Stable increase of organic loading rate in anaerobic membrane bioreactors working at ambient temperature

LUND UNIVERSITY

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Picture on front page: "The anaerobic membrane bioreactor" Photo by Jean Monhonval

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Preface

During my exchange year in Lund University, I have been given the exciting opportunity to realize my Master Thesis into the methanization field. As I've always been strongly attracted by renewable energy and environmental issue, I was glad to accept and join the project in the Water and Environmental engineering department. The subject turned out to be really interesting for me and gave me a unique experience on monitoring anaerobic membrane bioreactor. I've gained skills that I think will be definitely useful in my upcoming professional career.

This work would never have been successful without the help, advices and support of the wonderful people that has surrounded me during this year in Sweden.

First of all, I would like to thank my supervisor *Hamse Kjerstadius* for his kindness, for his advices, corrections and the very large amount of time he spent explaining me the biogas pilots. I'm grateful for the opportunity he gave me while proposing me this subject. I learned so much during those 20 weeks of collaboration with him, and *Hamse* was always attentive and ready to help me, even with the heavy amount of work that he has to deal with every day.

I wish to thank *Åsa Davidsson* for accepting being my examiner and for correcting my Master Thesis and gave me relevant advices to enhance it.

I would like to thank all the Chemical engineering department for its nice working environment and especially *Gertrud Persson* for her constant happiness that she always showed at the laboratory. It was always a pleasure to work with her.

I want to thank Lund University for accepting me as an exchange student for this academic year, which has been so fulfilling in many ways and has allowed me to discover so many different things. It's a fascinating experience to discover a country, a new way of living surrounded by other exchange students who become your friends.

I wish to thank my Swedish workmates *Lina*, *Nicolina* and *Hjalmar* for being so nice and helpful during the long working days at the lab and the office. I would like also to thank all my friends here in Lund, in Belgium and all over the world. You are so important in my eyes.

Finally, I would like to mention and say how I'm grateful to my family back in Belgium, especially my parents, for all the support and sacrifice they made for their children, but also for my sister *Claire*, and my brothers *Pierre* and *Clément*.

All the equipment were provided by Alfa Laval and an Erasmus grant was covering the accommodation and travelling costs.

Abstract

Anaerobic processes are an interesting alternative to treat wastewaters instead of aerobic treatments as it allows recovery of energy with the produced biogas while removing organic matter. In the industry, lots of low-strength industrial wastewaters are available and unused for biogas production due to their low concentration of organic material and high volumes (slaughterhouse wastewaters, dairies and beverages industries, oils and fats producers). However converting low-strength wastewaters into biogas is problematic because the need of expensive thickening and concentration steps for conventional continuously stirred tank reactors. Anaerobic membrane bioreactors (AnMBR's) are potentially a good method to convert low-concentrated wastewaters into methane, due to their ability to process large amount of influent using a small reactor volume.

Moreover using AnMBR's working at ambient temperature (25°C) instead of higher temperatures are interesting as it would improve the energy balance while processing wastewaters without any expensive heating and concentration steps. Nevertheless, use of industrial AnMBR's working at ambient temperature is still in its infancy and it is unknown how the process stability is affected by the step-increase in organic loading rate (OLR) during start-up.

Suitable OLR increase has then to be defined in order to know this critical operational parameter and perform the start-up of AnMBR as quickly as possible to reduce costs.

The aim of this work was to evaluate the stability of the anaerobic digestion process in ambient temperature (25°C) anaerobic membrane bioreactors (AnMBR's) while increasing the organic loading rates (OLR) during the start-up.

Two 180L AnMBR's fed with synthetic wastewater (milk powder) were used to realize upscales of the OLR, which were monitored by stability indicators, to determine if the biological process is stable or not according to the indicator's benchmarks. The stability indicators are constituted by the specific gas production, the pH, the alkalinity ratio, the volatile fatty acid concentrations and the membrane performance.

One reactor was first used to perform several step-increases of OLR after a long initial steady state. The other reactor was then used to reach directly the higher OLR reached by the first reactor after a short initial steady state, in order to figure out if a long initial steady state and a step-increase of OLR really were need in order to ensure the stability of the process.

The first reactor fulfilled all the stability indicators during its operation. The reactor handled the increase of the OLR without any failure in the anaerobic process. Nevertheless the membrane performance was not met since the membrane was fouled too often. The second reactor did not meet any of the biological benchmarks or the benchmarks for the membrane performance. Fouling of the membrane were investigated in order to enhance membrane performance.

Finally, one step-increase of OLR was successfully carried out while monitored by stability indicators, which found out to be powerful monitor tools to control the anaerobic digestion process.

Table of abbreviations

AD	Anaerobic digestion
AnMBR	Anaerobic membrane bioreactor
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
HRT	Hydraulic retention time
IA	Intermediate Alkalinity
IA:PA	Alkalinity ratio (ratio between intermediate and partial alkalinity)
MBR	Membrane bioreactor
MLSS	Mixed liquor suspended solids
OLR	Organic loading rate
PA	Partial Alkalinity
SD	Standard deviation
SRT	Solids retention time
TOC	Total organic carbon
VFA	Volatile fatty acid
WT	Wastewater treatment

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1 Introduction

1.1 Background

Anaerobic processes are an interesting alternative to treat wastewaters instead of aerobic treatments as it allows recovery of energy with the produced biogas while removing organic matter. In the industry, lots of low-strength industrial wastewaters are available and non-efficiently used (slaughterhouse wastewaters, dairies and beverages industries, oils and fats producers). Those wastewaters need to be treated by decreasing their organic contents in order to meet environmental requirements for wastewater discharge coming from the industry into the municipal sewer net. Anaerobic processes would allow to gain energy from those removal processes. Thus wastewater treatment has the potential to become net producer of renewable energy, by converting the organic chemical energy of pollutants into the useful energy carrier methane.

