inhibition would have caused drastic reduction of biogas generation or COD removal rates, as well as an abrupt diminution of pH values. Therefore, the lower pH tolerance (6.4) was actually more flexible than stated in literature (Khanal, 2008). The pH range of System 1 was between 6.2 and 6.8 with successful operation.

On the other hand, System 2 did have sudden pH drops, which caused inhibitory periods. The evolution of pH was similar to System 1 until day 24. The pH went below 6 in two occasions after, as it can be seen in Figure 4-8.

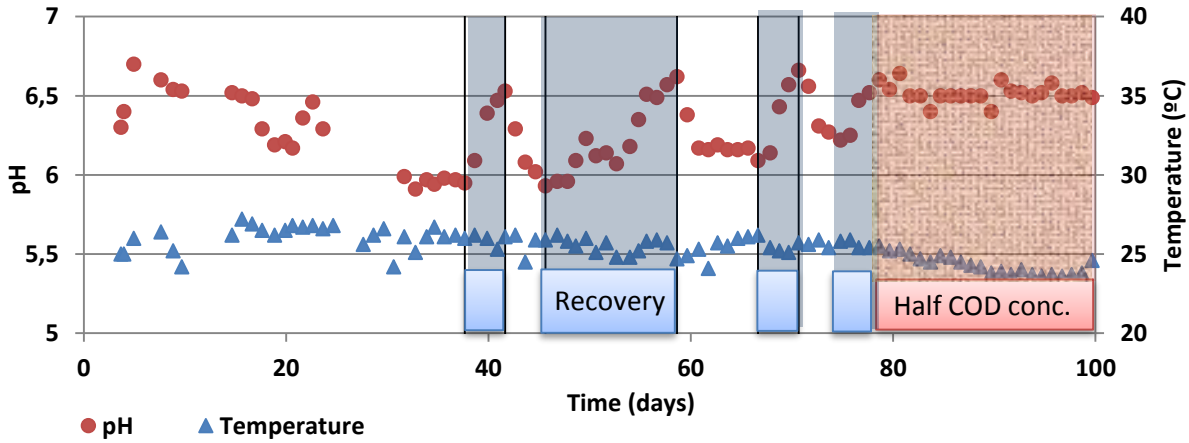


Figure 4-8. pH and Temperature evolution during the experiment in System 2.

As mentioned before, recovery modes ("Recovery" areas in Figure 4-8) were applied to improve methanogen activity. The recovery consisted of influent periods with only water and essential nutrients for microbial development. Therefore, no OLR was introduced in the digestion, and the excess of VFA in System 2 (see Figure 4-9) was either extracted within the effluent or consumed by methanogens. High concentrations of VFA can lead to lower pH values, because it acidifies the reactor liquid, like Sánchez et al. (2005) suggested.

Dissolved CO_2 also plays a role in the acid-base balance. A small proportion of the gas reacts with water to form carbonic acid, and it tends to lower the pH. Therefore, the presence of dissolved CO_2 might have been influential to pH in this experiment, due to the higher solubility of CO_2 when temperature is decreased. However, this cause was not specifically studied.

Another cause of low pH was from substrate degradation in the substrate tank, caused by the development of aerobic microorganisms. This microbes would have favorable conditions due to accidental aeration when mixing the substrate. Furthermore, constant mixing caused an increase of substrate temperature, which would facilitate aerobic reactions and form fatty acids.

The substrate pH could go from pH 10 to 6 in 24h depending on substrate tank temperatures (i.e. mixing time) and the aeration (bubbling of the mixing). Also, if no Na_2CO_3 was added, it could drop down to pH 5. This influence was very important in the first days of the experiment, when none of the substrate conditions was rectified for a higher pH.

Between days 23 and 30, results were disregarded due to an erroneous calibration of the pH meter. After the new calibration was done, results from day 31 showed quite different values from days before (deviations showed pH = 7 when it was actually 6 or less). Since the feeding tank and the reactor were measured with the same pH meter, both of them could have been at pH under 6 for several days. Therefore, System 2 might had been exposed to total inhibition of methanogens during a week. Furthermore, granulation of milk fat and proteins occurred in the feeding tank at low pH, causing disturbances in the influent pumps (clogging).

Conversely, System 1 did not have a pH drop below 6, although the results were equally disregarded as like System 2. System 1 was capable to keep a pH that allowed normal operation, mostly because there was less risk of acidification caused by VFA concentration. System 2 had substantially higher VFA levels compared to System 1.

After this experience, from day 35 the substrate was prepared with systematic addition of buffering agent (sodium carbonate). Furthermore, mixing times were reduced to minimize temperature increase in the substrate tanks. Also, the mixing was adapted to produce the least aeration possible, in order to minimize growth of aerobic microbes. The tanks were cleaned once a week to remove any rests of aerobic microorganisms attached to the tank surface.

These measures improved substantially the pH stability from substrate tanks. However, although they were applied gradually, it was found much more effective when all of them were used. At the end, substrate pH was increased to 10 to help neutralize pH of reactors.

The concentration of fatty acids in the reactors is shown in Figure 4-9. The only data point that matched in both systems is the first one. This point could suggest a normal concentration of the digestion since the starting day. However, no earlier data is available (VFA was added the 2nd day of laboratory analysis) and nothing can be stated from previous days. From the

second data point, System 2 became unstable and VFA concentration started to increase, whereas System 1 achieved a steady trend at very low VFA concentration.

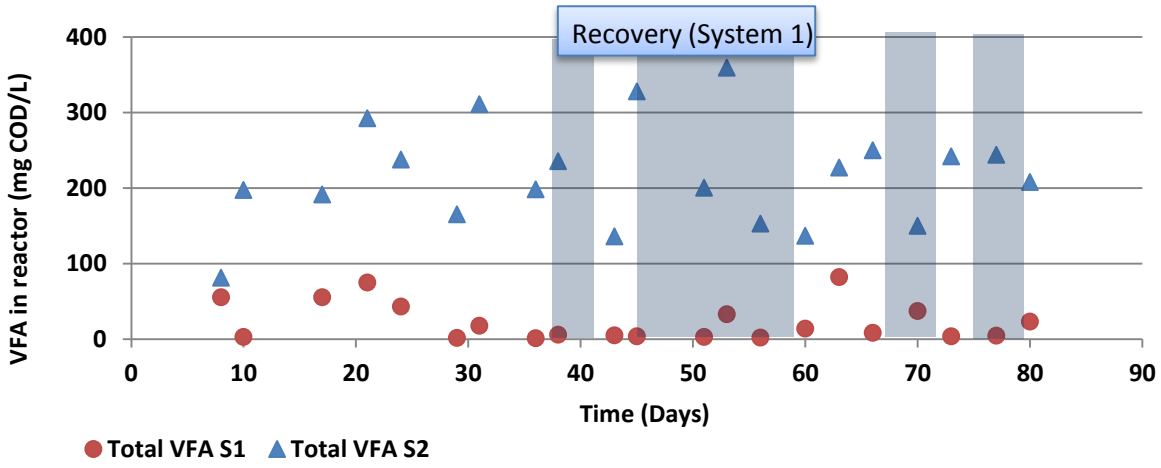


Figure 4-9. Total VFA in reactors.

However, a more deep evaluation of VFA influence is required to state its importance in pH of the systems. The concentration of VFA can be compared to the alkalinity of the reactor to assess how much VFA influenced the buffering capacity, and cause pH decrease. Alkalinity can determine the buffering capacity of the reactor, and how it can absorb the acidification of the fatty acids. A good indicator of this is the TVFA – Alkalinity ratio.

According to Sánchez et al. (2005), a well-functioning reactor is characterized by TVFA-alkalinity ratios lower than 0.3-0.4. If the reactor is above these values there is risk of acidification, and corrective measures should be applied. From ratios above 0.8, there will be total inhibition for methanogenesis, with significant acidification of pH that disable microbial development.

Figure 4-10 represents the TVFA:Alkalinity ratio during the days of experiments in both systems. As it is observed in the figure, unstable conditions in System 2 appeared from day 30 until the end of analysis (no data is available in the last 20 days due to instrumental problems in laboratory analysis). There was not sufficient buffer capacity to balance the excess of VFA, because the ratio exceeded 0.5 frequently. Hence, the reactor suffered from acidification.

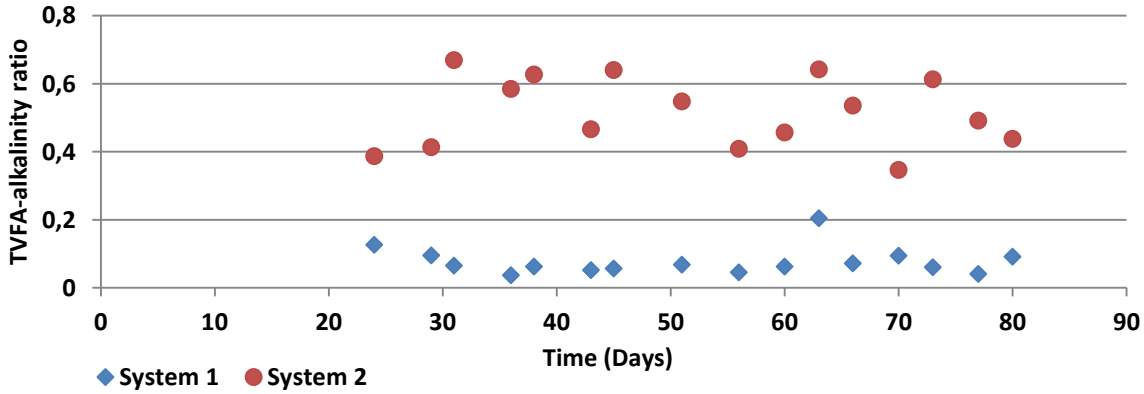


Figure 4-10. Total Volatile Fatty Acids (TVFA – in equivalents of Hac): Total alkalinity (equivalents of $CaCO_3$) ratio.

As for System 1, the ratio was stabilized under values of 0.1, ensuring minimum affection of VFA acidification on pH as exposed in previous figures. Comparing the pH and TVFA-alkalinity ratio of both systems, there was no increment of pH above 6.5 regardless of VFA concentration in System 1, even though the excess of VFA could decrease pH in System 2. A possible influence of dissolved CO_2 over pH could have caused the low pH.