Nevertheless, converting low-strength wastewaters into biogas is problematic because the need of expensive thickening and concentration steps needed for conventional treatment in continuous stirred tank reactors (CSTR's). Recent improvement of anaerobic membrane bioreactors (AnMBR's) in the last decade could remove these expensive steps by allowing the separation between the solids retention time (SRT) and the hydraulic retention time (HRT). The uncoupling of SRT and HRT allows the retention of micro-organisms and thus allows the substrate to be fed directly into the reactor even at low concentration while avoiding washing out of the micro-organisms. Therefore AnMBR's are potentially a good method to convert low-concentrated wastewaters into methane, while processing large amount of influent in a relatively small reactor volume.

However, even if anaerobic digestion is a well-established technology to process wastewaters, the energy balance of the anaerobic digestion (AD) plants is still improvable, due to need to heat the digester or wastewaters for usual mesophilic (30-40°C) digestion. One approach to lower the energy cost of the AD is to operate the bioreactor at low temperature. The ambient methanization (methanization occurring at ambient temperature, 25°C) would be interesting as it would allow to process wastewaters on their released temperature.

Nonetheless, use of industrial AnMBR working at ambient temperature is still in its infancy and it is unknown how the process stability is affected by the step-increase in organic loading rate (OLR) during start-up. The optimal rate for OLR increase during the start-up of an ambient AnMBR is still unknown. This is critical for the start-up of an anaerobic process at an industry. In one hand, a too rapid increase of loading rate will lead to the failure of the system, due to overloading of the un-adapted microbiological process. On the other hand the shortest possible start up time is wanted in order to be cost-effective. Then the best compromise, the quickest OLR increase while still keeping the process stable has to be found.

1.2 Aim

1.2.1 Objective

The aim of this work was to find suitable organic loading rate (OLR) increases in ambient temperature (25°C) anaerobic membrane bioreactors (AnMBR's). The stability of the anaerobic digestion process was evaluated while increasing the organic loading rates during the start-up.

The goal of the study was to achieve results that would allow giving recommendations on how to manage the start-up in similar processes in industry.

1.2.2 General method

In order to reach the aim two 180L AnMBR's fed with synthetic wastewater (milk powder) were used to realize the upscale of the OLR with a different method.

One reactor was first used to perform several step-increases of OLR after a long initial steady state. The other reactor was then used to directly reach the higher OLR reached by the first reactor after a short initial steady state, in order to figure out if a long initial steady state and a step-increase of OLR really were needed in order to ensure the stability of the process.

Stability of reactors was monitored by several stability indicator (found in the literature).

1.2.3 Delimitation

Only two different startup methods were investigated. Thus this work can only say which one of these is the better method for start-up of similar processes; however there might be other start-up regimes that are better but not investigated here.

2 Literature study

2.1 Anaerobic digestion

The anaerobic digestion (AD) is a very common process in nature, where it allows organic matters to be reduced and broken down by micro-organisms in absence of oxygen. This process is also used in industry in order to remove organic compounds from wastes and to recover energy, as methane (CH_4). Compared to aerobic processes, the costs of treatment and sludge handling are much lower as there is no need for aeration and less sludge is produced in the anaerobic process (Lin et al., 2013). Anaerobic digestion is a series of biochemical reactions leading to the catalysis of organic matters in absence of oxygen as electron acceptor. The organic matters act thus both as electron donor and acceptor, leading to formation of gas, mainly methane (CH_4) and CO_2 (Gerardi, 2003).

These step-reactions involves four different metabolic groups of micro-organisms: hydrolytic, acidogenic, acetogenic and methanogenic micro-organisms. These four different groups are needed in order to achieve the biomethanation process, and have specific keys roles in the AD (Khanal et al., 2008). The four step process stages are illustrated in Figure 1. Each step-process corresponds to one group of micro-organism (Gerardi, 2003).

2.1.1 Hydrolysis

The first part of the AD is the hydrolysis of large organic polymers into their smaller constituent parts. These released monomers, such as e.g. sugars, amino acids or fatty acids, are then ready to be metabolized by other bacteria. Nevertheless, other molecules as volatile fatty acids (VFA's) longer than acetate need further steps of catabolism to be converted into compounds directly usable by the methanogens.

2.1.2 Acidogenesis or fermentation

The acidogenic bacteria continue further the breaking of the remaining compounds. VFA's are produced, along with ammonia, CO_2 and hydrogen sulfide (H_2S).

2.1.3 Acetogenesis

Simple molecules produced in the acidogenesis are further processed and digested by acetogenic bacteria in order to produce mainly acetic acid, CO_2 and hydrogen.

Acidogenesis and acetogenesis play an important role in the AD process as they can lead to the acidification of the solution. The acidification is the release of acids (acetic acid and other VFA's) during those two steps. This acidification mustn't exceed a certain level because of the risk of inhibition of the methanogenesis, the last step of AD, by a low pH.

2.1.4 Methanogenesis

During the last step of the AD, methanogens use the previously released acetic acid, CO_2, and H_2 and convert them into methane and CO_2. The produced gas mixture, containing mainly CH_4, CO_2 and with trace of H_2S, is called biogas. Methanogenesis is sensitive to both high and low pH and thus occurs only in the pH range of 6.5-8 (Gerardi, 2003). Thus the acidogenesis rate mustn't overload the methanogenesis, otherwise the VFA's accumulation will lead to a pH drop, and then a failure of the AD general process. The remaining of the organic matters and compounds, indigestible by the anaerobic bacteria, plus the dead bacterial constitute the remaining digestate in the reactor.

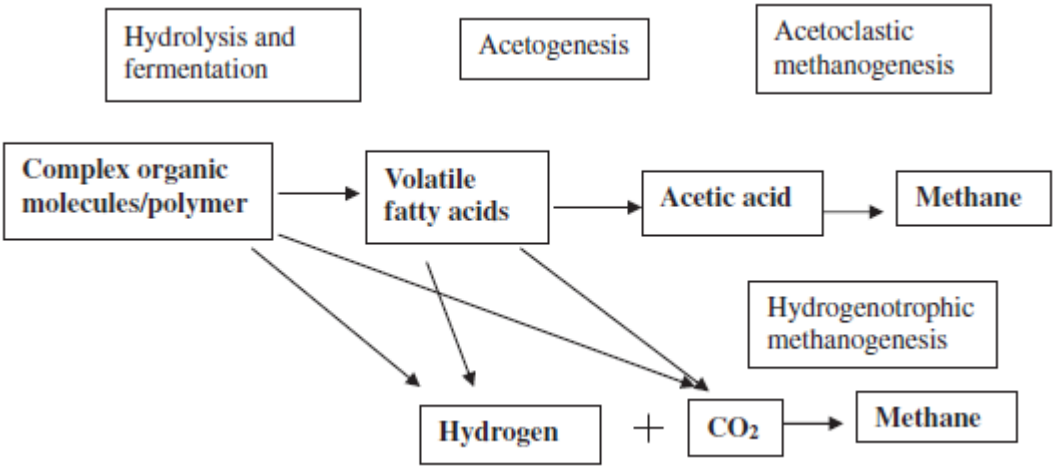


Figure 1: Schematic representation of the decomposition pathways of excess activated sludge (and other organic material) by anaerobic digestion (Dhaked et al., 2010). Four step-reactions degrade the organic matters into methane. Used with permission of Elsevier.

2.2 Anaerobic membrane bioreactor

In the last decade, the use of anaerobic membrane bioreactors (AnMBR's) for the treatment of wastewater has been increasingly studied as it seems to be an interesting and promising alternative to conventional wastewater treatment. The main characteristic of AnMBR's is that they allow the separation between the solids retention time (SRT) and the hydraulic retention time (HRT) with the help of membrane micro- or ultrafiltration.

This uncoupling of SRT and HRT allows the substrate to be fed directly into the reactor even at low concentration while avoiding washing out of the micro-organisms thanks to the high SRT, because of the retention of micro-organisms and particles due to the membrane. This selective retention has solved the biomass wash-out which is an issue related to other anaerobic processes. In fact, because of the slow growth of the anaerobic micro-organisms compared to aerobic processes (Chan et al., 2009), their retention by the membrane allows them a sufficient SRT, especially for the methanogens and thus avoid a loss of valuable biomass in the effluent. Therefore AnMBR's are a good method to convert low concentrated wastewater into methane, while processing large amount of influent in a relatively small reactor volume.

Figure 2 represent a general process of an AnMBR. In summary, low concentrated influent wastewater is fed directly (or after pretreatment) into the main vessel where the biomethanation occurs, producing the biogas. Sludge is constantly recirculated into the separation tank, where a membrane filter out a high quality (with a low COD concentration) effluent from the system. Note that the biogas can also be recirculated to scour the membrane surface and minimize the membrane fouling rate. Finally, biogas can then be used e.g. as energy in power generating facility, while effluent and sludge can undergo additional treatments (Christian et al., 2011).

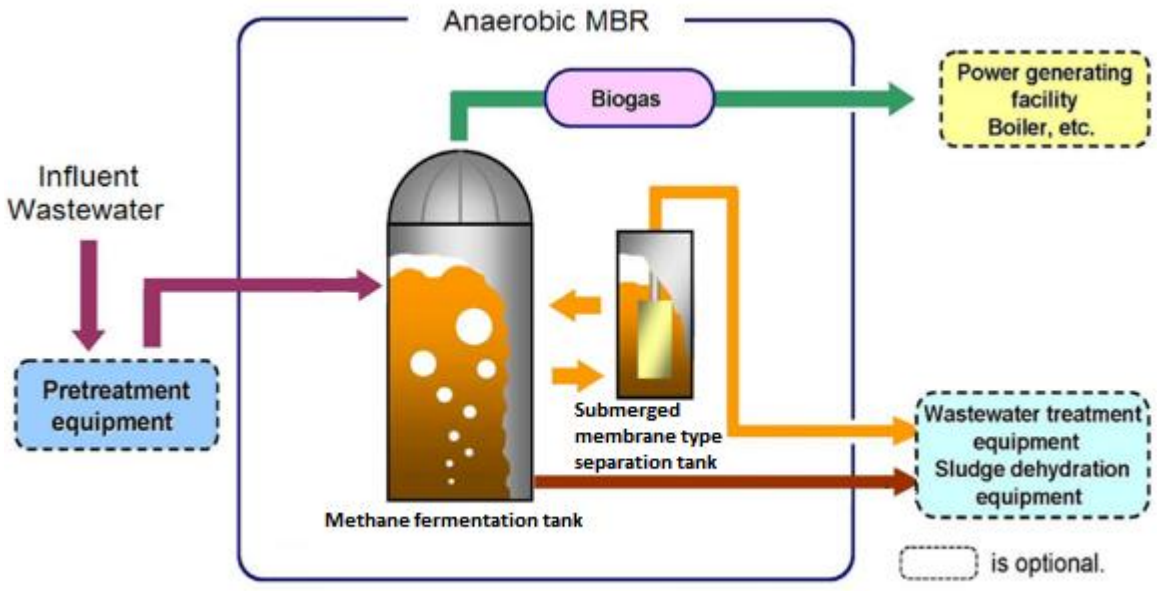


Figure 2: General process flow diagram of the AnMBR process, modified from Christian et al., 2011. Used with permission of Elsevier

The main advantages of AnMBR's are that they are cost-effective (Christian et al., 2011) in order to produce net energy with the biogas released, they have a small reactor size compared to conventional CSTR systems, they don't need the expensive aeration step of aerobic processes. They also have a low sludge production, release nutrient rich and solids free effluent and have high

pathogens removal to the effluent (Ozgun et al., 2013 ; Lin et al., 2013). Moreover, AnMBR technology is suitable to different kind of streams, especially for liquid food wastes and industrial wastewater (Skouteris et al., 2012 ; Lin et al., 2013).

Table 1 compares the conventional aerobic treatment, anaerobic treatment, aerobic MBR and AnMBR. As seen, AnMBR combines advantages of anaerobic treatment and membrane bioreactor (MBR) technology.

Table 1: Comparison of conventional aerobic treatment, anaerobic treatment, aerobic MBR and AnMBR (Lin et al., 2013). Used with permission of Elsevier.