Although it is important to follow the TVFA – Alkalinity ratio rather than alkalinity itself (Appels et al., 2008), the levels of alkalinity should be assessed with the pH of reactor, and it should be high enough to balance the potential acidification from a sudden increase in VFA.

4.2.2 TSS and VSS

The total and volatile suspended solids represent the amount of microbial population in the system. Therefore, suspended solids are a good indicator of biomass presence in the reactor. The evolution of TSS and VSS are shown in Figure 4-11 and Figure 4-12 for System 1 and 2, respectively. VSS/TSS ratio was also plotted to evaluate sludge age and biomass quality.

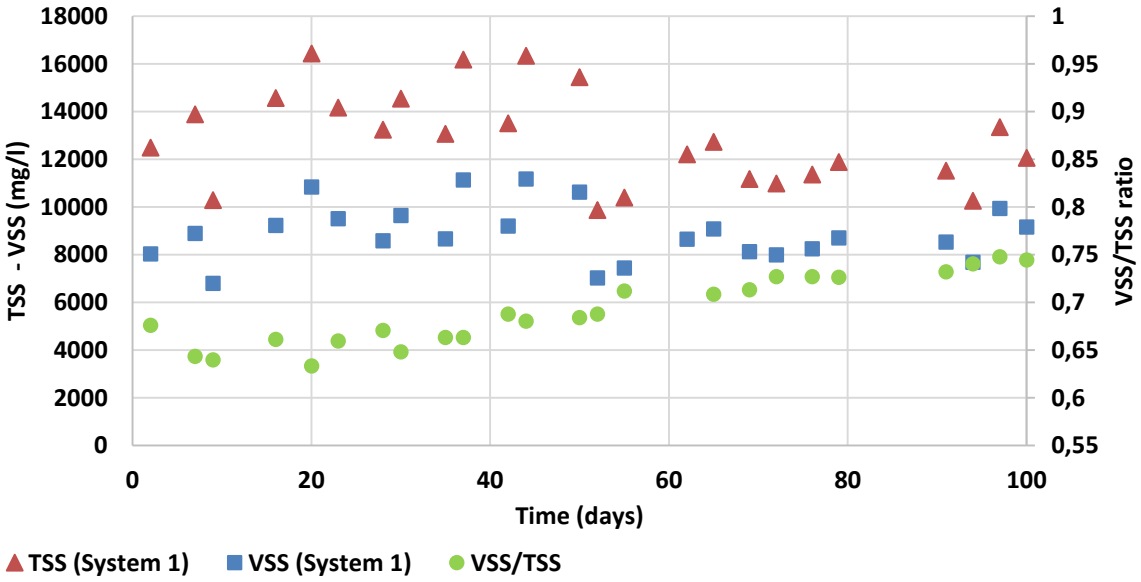


Figure 4-11. Total Suspended Solids and Volatile Suspended Solids in System 1.

Biomass development in System 1 was characterized by slowly increasing until day 50, followed by a sudden decrease. This decrease happened in the same period of the loss of performance mentioned before in System 1, in terms of methane content and COD in effluent. Less amount of VSS is directly related to less methanogen activity, knowing that approximately 90% of VSS corresponds to active biomass (Tchobanoglous et al., 2003). The remaining 10% is attributed to dead cell debris and non-biodegradable volatile solids.

Given the biomass immobilization characteristic in AnMBR, an increasing trend would be expected in VSS concentration, showing a good health of microbial population. In this perspective, a more stable period was experienced until the end of the experiments. The overall VSS slowly increased from 8000 mg/l to 9000 mg/l (11%). On the other hand, sample withdrawal supposed a reduction of 8% of total suspended solids in all the experiment time (100 days). That means a removal of ~1g TSS per day.

As like other indicators, the evolution of TSS and VSS in System 2 seen in Figure 4-12 is quite different from System 1. Until day 20, System 2 seemed to develop even better than 1 in

regards to biomass growth. However, a constant decrease of VSS was experienced until day 55, losing about 50% VSS (from 12000 mg/l to 6000 mg/l). The biomass loss was very probably the trigger for a cascading failure of other parameters: low methane production, VFA overload, pH decrease and high COD effluent.

The biomass leakage was caused by an intermittent failure of the membrane gaskets, allowing a direct escape of the reactor fluid without passing through the membrane. The leakage started on day 20, just before methanogen activity decreased in terms of low methane production and VFA overload. Right after, pH dropped down below 6, causing total inhibition of methanogenic archaea.

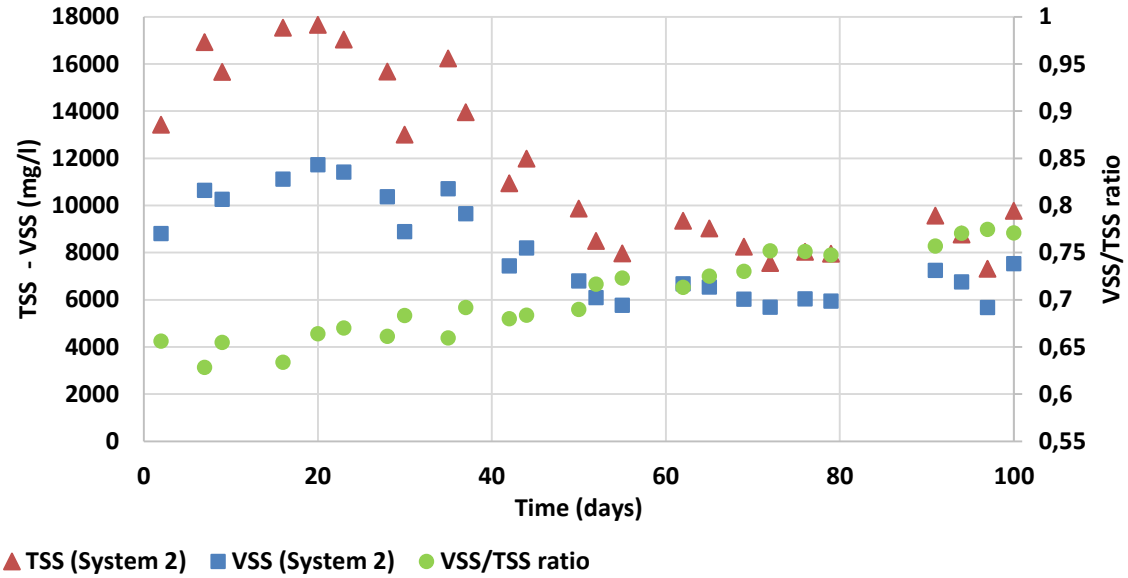


Figure 4-12. Total Suspended Solids and Volatile Suspended Solids in System 2.

After the decrease period, the biomass leakage was fixed on day 53 by the substitution of gaskets for bigger ones, improving insulation of the membrane when filtrating effluent. A stable VSS concentration remained until the end of experiment, at around 6000 mg/l, with very little biomass recovery. This situation was not a surprise, considering that even at a higher VSS concentrations there was hardly any biogas production.

On the other hand, the VSS/TSS ratio increased constantly in both reactors. Values from 0.7 to 0.85 are considered normal to operation, and they show a good reflect of biomass growth and quality (Rizvi et al., 2013).

4.3 Alkalinity tests

In this experiment, the APHA (1999) method for total alkalinity measurement was compared to Moosbrugger et al. (1993) method. The method of Jenkins et al. (1983) and Ripley et al. (1986), were also compared to Moosbrugger et al. (1993).

The last two are also capable of measure carbonate and VFA alkalinity (partial and intermediate alkalinity). However, they differ in the calculations, as the Moosbrugger et al. (1993) utilizes more complex equations and complementary deviations compared to Jenkins et al. (1983).

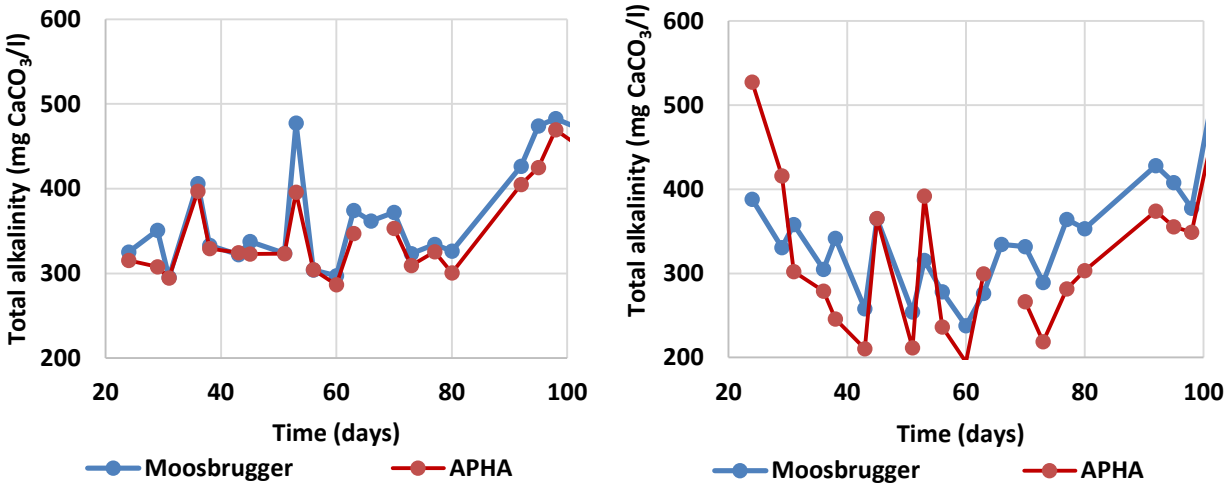


Figure 4-13. Total alkalinity measured with APHA and Moosbrugger. System 1 (left) and 2 (right).

In Figure 4-13, total alkalinity was found to be truly similar when monitoring system one. The APHA and Moosbrugger methods gave roughly the same results, with deviation less than 5%. However, a slight difference was shown when comparing data from System 2. It could be explained by the effect of interferences with other compounds, especially VFA, none of which are considered in the APHA method.

It is worth mentioning the increasing trend during the last days, where systematic addition of buffering compound was done, in order to stabilize pH of reactors and substrate.