Feature	Conventional aerobic treatment	Conventional anaerobic treatment	Aerobic MBR	AnMBR
Organic removal efficiency	High	High	High	High
Effluent quality	High	Moderate to poor	Excellent	High
Organic loading rate	Moderate	High	High to moderate	High
Sludge production	High	Low	High to moderate	Low
Footprint	High	High to moderate	Low	Low
Biomass retention	Low to moderate	Low	Total	Total
Nutrient requirement	High	Low	High	Low
Alkalinity requirement	Low	High for certain industrial stream	Low	High to moderate
Energy requirement	High	Low	High	Low
Temperature sensitivity	Low	Low to moderate	Low	Low to moderate
Start up time	2–4 weeks	2–4 months	<1 week	<2 weeks
Bioenergy recovery	No	Yes	No	Yes
Mode of treatment	Total	Essentially pretreatment	Total	Total or pretreatment

Notwithstanding these advantages, there are still severe drawbacks to the widespread of the AnMBRs. The membrane fouling, is the key problem to solve before the upscale into industrial implementation, especially in anaerobic conditions where it seems much more severe (Skouteris et al., 2012). The reasons are the costs and time needed in order to clean or replace membranes. Others drawbacks are the need of frequent cleanings (to prevent membrane fouling) and the costs of operations (Lin et al., 2013).

2.3 Ambient methanation

Even if anaerobic digestion is a well-established technology to process wastewaters, the energy balance of the AD plants is still improvable, as the need to heat the digester for usual mesophilic (30-40°C) digestion is quite high. One approach to lower the energy cost of the AD is to operate the bioreactor at low temperature. The ambient methanation (methanation occurring at the ambient temperature of the wastewter) would be interesting as it would allow to process wastewater on their released temperature. Nevertheless, although the methanation process is well understood at mesophilic (30-40°C) and thermophilic ranges (45-60°C), not much is known about the psychrotrophic (20-30°C) or psychrophilic (under 20°C) processes (Dhaked et al., 2010). The methanogenesis process is a highly sensitive process to temperature, and temperature change affects the activity and structure of the microbial community and thus modifies the degradation pathways of organic matter (Chin and Conrad, 1995 ; Kotsyurbenko, 2005, Nozhevnikova et al., 2007). The mesophilic AD process is thus slightly different than the ambient process and do not react exactly in the same way.

2.4 Membrane fouling

The membrane fouling is a phenomenon whereby particles are deposited on the surface or on the pores of the membranes leading to the degradation of its performance. The flux can be severely decreased as well as the quality of the released water. Intense chemical cleaning are often needed in order to recover an efficient membrane, increasing the cost of operation of the filtration treatment. In order to evaluate the fouling, flux and transmembrane pressure (TMP) are the most used indicators. The flux is the flow divided by the surface of the membrane while the TMP is the measurement of the pressure gradient through the membrane. As shown in Figure 3 (a), under constant flux operation, the TMP will slowly increase to compensate for the fouling.

As membrane fouling remains the main key factor limiting for a larger use of AnMBR (Skouteris et al., 2012), it seems important to get its theoretical explanation. According to Jeison et al. (2007), fouling is a complex mechanism that can be explained by the contribution of several processes as the pore plugging/clogging by colloidal particles, the adsorption of soluble compounds, biofouling (incrustation of micro-organisms) and finally deposition of a solid cake layer onto the membrane.

Most commonly membranes used in AnMBR's are operating at constant flux (Lin et al., 2013). When used in that way, a three-stage transmembrane pressure profile can be observed during the running of the reactor, as seen in Figure 3 (a). First stage consists of an initially short term rapid TMP rise, which is quickly followed by an extended slow TMP rise period. Finally, the third stage is a quick TMP rise period, until the TMP values reaches its maximum (Lin et al., 2013). Each stage mechanisms are illustrated in Figure 3 (b, c, d). Basically, two opposite forces control the deposition of sludge contents on membrane surface; the permeate drag, created by the suction of the effluent through the membrane, and the shear force, created by gas scouring (due to the recirculation of the biogas in the filtration tank) which decrease the speed of fouling. Briefly, during the stage 1, colloids and soluble products (generally with length under the pore sizes) that can't be detached by the shear force are deposited onto the membrane by the permeat drag force; that deposit explains the TMP rise until stage 2. Second stage correspond to a gradient developing sludge cake that prevent the penetration of more foulants. It corresponds to the slow TMP increase as more pressure is needed in order to keep the flux constant. According to Lin et al. (2013), the interpretation of the second TMP jump is still unclear and debated. The most accepted interpretation would be the "local flux theory", which is the hypothesis that there are changes in the local flux due to unbalanced distribution of foulants onto the membrane, leading to local TMP jump and fouling.

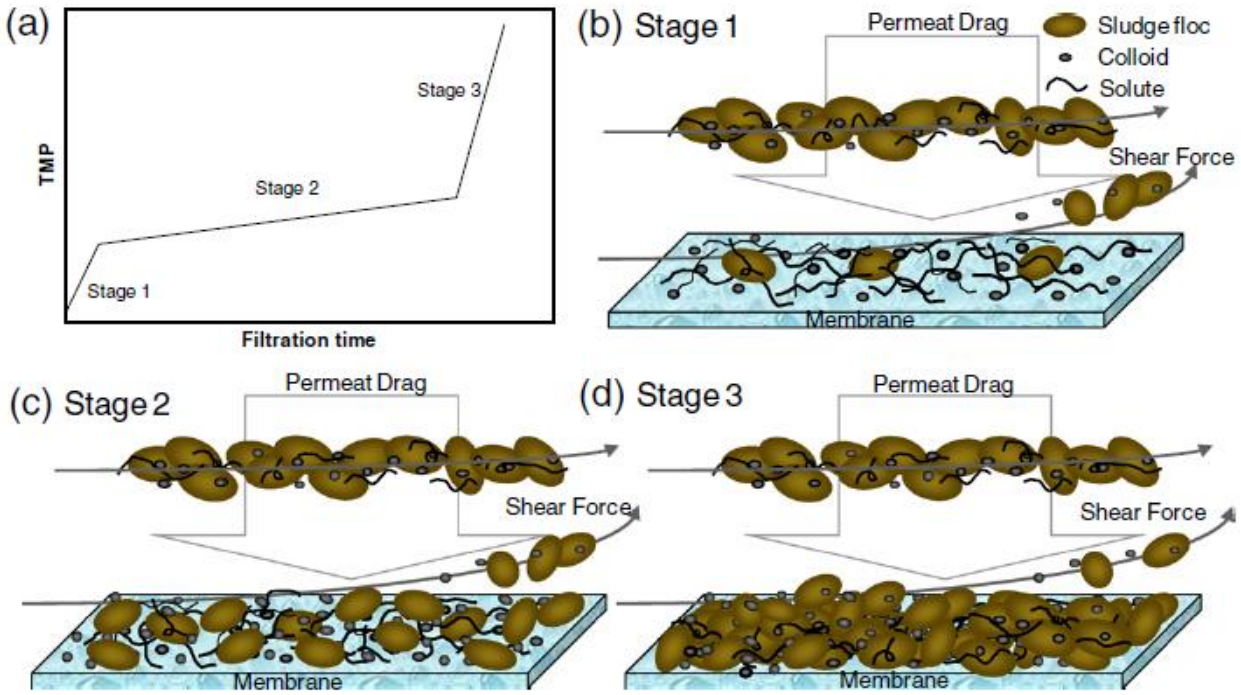


Figure 3: Schematic illustration of the three-stage TMP profile and its fouling mechanism. Working at constant flux, a three-stage transmembrane pressure profile can be observed during the running of the reactor (Lin et al., 2013). The combination of sludge flocs, colloids and solute compounds with the permeat drag force cause the fouling while shear force prevent and decrease the membrane fouling rate. Used with permission of Elsevier.

2.5 Biological stability indicators

In order to monitor the stability of the anaerobic process during the increase of the organic loading rate (OLR) in AnMBRs, it is necessary to use and control some parameters. The stability indicators are parameters that have been chosen from literature references in order to reach the aim of this work. For each stability indicators, limits have been taken as benchmarks in order to determine if the process is stable or not. Thus measurements of these stability indicators during the process was compared to the benchmarks to determine the stability of the anaerobic processes during the experiment. These stability indicators were the specific gas production, pH, alkalinity ratio, and the volatile fatty acid concentration. According to Bjornsson et al. (2001), these parameters are the most commonly used in order to monitor the process failure.

2.5.1 Specific gas production

One of the most important stability indicators for an anaerobic bioreactor is production of methane, which is the most valuable energetic product released by the system. The stability indicators proposed by Sanchez et al. (2005), consists of the ratio between the methane going out of the system and the inflow of COD (see Equation 1). According to these authors, specific biogas production rate must be ≥ 0.5, meaning that at least half of the COD going in the system must go out in form of methane, and thus consisting in a positive stability criterion for the system. On the contrary, a specific biogas production rate under 0.5 would mean that the reactor process is not stable but rather in stressed operations.

$$Specific\ biogas\ production\ rate = \frac{g(CH_4 - COD).L^{-1}.d^{-1}}{gCOD.L^{-1}.d^{-1}}$$

Equation 1: Specific gas production

2.5.2 pH and Alkalinity

pH measurement consists on the measurement of the H^+ concentration in an aqueous solution. This is one of the basic monitoring tools to check the stability of anaerobic processes. According to Bolzonella (2011), the optimal recommended pH range for AD (anaerobic digestion) is generally between 6.5 – 7.5. Methanogenic micro-organisms are actually the most sensible to an eventual pH drop in the bulk by anaerobic digestion steps, with a working pH range of only 6.5-8.0. pH drops occur because of acidification, which is the release of acids (acetic acid and other VFA's) by the acidogenesis and acetogenesis steps of the AD pathways. The pH monitoring is thus an important stability indicator to control the AD.

Since the reactor sludge is a buffered suspension, it is difficult to monitor the acidification process occurring during the running of the reactor only with the pH, as it won't be able to predict a system failure just by itself. If the acidification performed by acidogenic and acetogenic bacteria during the AD of a reactor outruns the down-stream processes of the methanogenesis, the pH drop can be sudden after overpassing the buffering capacity of the solution, leading to the failure of the anaerobic process. Then another criterion, the alkalinity, must be used with pH in order to monitor the anaerobic degradation and prevent the failure on time.

Alkalinity is the buffering capacity of the system and is more sensitive to process changes than the pH. The alkalinity ratio, is the ratio between two buffering systems, the intermediate alkalinity and the partial alkalinity (IA:PA). The intermediate alkalinity (IA) measures the buffering capacity of volatile fatty acids (VFAs) of the solution while the partial alkalinity (PA), measures the buffering capacity of the carbonate species OH^-, HCO_3^- and CO_3^{2-}. Alkalinity is the measurement of the capacity of a solution to neutralize acids (Bolzonella, 2011). Practically, by the titration of the supernatant of a centrifuged sludge sample by an acid to the pH 5.75 (corresponding to partial alkalinity) and then pH 4.3 (for intermediate alkalinity), it is possible to determine the contribution to the buffer of the bicarbonates and of the VFA's. The alkalinity ratio is useful in monitoring process stability since a decrease in the ratio could identify a potential incoming process failure before the pH drop. The alkalinity ratio is an indirect measurement of VFA's accumulation which often happens before the pH drop and the reactor failure.

Figure 4 illustrates the difference of sensibility of the pH and the Alkalinity while an increase of VFA concentration. It can be clearly seen that Alkalinity is much more sensible to the concentration of VFA than the pH, and thus is a better indicator in order to evaluate the VFA-buffer capacity of the solution and then monitor the process stability.