In any case, the deviation between methods was not severe, and patterns of alkalinity change were identical. This fact is the most important one, rather than the absolute value of alkalinity.

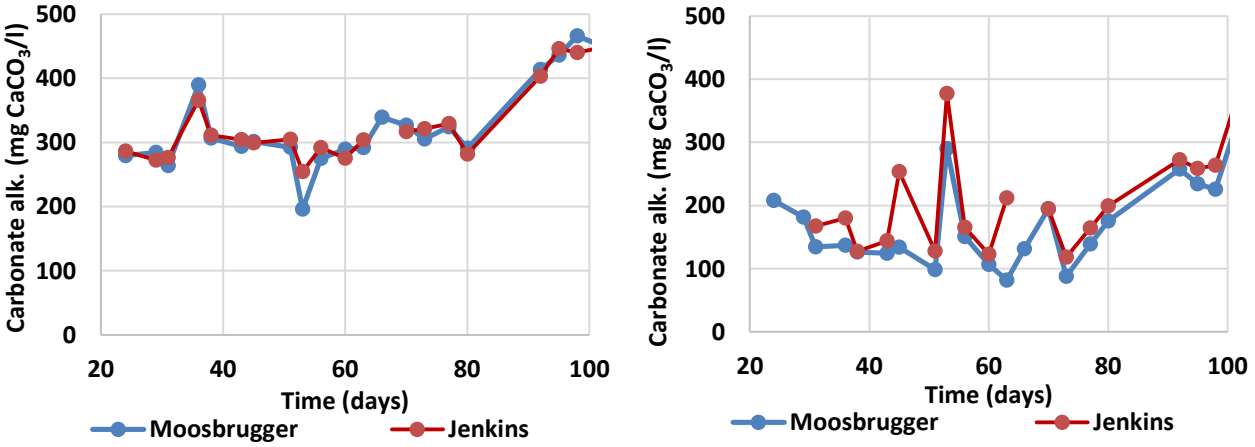


Figure 4-14. Carbonate alkalinity measured with Jenkins and Moosbrugger methods. System 1 (left) and System 2 (right).

Carbonate alkalinity measured the influence of carbonate species in the reactor. They represent the main compounds that cause buffer capacity of the system, as it can be seen for System 1 in Figure 4-14 (left) values compared to Figure 4-13 (left), which are similar.

The same cannot be said of System 2, where carbonate species represent approximately half of the total alkalinity. This indicates a clear sign of process failure, and the vulnerability of System 2 to a sudden change in pH, concretely caused by VFA concentration. The pH had similar evolution as alkalinity in low (and disparate) values. pH also stabilized at 6.5 when carbonate alkalinity started to increase from day 60 due to buffer addition.

As for the comparison of the methods, they also give very similar results. Again, System 1 is almost identical for both methods, and System 2 also gives fairly the same results.

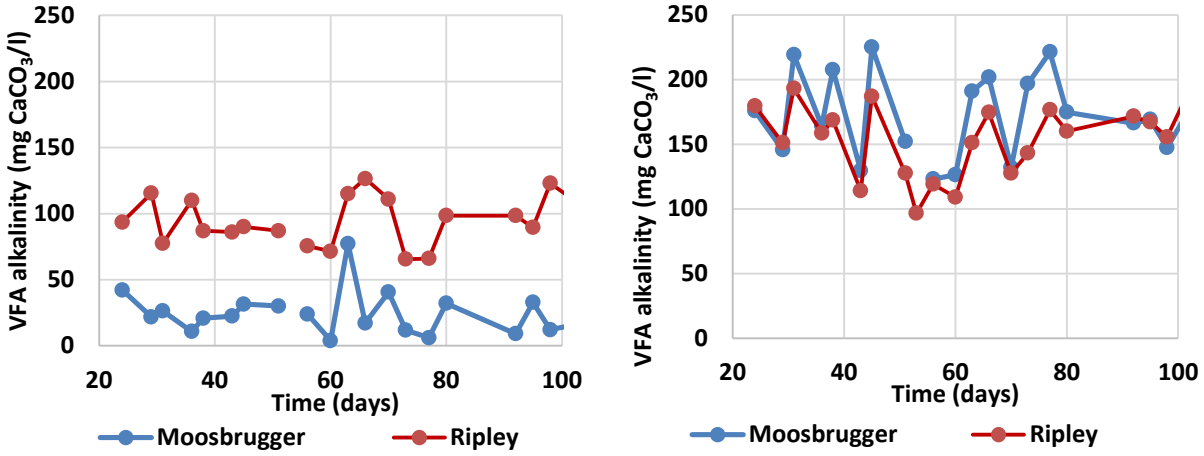


Figure 4-15. VFA alkalinity. Ripley and Moosbrugger methods. System 1 (left) and System 2 (right)

The last comparison is about VFA alkalinity. This measurement is the most differential between the methods compared. In System 1 (Figure 4-15 left), all the values have bigger differences between methods due to very low concentration, which reduced precision. Figure 4-15 right shows System 2 values, where both Ripley and Moosbrugger methods showed similar results. Note that the effect of VFA over total alkalinity is substantial in System 2, as mentioned when showing Figure 4-14 right, suggesting a digestion upset.

The overall results of the alkalinity experiments are summarized in Table 4-2. As it was seen in the previous figures, most of the alkalinity parameters had the same results using one or another method. According to Lahav and Morgan (2004) and Vannecke et al. (2014), Moosbrugger et al. (1993) have the most accurate titration method among the others. Indeed, the advantages of this method are characterized by the incorporation of other substances that may participate in the alkalinity results, such as ammonia or sulfate concentration as buffers. In this study, interfering substances did not play an important role in alkalinity due to their low concentration in the reactor liquid. Only VFA was of remarkable concentration in System 2, but this parameter is already included in Jenkins, S.R., Morgan, J.M. (1983) and Ripley et al. (1986) methods.

Altogether, Moosbrugger et al. (1993) does not appear to give improved results compared to the others. Furthermore, it requires to analyze concentration of other compounds that may provoke alkalinity deviations. Considering the scope of this study, it would be recommended to utilize this method only in high strength or sulfate rich wastewaters, for instance.

Moreover, even if the deviation occurs, the patterns of alkalinity change were followed in both systems, so that basic alkalinity methods could also be useful for any change of stability in the reactors operation. Therefore, APHA method was appropriate to be used in this experiment to give enough information about alkalinity, and Jenkins & Ripley methods for VFA and Carbonate alkalinity.

Table 4-2. Matrix of alkalinity values measuring total, carbonate or VFA within the different methods, for each system.

Method	Total		Carbonate		VFA	
	S1	S2	S1	S2	S1	S2
Moosbrugger (1993)	362 (±60)	337 (±62)	331 (±64)	162 (±61)	24 (±17)	171 (±33)
APHA (1999)	350 (±55)	328 (±110)	n/a	n/a	n/a	n/a
Jenkins (1983) & Ripley (1986)	383 (±96)	296 (±103)	327 (±61)	205 (±77)	95 (±19)	152 (±28)

Values are the mean ± StDev in mg as $CaCO_3$/l.

4.4 Membrane performance

Like in all AnMBR's operation, membranes are responsible for biomass retention, and play a major role on providing a high quality effluent. However, membranes are vulnerable to fouling, by blocking and clogging the pores internal or externally.

Among other parameters explained in literature, fouling depends on the operating flux that passes through the membrane. The flux relates directly to the driving force, which is the transmembrane pressure (TMP). The critical flux (the highest flux at which no increase transmembrane pressure is observed (Martin-Garcia et al., 2011)) is a useful value that helps deciding a range of membrane operation (Chang, 2014).

However, although being a wide term extended among researchers and industry, some experiments questioned the existence of a real limit for TMP, because it will always increase until the physical failure of the membrane material (Andreottola and Guglielmi, 2003).

Both parameters (flux and TMP) were taken into account to evaluate the membrane performance. The flux was set to 8 liters per m^2 per hour (LMH) during filtration mode, based on Smith et al. (2012).

Figure 4-16 shows the flow vs TMP values over time in System 1, and one period of membrane chemical cleaning. In general, this membrane operated well without rapid signs of membrane fouling. The TMP slowly increased from day 0 until the cleaning day. The only cleaning was performed to prevent any risk of sudden TMP increase during Christmas vacation period, because TMP was 80 mbar and close to the limit (100 mbar).

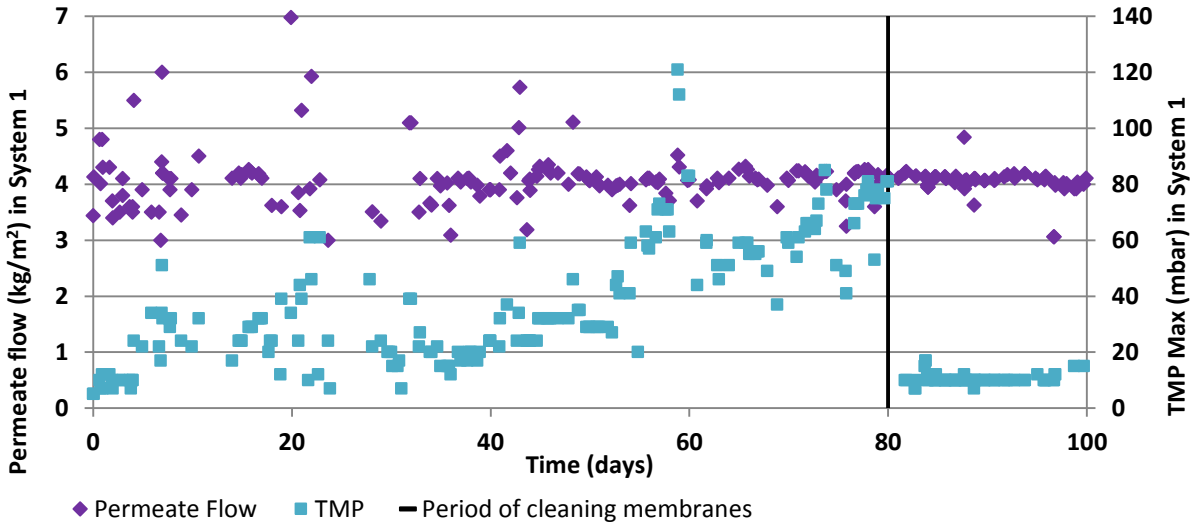


Figure 4-16. Flow vs maximum TMP in System 1.

If the first and last days of the experiment are compared in Figure 4-16, high variability in permeate flow lead to uncontrolled TMP during the first days, causing a lot of stress to the membrane. However, improvements in the equipment operation allowed a more precise flow, which resulted in a much more stable TMP. This was especially noted in the last days of the study.

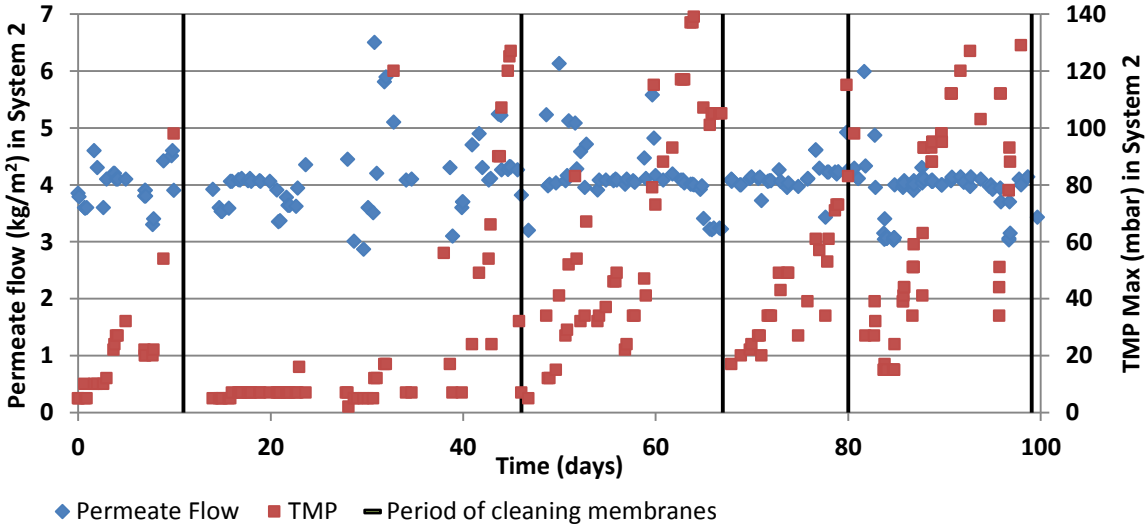


Figure 4-17. Flow vs maximum TMP in System 2.

Figure 4-17 shows that membrane performance in System 2 appeared to be totally different from System 1. During the first 10 days, one cleaning had to be performed. From day 10 to 40, there was a relative stable operation, followed by a sudden increase in TMP that obliged a second cleaning. From there, the membrane become increasingly sensible to fouling over time. Three more cleaning procedures were performed until the end of the study.

Considering that TSS concentration was similar in both reactors during the first days, no relation can be found in the first fouling of membrane 2. Also, TMP increased from 20 to over 100 in 2 days, with no other parameters changed. On the other hand, a technical problem with

the filtration pump left the filtration tank sucked dry for hours on day 9. In this regard, membrane was most likely affected by this issue, and it presumably had a negative effect on fouling for rest of the experiment.

The problems affecting the membrane in System 2 may be a cause for its reduced effluent quality, with a higher COD compared to System 1. However, it was previously stated that an important proportion of the COD effluent was from VFA. Precisely, Zacharof and Lovitt (2012) assured that only nanofiltration is capable of VFA retention, although other non-physical factors can help in retention like charge and iso-electric points of the compounds as well as pH and temperature. All in all, both membranes did not retain any VFA, as it had very similar levels in reactor and effluent samples.

Finally, keeping a steady and successful operation helped in reducing the number of cleanings in System 1. On the other hand, it is out of the scope of this thesis to analyze in depth which where the causes for excessive and frequent fouling in System 2, although the most plausible cause was the membrane drying. Also, the chemical cleaning of membranes offered satisfactory results when put back to operation, showing an effective reduction of TMP.

4.5 Inhibitory conditions

Inhibition refers to the microbial inactivity of any part of the anaerobic digestion (normally methanogenesis, but also hydrolysis or acetogenesis), caused by conditions like toxic concentration of a determined compound and/or external factors as pH and temperature.

Actually, temperature does not intrinsically inhibit methanogen activity (Bialek et al., 2012; O'Reilly et al., 2009; Smith et al., 2013). However, it reduces the reaction rate and slows down the process requiring a longer period of acclimation to adapt the biomass. This period was accomplished by both reactors, with stable operation in System 1 after the first weeks since startup at temperature of 25±1ºC. In System 2, other conditions were responsible for failure, and temperature was not a cause for inhibition.

The pH was a limiting step for methanogenic reactions, as mentioned before. Considering that pH was below 6 for several days in System 2, it could have affected microbial recovery during the experiment. In System 1, the pH averaged 6.3 – 6.5 with no direct relation of inhibition, conversely to what Khanal (2008) stated about total inhibition under pH 6.4.

Dissolved oxygen (DO) in the influent may have also been a source of inhibition, introducing aerobic conditions in the reactor. According to Henry's law, the potential concentration of DO was 9.1 mg/l in influent (atmospheric pressure, 20ºC). Indeed, Lettinga (1995) mentioned that elevated concentrations of DO were common in low-strength wastewaters, up to 10 mg/l. However, there is no serious risk of inhibition and methanogens remain active. On the other hand, aerobic organisms would grow and consume available oxygen, causing a deterioration of granular sludge but protecting methanogens from DO. Additionally, an improper mixing could enhance accidental aeration in the substrate tanks, also promoting aerobic microbial growth.

Short chain fatty acids (VFA) can also inhibit methanogens at high levels. They represent a key intermediate in the anaerobic digestion, and its increase in the reactor can represent an overload of organic loading. Acetic acid (acetate ions) is usually present in higher concentration than other VFA (Weiland, 2010). In both reactors, however, the concentration of acetate

was similar to propionate. Overall, VFA concentration was not as high as to cause direct inhibition, usually between 2 – 4 g/l in the reactor (Ward et al., 2008)

However, even if the fatty acids concentration was not hazardous, it was high enough in System 2, compared to System 1, to lower the pH and inhibit methanogens. Buffering capacity was crucial to control this undesired effect, at which alkalinity values in both reactors was low (under 1000 mg/l as $CaCO_3$) in comparison to other literature, above 1500 mg/l as $CaCO_3$ (Dupla et al., 2004; Sánchez et al., 2005). Low pH conditions in both reactors could likely be explained by this effect.

Ammonia and sulfide are also indicators of inhibition at a certain concentration. Although dairy wastewater is characterized by containing ammonia close to inhibition levels (80 mg N/l as TAN) (Vidal et al., 2000), it ranged roughly between 20 – 40 mg N/l as TAN in both reactors. Also, sulfide was not detectable at the first days of the study, but during the last days it was detected in lower concentrations than inhibition, at approximately 250 mg S/l (Chen et al., 2008; Lens et al., 1998). At the end of the experiment, an error in sulfate analysis was detected, after which revealed a much lower concentration of sulfate in the reactor.

Because the OLR was maintained at a low level with synthetic substrate, other typical inhibitory substances were not hazardous for the anaerobic digestion. For instance, concentrations of phosphate, light and heavy metals were under toxic values or assumed trivial (Chen et al., 2008). In the analysis results section more information is given in regards to substance concentrations.

4.6 Mass and COD balances

Unlike aerobic digestion, it is relatively simple to do a COD balance in anaerobic systems. Most of the organic compounds are digested and broken down to intermediates and eventually CH_4 and CO_2. Therefore, a complete mass balance in terms of COD can be used as a tool for monitoring AnMBR.

Traceability of the COD must be assessed to understand which elements are necessary to include in the balance. To facilitate the concept of the inputs and outputs of the balance in the reactor, Figure 4-18 shows a basic schematic version of the AnMBR type with the balance fractions.

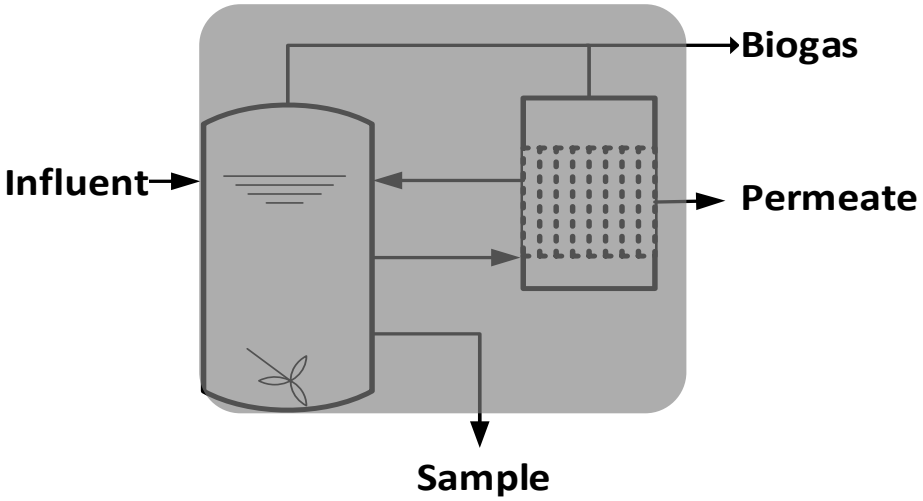


Figure 4-18. System boundaries for the COD balance.

Table 4-3 summarizes the majority of the fractions in the COD balance. In Figure 4-18, dissolved methane in effluent was not included in the figure to stress the most remarkable fractions. All of the fractions were calculated according to the operational parameters in reactors, except dissolved methane, which was calculated using methane solubility in water.

Table 4-3. Daily amount and proportional fraction of each part of the COD balance.*

Type	S1 (g COD/day)	Fraction (%)	S2 (g COD/day)	Fraction (%)
Influent (in)	97.3 (31.4)	100	58.5 (43.6)	100
Biogas (out)	59.9 (42.1)	61.6	4.8 (12.6)	8.2
Permeate (out)	3.1 (2.7)	3.2	19.0 (9.1)	32.4
Sample (out)	1.7 (0.4)	1.7	1.6 (0.3)	2.7
Dissolved CH_4 (out)	2.9 (0.9)	3	2.8 (1.1)	4.8
Balance (should be 0)	29.7	30.5	30.3	51.9

*Values in parenthesis are StDev.