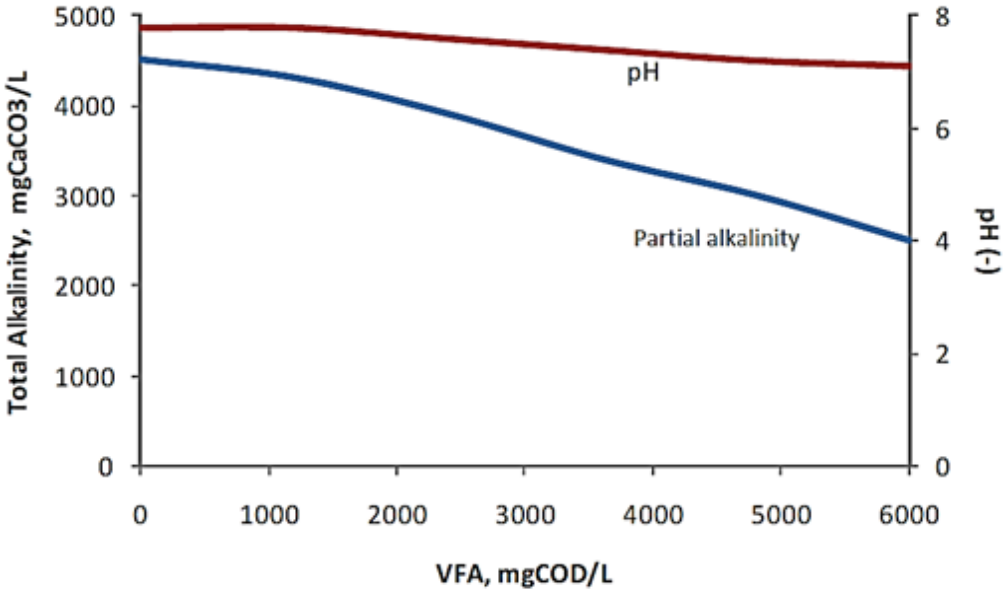


Figure 4: Difference of sensitivity between the Total Alkalinity [mg $CaCO_3$/L] and the pH according to the VFA concentration [mg COD/L] (Bolzonella, 2011). Partial alkalinity is more sensitive to VFA accumulation than the pH. Modified and used with permission of Bolzonella.

According to Ripley et al. (1986), optimal digestion of poultry manure occurred with an Alkalinity ratio (IA:PA) of 0.3. Another source recommend to maintain the ratio lower than 0.3-0.4 to assure a stable reactor performance (Franco et al., 2007). Nevertheless, for Carballa et al. (2011) and Sanchez et al. (2005), an alkalinity ratio of 0.5 is the limit for a good process, as a higher level define imminent failure of the anaerobic system. The limit used as benchmark in this work will then be a IA:PA of <0.5.

2.5.3 VFA

During the biomethanation process, micro-organisms hydrolyze organic polymers into acid compounds as volatile fatty acids (VFAs). If the concentrations of VFA's increases, this accumulation can lower the pH and then inhibit the biomethanation. Accumulation of VFAs reflect a kinetic uncoupling between acid producers (acidogenic and acetogenic bacteria) and consumers (methanogens). This uncoupling is typical from a stress situation (Hill et al., 2014). It is thus interesting to include the acetate and propionate concentrations into the stability indicators, in order to monitor their removal.

According to Chen et al., (2008) VFA's accumulated until the concentration of 316 mg/L during the anaerobic digestion of liquid piggery manure. An adjustment of pH was needed in order to recover better performance. As the values of 316 mg/L of VFA's was specific to a stressed process, it will be used as a benchmark limit for VFA's concentration, even though this value was not commonly used in the literature.

2.6 Other parameters

Another parameter was used as informative in order to monitor the process, the conductivity.

2.6.1 Conductivity

As electrical conductivity measures the ions content in a solution, it can also be used for the measurement of the concentration of VFA's and bicarbonate concentrations, as they are ions present in the sludge during the anaerobic fermentation process (Aceves-Lara et al., 2012). The monitoring of the concentration of the total ions content can thus be done by the conductivity measurement, and can then be used as early warning indicators of process failure due to the acidification (Aceves-Lara et al., 2012 ; Lei et al., 2014).

However, according to De Vrieze (2014), based on the results of AD of molasses, it is recommended to stay below a conductivity of 20-25 $mS.cm^{-1}$. These experimental limit values were set after observations. The failure after these limits could actually be explained by severe VFA accumulations. For information, it is also found in the literature that methanogens are especially susceptible to conductivity level higher than 30 $mS.cm^{-1}$ (De Vrieze et al., 2012).

3 Materials and methods

In order to evaluate the stability of the AD (anaerobic digestion) process, monitoring tools were needed to evaluate the stability process in ambient temperature AnMBR during the increase of its organic loading rate (OLR) during start up. Several stability indicators were used: The gas production, the pH, the alkalinity ratio, and the volatile fatty acid concentrations (VFA) as they are considered the most common parameters used in order to monitor AD processes (Bjornsson et al., 2001). Other reactor parameters were also monitored while analyzing the reactor, but were not used to establish the stability of the reactor and are thus more secondary (see 3.3.1).

The study assessed OLR increases during 190 days of operations in two AnMBR's. After reaching steady state at an initial low OLR, step-wise increases were performed, during which AD process of both reactors were monitored by the selected stability indicators.

3.1 Anaerobic membrane bioreactor

Two identical AnMBR systems (labelled AnMBR#1 and AnMBR#2) with external membrane configuration were used during the experimental work. Reactors were located in the Chemical engineering department (VA-Teknik), Lund University, in Lund (Sweden). As seen in Figure 5 (a. and b.), each system was composed by a main stainless steel vessel of 180L, a CSTR (continuously stirred tank reactor), which is coupled with a filtration tank of 35L where the membrane is located. A substrate tank (270L) was connected to the main vessel with a hydraulic pump in order to perform the feeding. Biogas scouring was done over the membrane to reduce membrane fouling.