The most remarkable COD fraction was the biogas in System 1 and permeate in System 2. The rest represented less than 15% together. The influence of COD accumulation in the reactors represented 0.1 g COD/day, considering total COD and VSS content. H_2S production from sulphate reducing bacteria was also minimal, according to sulphate concentration found in reactors, representing 0.4 g COD/day.

Permeate was calculated using data from two sensors, level indicator and permeate flow sensor. That is to minimize error when calculating the daily amount of permeate produced. Cleaning procedures for level indicator revealed that there was up to 30% of deviation from the real values. Also, the variability in permeate flow made it difficult to calculate a daily average. For this reason, the results from each sensor were combined when calculating the permeate fraction.

In Figure 4-19, a 24h COD balance is shown for each reactor. The inlet was set as 100% reference, and data points correspond to the sum of all outlets mentioned before (on a daily 24h basis). In this regard, both systems had high variability in the balance, showing important gaps that do not close the COD balance. Furthermore, there was an overbalance to 100% in some cases. In the last days of experiment, the COD balance matched better in a stable period, more close to 100% than the first values.

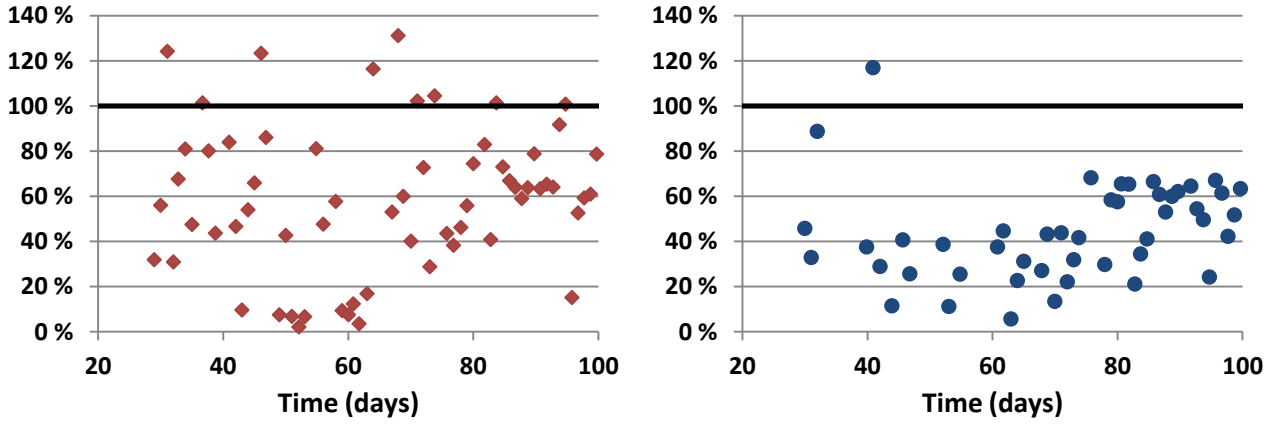


Figure 4-19. COD balance on 24h basis: System 1 (left) and System 2 (right)

A first interpretation of Figure 4-19 should consider severe gaps in both reactors. For instance, important influence from other electron acceptors rather than sulfate already mentioned (oxygen from aerobic microorganisms, nitrate or iron). However, none of these had relevant presence in the reactor, because other indicators would already point at them too (reactor performance or gas production). A different scope is needed to use the COD balance results.

Making a raw mass balance can reveal the most probable reason for the differences in the COD balance. Figure 4-20 shows the comparison between effluent outlet calculated from reactor level (considering the inlet) and the flow sensor, in terms of reactor volume accumulation. It can be seen that there were frequent accumulation levels of up to 40 L. Also, the average variation of System 1 (15 L) and System 2 (10 L), with similar standard deviations, was noticeable.

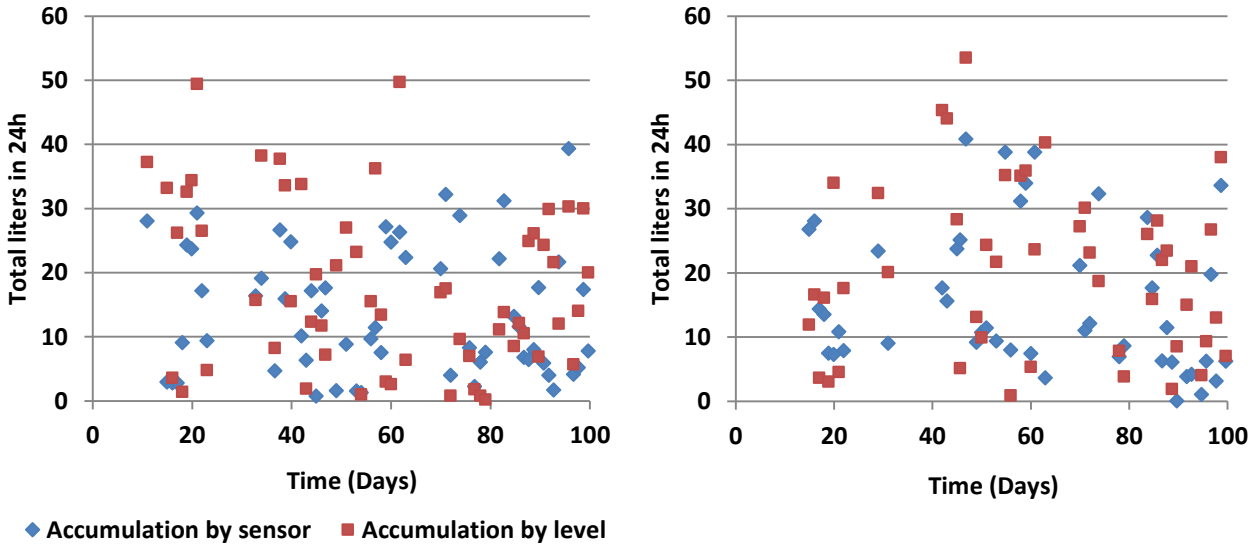


Figure 4-20. Comparison of daily reactor accumulation between sensor flow and level indicator. System 1 (left) and System 2 (right).

These two aspects could explain the big COD gaps exposed in the previous balance, because they affect daily biogas production and effluent production with unstable values. Therefore, a 24h basis COD balance was not suitable to evaluate with certainty what was the unknown COD fraction in the balance, if any. Additionally, it is worth mentioning that both Figure 4-19 and Figure 4-20 in each reactor tend to approach to nominal expected values of 100% balance and less volume accumulation, respectively.

A better approach of the COD balance can be seen in Figure 4-21 and Figure 4-22 for each reactor, with much more clear interpretation that can be done to assess the balances. From reactor one, two noticeable periods can be seen that do form a COD gap. Similarly to Figure 4-1, on days 50 and 60 there was a slight difference in the balance that produced the gap, due mainly to the null gas production (flat data points marked in Figure 4-21). After, the COD gap gradually increased until the end of the experiment.

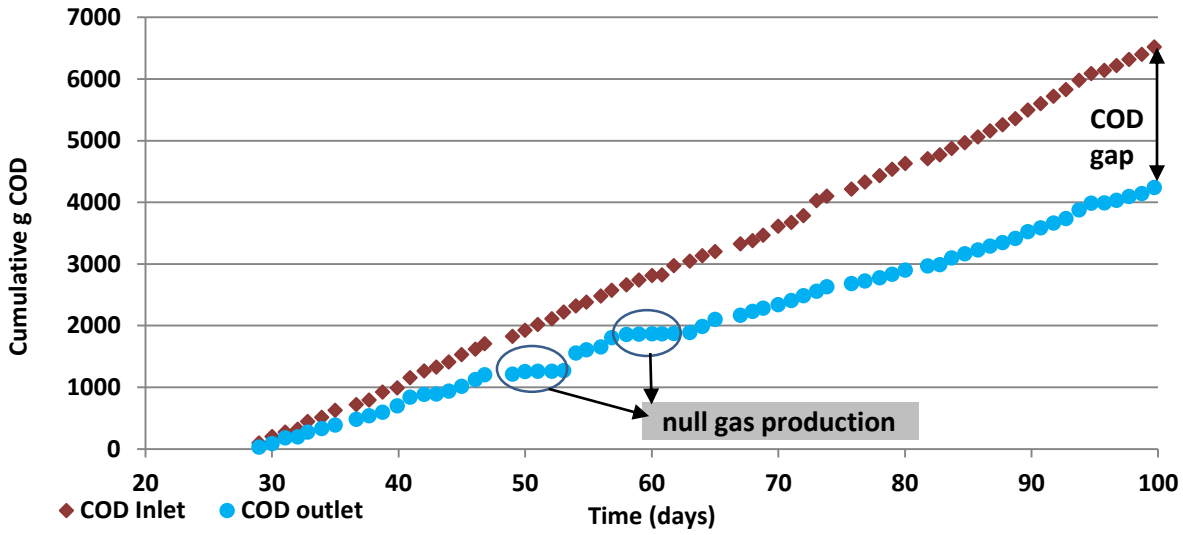


Figure 4-21. Cumulative COD balance of System 1.

If the other parameters during the days of the COD gap are analyzed, they reveal, for instance, a decrease in the biomass fraction (VSS in Figure 4-11) as well as $\%CH_4$ reduction in the biogas (in Figure 4-4). A slight increase in VFA is also notable, especially on day 60 (Figure 4-9), as well as OLR stress (shocking loads three days before and after day 60, in Figure 4-2). Last, the level indicator showed a deviation of 30% from real reactor volume in day 50.

All in all, the events mentioned above along with instrumental imprecisions or errors in measurements could be the major reasons for the COD gaps. Another source of COD outlet could be other oxidizable gas compounds and the accumulation of milk fats or Long Chain Fatty Acids (LCFA) in the system (Lier et al., 2008).

As for System 2, there was more important COD gaps than in System 1. They also revealed the recovery periods of the system (marked in Figure 4-22). The COD balance was stable until day 40 (no available data from 32 to 38). From there, the gap between inlet and outlet COD started to increase. After the recovery periods, the balance gradually increased its COD gap.

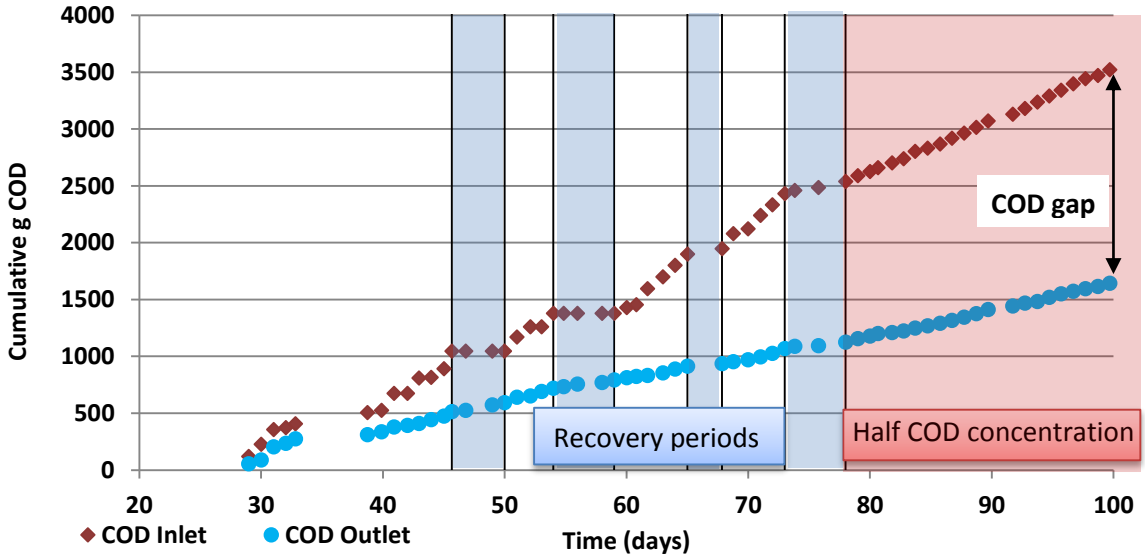


Figure 4-22. Cumulative COD balance of System 2.

According to the other monitoring parameters, the periods of COD gaping correspond to hardly any activity in the anaerobic methanogens. During this time, most of the parameters stabilized after an important decrease in terms of VSS and methane production levels. On the other hand, VFA was increased and stayed at high levels in the effluent and in the reactor.

Each time the reactor was fed again with organic matter (inlet), there was no response observed in biogas or effluent COD. All of the organic load pointed to other sources of exit or accumulation in reactor. The most important COD gap happened between days 60 and 73, where it was tried to recover the system using default OLR.

Due to the late implementation of analysis of sulfate, and the lack of information about other possible electron acceptors in the intermediates of anaerobic digestion, the COD gaps can only be explained similarly as System 1, with instrumental imprecisions or errors in measurements, not analyzed oxidizable gas compounds and accumulation of biomass in the membranes. This accumulation could be acute in System 2 knowing that it suffered from fouling more frequently.

5 Conclusions

A successful startup of AnMBR at room temperature and low OLR while keeping stable process was accomplished in the first system, but the second failed to stabilize. In order to find a method to evaluate all the important process parameters that are crucial for a stable startup, the following conclusions were drawn according to the aim and results obtained:

- Both reactors showed a delicate stability at 25 ºC and low OLR, especially during the first weeks of startup. The temperature did not affect methanogen activity, as it was stated in literature.

- Even short-term changes of stability indicators can lead to system failure during startup. Corrective measures are necessary to fix a digester upset as soon as possible.

- The process was totally inhibited under pH of 6.0, but it could work above it. Therefore, the normal pH limits reviewed in literature are actually more flexible than expected, at least in an AnMBR at ambient temperature and low OLR.

- Failure of System 2 was provoked by multiple causes of inhibition: washout of biomass, high content in VFA from the beginning of operation, low alkalinity and poor performance of the technical equipment.

In a more general scope of AnMBRs, the following conclusions were outlined:

- The most relevant parameters for an evaluation of startup are pH, alkalinity (including VFA alkalinity), methane content, biomass content (TSS & VSS), effluent quality and biogas production rate. Almost all degradation steps of the digestion can be supervised by one of the mentioned parameters, hence it is crucial to monitor them to detect any alteration of the process.

- Low buffering capacity of a reactor makes it vulnerable to inhibition caused by sudden pH changes. Special attention to the substrate conditions is important to increase buffer capacity. Systematic addition of buffering compounds contribute to strengthen the constant pH conditions when the substrate has a low pH or high concentration of pH lowering compounds.

- There was hardly any recovery of System 2 after its failure. A much faster, measure is to re-start the AnMBR. If it is available, inoculum from other similar reactors can facilitate the restart and stabilization.

- Although a COD balance in AD is simpler than aerobic, the fractions to make the balance need to rely on very solid data in order to close the balance. Otherwise there will be high disparity in the balance.

- Membrane performance is dependent on reactor stability. Chemical cleaning combined with gas sparging seems to offer a reliable operation during all the experiment.

- Simple alkalimetric tests are as good as complex ones when measuring buffering capacity of the reactors, if the concentration of interfering compounds is not significant.

6 Future studies

Successful and reliable operation of AnMBR's is still a field with lots of potential to be discovered. More investigations should be made in terms of lower temperature (20ºC) applied in pilot scale reactors. The equipment utilized in this experiment could have incorporated specific temperature control to test performance under different temperatures and test acclimation periods.

A more thorough analysis should have been performed, taking into account unknown substances such as EPS or other gas contents (mainly H_2S). Analysis of dissolved methane in reactor and effluent is proposed, as it can be an important factor for COD balance. Moreover, centrifuge methods for measuring soluble COD and other analysis should be compared to filtration procedures, testing different pore sizes to calculate the margin errors for using a less precise technique.

Further investigation of operation at higher OLRs and/or fluxes is suggested. However, great attention should be paid when breaking stable conditions, performing more frequent analysis to rapidly evaluate reactor response.

Membrane performance could also be improved if different biogas gassing rates are tested, along with change in filtration/relaxation periods.

Testing with substrates containing real wastewater could help in the design and operation of full-scale digesters, based on the results in this experiment.

Also, it would be interesting to perform optimization tests with advanced software, combining the previous operational variables and finding the most appropriate combination for best performance.

7 References

Andreottola, G., Guglielmi, G., 2003. Critical flux determination in two MBRs for municipal wastewater treatment 1–6.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., 1976. Inhibition of Nitrification by Ammonia and Nitrous Acid. J. (Water Pollut. Control Fed. 48, 835–852.

APHA, AWWA, WEF, 1999. Standard Methods for the Examination of Water and Wastewater, Standard Methods for the Examination of Water and Wastewater.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781.

Bialek, K., Cysneiros, D., Flaherty, V.O., 2012. Low-Temperature (10^{o}C) Anaerobic Digestion of Dilute Dairy Wastewater in an EGSB Bioreactor: Microbial Community Structure, Population Dynamics, and Kinetics of Methanogenic Populations. Hindawi Publ. 2013, 10.

British Petroleum, 2014. BP Statistical Review of World Energy, June 2014. Nucl. Energy www.bp.com/statisticalreview.

Cao, X., Chen, H., Li, Y., He, Y., 2011. Influence of alkalinity and load on the start-up process of anaerobic reactors. In: ISWREP 2011 - Proceedings of 2011 International Symposium on Water Resource and Environmental Protection. pp. 1468–1472.

Chang, S., 2014. Anaerobic Membrane Bioreactors (AnMBR) for Wastewater Treatment. Adv. Chem. Eng. Sci. 04, 56–61.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 99, 4044–4064.

De Baere, L., Mattheeuws, B., 2011. State of the art of Anaerobic Digestion of Municipal Solid Waste in Europe. In: International Conference on Solid Waste 2011 - Moving Towards Sustainable Resource Management. Hong Kong, p. 416.

De Vrieze, J., Gildemyn, S., Vilchez-Vargas, R., Jáuregui, R., Pieper, D.H., Verstraete, W., Boon, N., 2014. Inoculum selection is crucial to ensure operational stability in anaerobic digestion. Appl. Microbiol. Biotechnol. 99, 189–199.

Demirel, B., Yenigun, O., Onay, T.T., 2005. Anaerobic treatment of dairy wastewaters: A review. Process Biochem. 40, 2583–2595.

Dereli, R.K., Grelot, A., Heffernan, B., van der Zee, F.P., van Lier, J.B., 2014. Implications of changes in solids retention time on long term evolution of sludge filterability in anaerobic membrane bioreactors treating high strength industrial wastewater. Water Res. 59C, 11–22.

Dupla, M., Conte, T., Bouvier, J.C., Bernet, N., Steyer, J.P., 2004. Dynamic evaluation of a fixed bed anaerobic digestion process in response to organic overloads and toxicant shock loads. Water Sci. Technol. 49, 61–68.

Fritzmann, C., Löwenberg, J., Wintgens, T., Melin, T., 2007. State-of-the-art of reverse osmosis desalination. Desalination 216, 1–76.

Ghyoot, W.R., Verstraete, W.H., 1997. Coupling Membrane Filtration to Anaerobic Primary Sludge Digestion. Environ. Technol. 18, 569–580.

Griffin, M.E., McMahon, K.D., Mackie, R.I., Raskin, L., 1998. Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. Biotechnol. Bioeng. 57, 342–355.

Hammer Sr., M.J., Hammer Jr., M.J., 2011. Water and Wastewater Technology, 7th Editio. ed. Prentice Hall.

He, Y., Xu, P., Li, C., Zhang, B., 2005. High-concentration food wastewater treatment by an anaerobic membrane bioreactor. Water Res. 39, 4110–4118.

Henze, M., van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D., 2008. Biological Wastewater Treatment - Principles, Modelling and Design. IWA Publishing.

Ho, J.H., Khanal, S.K., Sung, S., 2007. Anaerobic membrane bioreactor for treatment of synthetic municipal wastewater at ambient temperature. Water Sci. Technol. 55, 79–86.