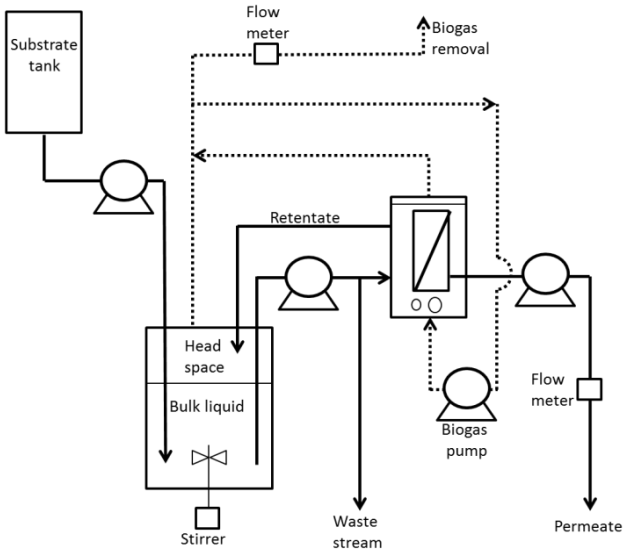
While running, the total reactor volume remained constantly at 150L thanks to the substrate and permeate pumps working on the same flow. Permeate outflow was exactly set to 4,13kg/h (with the help of a permeate pump and a permeate regulator resistance) in order to reach an operational flux of 8 LMH ([L/m^2*h]) with a hydraulic retention time (HRT) of 48 h. The daily flow was 71L a day. The reactors were operated with a cycle of 10 minutes filtration and 4 minutes relaxation to allow transmembrane pressure recovery while continuously scouring with biogas at $0.5 m^3*m^{-2}*h^{-1}$. All liquid pumps were provided by Quattroflow Company. The membranes were microfiltration flat sheets (the pore sizes are 0.2 μm) in polysulfone supplied by Alfa Laval (Figure 5 c.). They were immerged into the reactor sludge in the external filtration tank and their total specific surfaces were 0,5168 m^2.

Inoculum used for the start-up was collected from an industrial anaerobic CSTR treating dairy wastewater (BV Dairy, UK). Membrane cleaning was realized when transmembrane pressure (TMP) exceeded 100 mbars using commercial alkaline wash (P3-Ultrasil 10) at pH 11 subsequently for 2 hours. Finally, maintenance tasks (cleaning of the membrane, of the substrate tanks, pipes and pumps) were carried out frequently (see Table 2).

Table 2: Cleaning frequency of the reactor equipment

Equipment	Cleaning frequency
Membrane	When TMP exceed 100 mbars
Pump and pipes	Every month
Level indicator	Every 2 weeks
Substrate tank	Every 2 weeks

a.



b.

c.

*Figure 5: a. Process schematic of the anaerobic membrane bioreactor (AnMBR) b. Photo of the AnMBR#1 system (the main vessel is on the left, filtration tank is on the upper part of the shelf, above the recirculation, permeate and gas pumps). c. Photo of microfiltration flat sheets membrane provided by Alfa Laval. Dimensions:190*210*100 (mm).*

3.2 Feeding

Continuous feeding of the reactors was realized with milk powder used as synthetic dairy wastewater. To prepare the synthetic wastewater for the first OLR level (initial steady state), 112g of "Nido" milk powder (Nestlé) per 100L of tap water was prepared. Table 3 resumes the different amounts of milk powder used and the average COD (chemical oxygen demand) concentration of each OLR level when the substrate was mixed. Macro- and micro nutrients were added to the substrate according to Shelton and Tiedje (1984, see Table 4). pH was brought to pH 10 using 30 g of Na_2CO_3 to avoid pH drop due to degradation made by microbial activity. New synthetic wastewater was prepared every 24 hours and subsequently mixed 15 minutes per hour to avoid sedimentation using centrifugal pumps.

Table 3: Amount (g) of milk powder used and COD values of the different OLR levels

OLR level	**g of "Nido" milk powder (Nestlé) per 100L of substrate**	**COD values [mg COD*L^{-1}]**
Initial steady state	112	1856
Second level	224	3692
Third level	336	5532

Table 4: List of compounds for macronutrients and micronutrients stock solutions. In order to prepare 100L of synthetic wastewater, 1 ml of macronutrients stock solution and 0,01 ml of micronutrients stock solution were added according to Shelton and Tiedje (1984).

Macronutrients	**Concentration (g/L of stock solution)**	**Micronutrients**	**Concentration (mg/L of stock solution)**
KH_2PO_4	2,7	$MnCl_2$	50
K_2HPO_4	3,5	H_3BO_3	5
NH_4CL	5,3	$ZnCl_2$	5
$CaCl_2.{}_2H_2O$	0,75	$CuCl_2$	3
$MgCl_2.6H_2O$	1	$NaMo_4.H_2O$	1
$FeCl_2.4H_2O$[1]	0,20	$CoCl_2.6H_2O$	50
$NaHCO_3$	1,2	$NiCl_2.6H_2O$	5
		$Na_2.SeO_3$	5

3.3 Data collection and sampling

Data collection (gas flow, pH and temperature of the reactor, amount of substrate fed and its pH, reactor level and TMP) were performed every day. Sampling of reactor was carried out twice a week from the waste stream, using the recirculation flow (see Figure 5. A). Effluent samples were taken in the output of the permeate pipe and gas samples were taken before the flow meter. Samples were also directly taken from the substrate tank in order to measure the COD content of the substrate. Finally, samples of the biogas were taken through a rubber septum with a syringe and analysis of its methane content were carried out 3 or 4 times a week.

[1] $FeCl_3.6H_2O$ was used from day 91 to day 144.

3.4 Analysis and measurements

All analysis and measurements were performed in the Water and Environmental engineering lab in the Chemical engineering department (Lund University).

3.4.1 Reactor samples

After sampling the reactor samples were analyzed as quickly as possible in order to avoid degradation. Table 5 summarize the different analysis performed. Half of the reactor samples were centrifuged at 3900g for 15' in order to obtain the supernatant which was used in some of the analysis. Chemical compounds were analyzed spectrophotometrically using Hach Lange cuvettes (NH_4^+-N, TP, PO_4^{2-}-P, TN, SO_4^{2-}, S^{2-}, total and soluble COD). Total carbon and total inorganic carbon (TOC and TIC) were analyzed using Shimadzu TOC analyzer. Volatile fatty acids (acetate and propionate) were measured using gas chromatograph Agilient 6850 A equipped with a flame ionization detector (FID) and a 30 m (length), 0.53 mm (diameter), 1.0 µm (film) HP-FFAP column. pH and conductivity were measured with portable equipment (WTW Sentix). Alkalinity was measured using 5 point titration according to Vannecke et al. (2014). Alkalinity measurements were done by the titration of centrifuged samples (supernatant) using hydrochloric acid 0,05N. The pH limits needed to determine alkalinity were 6.7, 5.9, 5.75, 5.2, 4.5 and finally 4.3. For the TSS and VSS, "Munktell" filter papers of 1.6 µm were used. Filters were dried for 24h (at 105°C) and then ignited (550°C) with ovens.