Jenkins, S.R., Morgan, J.M., S.C.L., 1983. Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration.pdf. J. WPCF 55, 451–453.

Khanal, S.K., 2008. Anaerobic Biotechnology for Bioenergy Production: Principles and Applications, First. ed. Wiley-Blackwell, Hawai'i.

Kleerebezem, R., Macarie, H., 2003. Treating industrial wastewater: Anaerobic digestion comes of age. Chem. Eng. 56.

Kushwaha, J.P., Srivastava, V.C., Mall, I.D., 2011. An Overview of Various Technologies for the Treatment of Dairy Wastewaters. Crit. Rev. Food Sci. Nutr. 51, 442–452.

Labatut, R.A., Gooch, C.A., 2012. Monitoring of Anaerobic Digestion Process to Optimize Performance and Prevent System Failure. Got Manure Tradeshow Conf.

Lahav, O., Morgan, B.E., 2004. Titration methodologies for monitoring of anaerobic digestion in developing countries - A review. J. Chem. Technol. Biotechnol. 79, 1331–1341.

Lens, P.N.L., Visser, a., Janssen, a. J.H., Pol, L.W.H., Lettinga, G., 1998. Biotechnological Treatment of Sulfate-Rich Wastewaters. Crit. Rev. Environ. Sci. Technol. 28, 41–88.

Lettinga, G., 1995. Anaerobic digestion and wastewater treatment systems. Antonie Van Leeuwenhoek 67, 3–28.

Liao, B.-Q., Kraemer, J.T., Bagley, D.M., 2006. Anaerobic Membrane Bioreactors: Applications and Research Directions, Critical Reviews in Environmental Science and Technology.

Lier, J.B. Van, Mahmoud, N., Zeeman, G., 2008. Anaerobic Wastewater Treatment, Biological Wastewater Treatment : Principles, Modelling and Design.

Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., Zhang, Y., 2013. A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. Desalination 314, 169–188.

Martin-Garcia, I., Monsalvo, V., Pidou, M., Le-Clech, P., Judd, S.J., McAdam, E.J., Jefferson, B., 2011. Impact of membrane configuration on fouling in anaerobic membrane bioreactors. J. Memb. Sci. 382, 41–49.

McKeown, R.M., Hughes, D., Collins, G., Mahony, T., O'Flaherty, V., 2012. Low-temperature anaerobic digestion for wastewater treatment. Curr. Opin. Biotechnol. 23, 444–51.

McKeown, R.M., Scully, C., Enright, A.-M., Chinalia, F.A., Lee, C., Mahony, T., Collins, G., O'Flaherty, V., 2009. Psychrophilic methanogenic community development during long-term cultivation of anaerobic granular biofilms. ISME J. 3, 1231–42.

Moosbrugger, R.E., Wentzel, M.C., Ekama, G.A., Marais, G. v R., 1993. A 5 pH Point Titration Method for Determining the Carbonate and SCFA Weak Acid/Bases in Anaerobic Systems.

O'Reilly, J., Chinalia, F.A., Mahony, T., Collins, G., Wu, J., O'Flaherty, V., 2009. Cultivation of low-temperature (15 degrees C), anaerobic, wastewater treatment granules. Lett. Appl. Microbiol. 49, 421–6.

Obasohan, E., Agbonlahor, D., Obano, E., 2010. Water pollution : A review of microbial quality and health concerns of water , sediment and fish in the aquatic ecosystem. African J. Biotechnol. 9, 423–427.

Ostrem, K., 2004. Greening waste: anaerobic digestion for treating the organic fraction of municipal solid waste.

Ripley, L.E., Boyle, W.C., Converse, J.C., 1986. Improved Alkalimetric Monitoring for Anaerobic Digestion of High-Strength Wastes. Water 58, 406–411.

Rizvi, H., Ahmad, N., Abbas, F., Bukhari, I.H., Yasar, A., Ali, S., Yasmeen, T., Riaz, M., 2013. Start-up of UASB reactors treating municipal wastewater and effect of temperature/sludge age and hydraulic retention time (HRT) on its performance. Arab. J. Chem.

Sánchez, E., Borja, R., Travieso, L., Martín, a., Colmenarejo, M.F., 2005. Effect of organic loading rate on the stability, operational parameters and performance of a secondary upflow anaerobic sludge bed reactor treating piggery waste. Bioresour. Technol. 96, 335–344.

Seghezzo, L., Zeeman, G., Liel, J.B. Van, Hamelers, H.V.M., Lettinga, G., 1998. a Review : the Anaerobic Treatment of Sewage in Uasb and Egsb Reactors. Bioresour. Technol. 65, 175–190.

Shelton, D.R., Tiedje, J.M., 1984. General method for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. 47, 850–857.

Skouteris, G., Hermosilla, D., López, P., Negro, C., Blanco, Á., 2012. Anaerobic membrane bioreactors for wastewater treatment: A review. Chem. Eng. J. 198-199, 138–148.

Smith, A.L., Skerlos, S.J., Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water Res. 47, 1655–1665.

Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J., Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater: A critical review. Bioresour. Technol. 122, 149–159.

Speece, R.E., 1996. Anaerobic Biotechnology for Industrial Wastewaters. Archae Press.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., Eddy, M.&, 2003. Wastewater Engineering: Treatment and Reuse, McGraw-Hill higher education. McGraw-Hill Education.

United Nations, 2012. United Nations Environmental Programme Annual Report.

Van Lier, J.B., 2008. High-rate anaerobic wastewater treatment: Diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. Water Sci. Technol. 57, 1137–1148.

Vannecke, T.P.W., Lampens, D.R.A., Ekama, G.A., Volcke, E.I.P., 2014. Evaluation of the 5 and 8 pH point titration methods for monitoring anaerobic digesters treating solid waste. Environ. Technol. 1–9.

Vidal, G., Carvalho, a., Méndez, R., Lema, J.M., 2000. Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. Bioresour. Technol. 74, 231–239.

Visvanathan, C., Abeynayaka, A., 2012. Developments and future potentials of anaerobic membrane bioreactors (AnMBRs). Membr. Water Treat. 3, 1–23.

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928–7940.

Weiland, P., 2010. Biogas production: Current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849–860.

Yang, W., Cicek, N., Ilg, J., 2006. State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America. J. Memb. Sci. 270, 201–211.

Zacharof, M.P., Lovitt, R.W., 2012. The recovery of volatile fatty acids (VFA) from mixed effluent streams using membrane technology: a literature review. In: Fourth International Symposium on Energy from Biomass and Waste San Servolo, Venice, Italy; 12-15 November 2012.

Appendix I

Table a-1 summarizes the average values of relevant parameters measured in laboratory, from reactor samples. The high standard deviation in some values is either due to their low concentration or change during the experiment.

Table a-1. Average values of parameters measured in laboratory.

Parameter	System 1	System 2
Total COD (mg/l)	13682 (±2862)	14080 (±3018)
Soluble COD (mg/l)	1253 (±536)	1105 (±318)
Total Organic Carbon (TOC) (mg/l)	4882 (±1245)	4566 (±1113)
Total Inorganic Carbon (TIC) (mg/l)	100 (±27)	60 (±40)
Total Nitrogen (mg TNb/l)	700 (±182)	550 (±161)
Total Phosphate (mg P/l)	646 (±166)	602 (±227)
Soluble Phosphate (mg P/l)	9.6 (±2.2)	10.4 (2.4)
Conductivity (µS/cm)	799 (±91)	700 (±112)
Sulfate (mg SO_4/l)	239 (±32)	143 (±31)
Sulfide (mg S^{2-}/l)	1.63 (±1.06)	0.87 (±0.67)
Total Ammonia Nitrogen (TAN) (mg/l)	33.2 (±4.5)	27.7 (±9.8)
Total Suspended Solids (mg/l)	12556 (±2524)	11529 (±3657)
Volatile Suspended Solids (mg/l)	8729 (±1626)	7939 (±2100)
Total alkalinity (mg $CaCO_3$/l)*	368 (±63)	337 (±62)
Volatile Fatty Acids (VFA) (mg COD/l)	43.7 (±29.6)	218.1 (±64.6)

Values are the mean ± StDev. *Using 5 point titration

Figure 1-a collects the evolution of the most relevant parameters from table a-1 analyzed in laboratory.

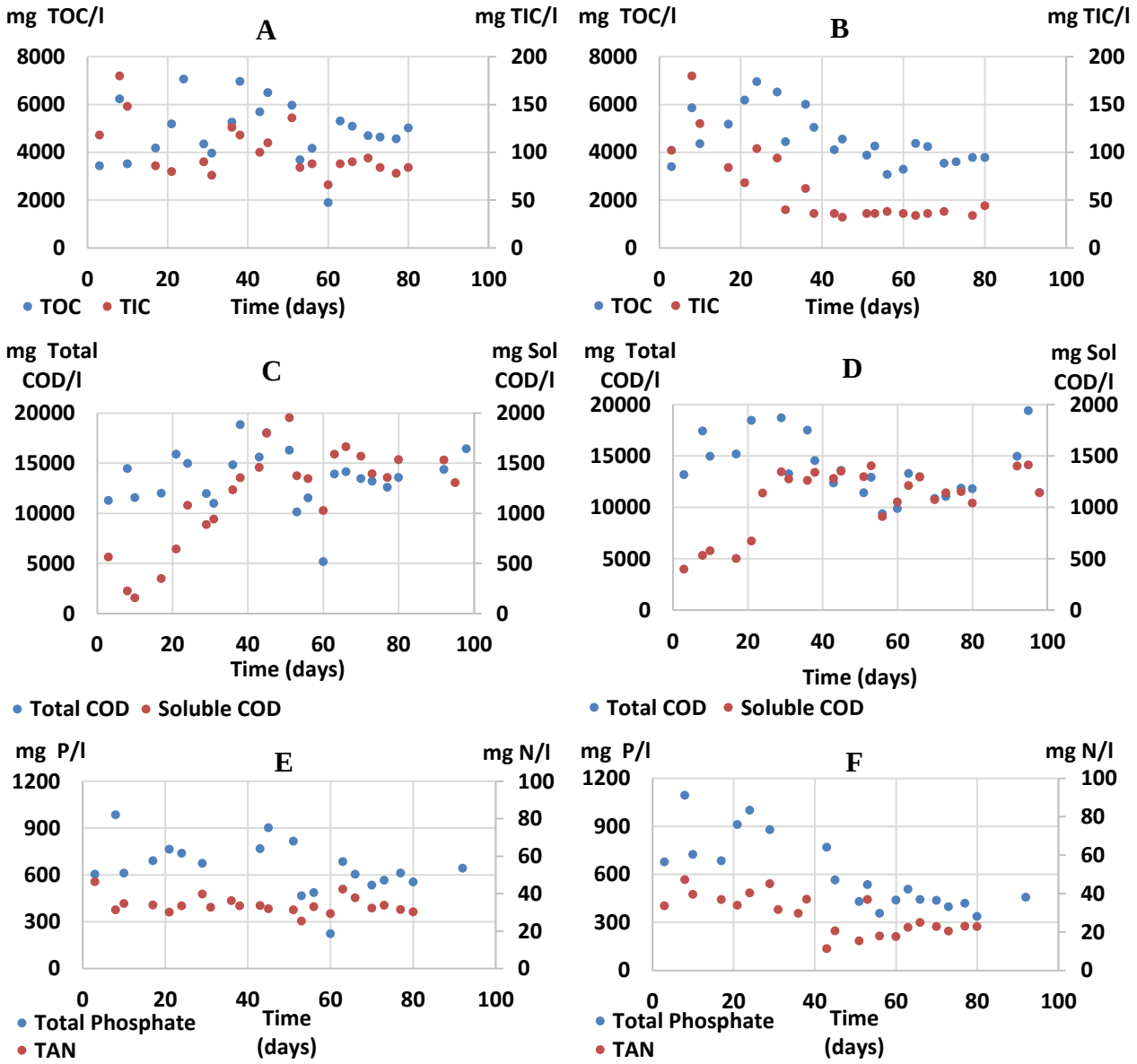


Figure a-1. A) & B) Total Organic and Inorganic Carbon (TOC / TIC). C) & D) Total and Soluble COD. E) & F) Total Phosphate and TAN. System one values on left and System two on right.

Total and soluble COD increased in reactor one until the middle of the study, then it remained around 14 g/l. Reactor two had a more unstable COD during all the experiment. Total COD increased in the first days, but decayed until day 60 to start increasing again.

Likewise, TOC analysis followed similar results as COD. TOC are considered secondary measurements of COD for monitoring organic matter content (Henze et al., 2008). Last, Total phosphate and TAN remained fairly constant in System 1. As for System 2, a clear decrease was found until day 50, more acutely in phosphate. At the end these parameters also stabilized.

Appendix II

In laboratory analysis, samples were centrifuged at around 10.000 G for some of the tests. The general equation to calculate the RPM of centrifugation is:

$$G = R * 1{,}118e^{-5} * RPM^2$$

Where:
G = The centrifuged force (10.000)
R = Radius of the centrifugation instrument
RPM = speed setting for the centrifugation

Dilutions of reactor samples that were not centrifuged were made with piston-driven pipettes and graduated cylinders as tools of acceptable precision. They were:

- 1/10 for total COD test.
- 1/20 for TC/TOC test.
- 1/100 for TNb & TP test.

Procedure for TSS and VSS analyses were done using the standard methods. For TSS, a sample of 4 ml was filtrated using a glass fiber filter previously weighted. The filter was then placed in an oven at 105 ºC for 24 hours. After this, the container was weighted again, and following next equation to calculate TSS. For the VSS, the same sample as TSS was then burned at 550 ºC for two hours, and weighted again:

$$TSS\ \left(\frac{mg}{l}\right) = \frac{m - m_0}{V}$$

$$VSS\ \left(\frac{mg}{l}\right) = \frac{m_0 - m_{550}}{V}$$

Where:
m = mass weighted after the oven 105 ºC
m_0 = mass weighted before filration
m_{550} = mass weighted after oven 550 ºC
V = volume of sample used

For the calculation of TAN, the concentration of ammonia was used along with pH and temperature according to Anthonisen et al. (1976)

$$TAN = [NH_3] + [NH_4^+] \qquad [NH_3] = [NH_4^+] * \frac{10^{pH}}{e^{\frac{6344}{273+T}}}$$

Where:
$[NH_3]$ = Concentration of ammonia
$[NH_4^+]$ = Concentration of ammonium
pH = pH of the sample
T = Temperature of the sample

As for alkalinity, the calculation for total alkalinity as standard method is:

$$Alkalinity\ (as\ mg\ CaCO_3/l\ )\ =\ \frac{V * N * 50 * 1000}{V_0}$$

Where:
V_0 = volume of sample (usually 50 ml)
V = Volume of titration down to pH 4.5
N = Normality of the acid compound.

The excel sheet used for calculation of alkalinity with Moosbrugger method is shown in figure a-2.

			Sample Data			Titration Data		
	(diluted or undiluted)	undiluted	Initial pH	6,7	6,505	0,05	Acid normality	
		Volumes	before dilution	50		23	Temperature °C	
		(ml)	After dilution	50			Titration	
	Conductivity Correlation		FSA (mgN/L)	22,84		mL Acid	pH	Corrected pH
	Ionic strength /EC25	Temp coeff	OrthoP (mgP/L)	7,44		0	6,7	6,505
	7,22E-05	0,0198	Sulphide (mgS/L)	0,457		2,71	5,9	5,705
	EC_T (mS/m)	75,5				4,43	5,2	5,005
	EC_{25} (mS/m)	78,61	Ionic strength	0,005676		6,39	4,3	4,105
								-0,195

Fill in all the input data (blue cells), then click the Calculate button.

Results

	Molalities	Alkalinities (mg CaCO3/l)		VFA	
$CO_3^{=}$	0,005847875	Carbonate+H2O	175,80	212,9178	(mg/L as acetate)
NH_4^{+}	0,001630613	Ammonia	0,14		
PO_4^{-3}	0,000240201	Phosphate	2,44		Colour key
HS^{-}	1,42519E-05	Sulphide	0,18		Inputs — Calculate
CH_3COO^{-}	0,003548156	VFA	174,69		Outputs
		Total	353,25		Status
					Could not correct for pH error
H^{+}	0,008679662				

This calculation deviates from the original TITRA5 in two respects:
1) The initial pH is assumed to that of the un-diluted sample, whereas the original applied the same dilution factor to all pH values.
However, both options are availalble by entering the keywords "diluted" or "undiluted". The keyword applies to the initial pH only.
For the other pH values, if there was no dilution, set the volumes before and after dilution the same.
2) The Ionic strength is calculated from the measured conductivity (EC_T mS/m) according to the correlation provided by Bhuiyan et al. (2009).
In the original TITRA5, ionic strength was estimated from TDS, with an option to calculate TDS from conductivity.
However, the original correlation was not specific to anaerobic digestion liquors.
The algorithm here uses the ionic strength value in E9, irrespective of how it gets calculated.

Reference: Bhuiyan IH, Mavinic DS and Beckie RD, Determination of Temperature dependence of electrical conductivity and its relationship with ionic strength of anaerobic digester supernatant, for struvite precipitation. Journal of Environmental Engineering 2009 135:1221-1226

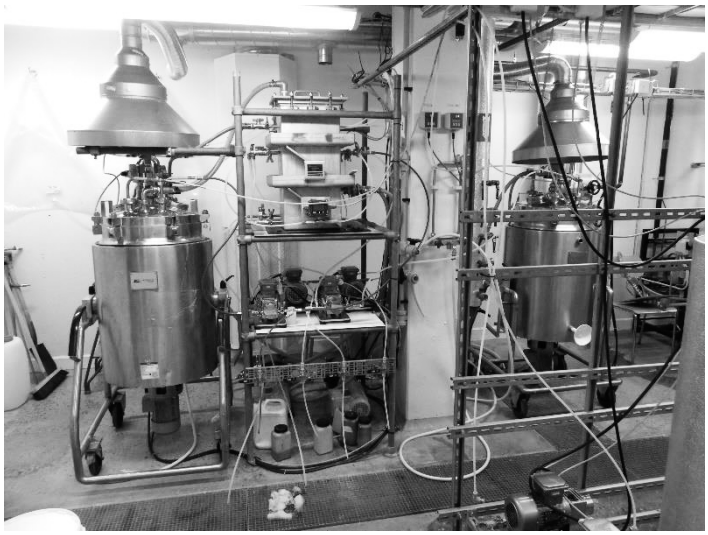
Figure a-2. Screenshot of the excel programme for Moosbrugger alkalinity from Chris Brouckaert.

Popular science article

Mission impossible: starting up anaerobic membrane bioreactors (AnMBR)

Why should we keep polluting rivers and lakes when we have AnMBR? Those fantastic systems with microbes that eat waste, produce biogas and leave a cleaned water. They don't even use air to live! It is a good idea, if starting up one of these engineering wonders was as easy as riding a bicycle. Actually, it is like riding a bicycle, but on ice.

AnMBR have lead the research in wastewater treatments for the last decades, but there has been hardly any approach into the start up period. This period is very sensible when the reactor works without temperature control, because those microbes love warmer waters. The idea was to setup two AnMBRs and try to make a successful startup and stable operation, but at ambient temperature and low polluted water: challenge accepted.



Imagine that you are one of those microbes. You live in a warm and poor country but you are offered a new job in the northern Europe. You definitely go for it, but you realize it is cold up there and you need some time of acclimation, not to mention the lack of food available. Well we found that 100 days of full operation were enough to say that one of our AnMBR friends did a good job and managed to work. Sadly, the other mate couldn't stand the new conditions and decided to strike after a few weeks from start until the end.

But what really made the second AnMBR fail were other working conditions apart from temperature or food. The insufferable acid pH, the excess of toxic species and the low performance of filtration were the real strong reasons for unpleased microbes. The AnMBR can retain most of the solids and provide a good place to live for the microbes. But these solids also block the filtration slowly and the water can be less clean over time.

We have learned that for a Good (AnMBR) Samaritan, the bible of starting up includes: good monitoring of pH and biogas, how cleaned is the water compared to the wastewater, and how pleased are microbes in the reactor. The excess of different toxic species is also risky, because microbes may have an indigestion. But what will happen if we give the microbes a good meal? A more polluted water will make them happier?