Table 5: Summary of the different analysis performed for reactor samples

Analysis	Units	Stability indicators	Use of supernatant	Method
Total COD	mg $COD*L^{-1}$			Hach Lange
Soluble COD	mg $COD*L^{-1}$		Yes	Hach Lange
Conductivity	µS$*cm^{-1}$		Yes	WTW apparatus
Alkalinity	mg $CaCO_3*L^{-1}$	Yes		Vannecke et al.
Volatile fatty acids	mg $COD*L^{-1}$	Yes	Yes	GC-FID
TC and TOC	mg $C*L^{-1}$			Shimadzu analyzer
Total nitrogen (TN)	mg $Nb^{(2)}*L^{-1}$			Hach Lange
Total phosphate (TP)	mg PO_4^{2-}-P$*L^-$			Hach Lange
Ammonium (NH_4^+-N)	[1]		Yes	Hach Lange
pH	mg NH_4^--N$*L^-$	Yes		
Phosphate (PO_4)	[1]		Yes	Hach Lange
Sulfate (SO_4^{2-})			Yes	Hach Lange
Sulfide (S^{2-})	mg PO_4^{2-}-P$*L^-$ [1]		Yes	Hach Lange
	mg $SO_4^{2-}*L^{-1}$			
	mg $S^{2-}*L^{-1}$			
Total suspended solids (TSS)	mg$*L^{-1}$			SS-EN12879
Volatile suspended solids (VSS)	mg$*L^{-1}$			SS-EN12880

[2] Total nitrogen (including all N species such as ammonium, nitrate, nitrite nitrogen, organic and inorganic compounds)

3.4.2 Effluent samples

COD, VFA and turbidity analysis were carried out on the effluent samples.

3.4.3 Gas sample

Biogas flow was measured using thermal mass flow meter (Vögtlin); it was calibrated for a gas composition of 60% CH_4 and 40% CO_2 and a maximum flow of 600 ml/min. The methane content of the released biogas was measured with gas-chromatograph – Varian 3800 Gas Chromatograph analyzing with TCD (thermal conductivity detector) and a column with dimensions of 2.0 m (length), 1/8" (diameter), 2.0 mm (film) HAYESEP mesh column.

3.5 Stability indicators

In order to monitor the stability of the anaerobic process in the reactors (while increasing the OLR), 5 Stability indicators were chosen. The stability indicators include four stability indicators concerning biological processes: The pH, specific gas production, volatile fatty acids (VFA's) and alkalinity ratio and one last concerning the membrane efficiency (to account the membrane fouling), named membrane performance. The four first stability indicators were chosen as they are the most common used criteria found in the literature in order to monitor AD processes (Bjornsson et al., 2001). The last membrane performance indicator was added in order to take account for the operational issues and the excessive working load needed in order to monitor the membrane fouling. As the membrane cleaning is a heavy operation (6 hours of work, while the reactor is shut-down), it has been decided that a shorter cleaning cycle than 7 days would mean that the process is not stable and sustainable. Table 6 resumes all the stability indicators used in this work.

Table 6: Summary of the stability indicators for monitoring anaerobic bioreactors

Stability indicators	Unit	Benchmark	Reference
Specific biogas production rate	$\frac{g(CH_4\text{–}COD).L^{-1}.d^{-1}}{gCOD.L^{-1}.d^{-1}}$	**≥0.5**	Sanchez et al. (2005)
pH	/	**6.5 – 7.5**	Bolzonella (2011)
Alkalinity ratio (IA:PA)	$\frac{Equivalents\ acetate}{Equivalents\ HCO3^-}$	**≤0.5**	Carballa et al. (2011) and Sanchez et al. (2005)
VFA	$mg*L^{-1}$	**≤316**	Chen et al., 2008
Membrane performance	Cleaning cycle	**≤7 days**	None

3.6 Organic loading rate increase

One reactor was first used to perform several step-increases of OLR (kg COD/m^3*d) after a long initial steady state (see Table 7). The other reactor was then used to directly reach the higher OLR after a short initial steady state, in order to figure out if a long initial steady state and a step-increase of OLR really were needed in order to ensure the stability of the process. Finally, a minimum time limit of 3 weeks have been set for each OLR levels in order to have a sufficient number of analysis of the reactor content. After that time, if all the stability indicators are met, the increase to the next OLR level is initiated.

3.7 Experimental plan

In order to evaluate the stability during the increase of the OLR, two reactors with the same setup were used, AnMBR#1 and AnMBR#2. As seen in Figure 6, AnMBR#1 was fed on steady state for more than 100 days before the first increase of the OLR was performed. Finally, AnMBR#1 was operated with 3 different OLR's (see Table 7). Concerning AnMBR#2, the reactor was first fed 3 weeks at steady state (in order to assure that biological processes is stable and ready for the up-scale) and then the OLR was increased until the final level reached by AnMBR#2 in order to figure out if the stepwise increases of AnMBR#1 was really needed. Thus the reactor was fed to the final third OLR level reached previously by the AnMBR#1 directly from the initial steady state, and without any intermediate states. Results obtained before day 80 were collected by the supervisor and another Master thesis student working on the reactors during the autumn semester (for further information: Benito C., 2015).

*Table 7: Summary of the starting experiment day of the different OLR phases used for both reactors and their aim OLR [kg COD/m^3*d]*

OLR Phase	Experiment day for AnMBR#1	Experiment day for AnMBR#2	Aimed OLR [kg COD/m^3*d]
1 (steady state)	1	151	0,9
2	103		1,8
3	126	179	2,6

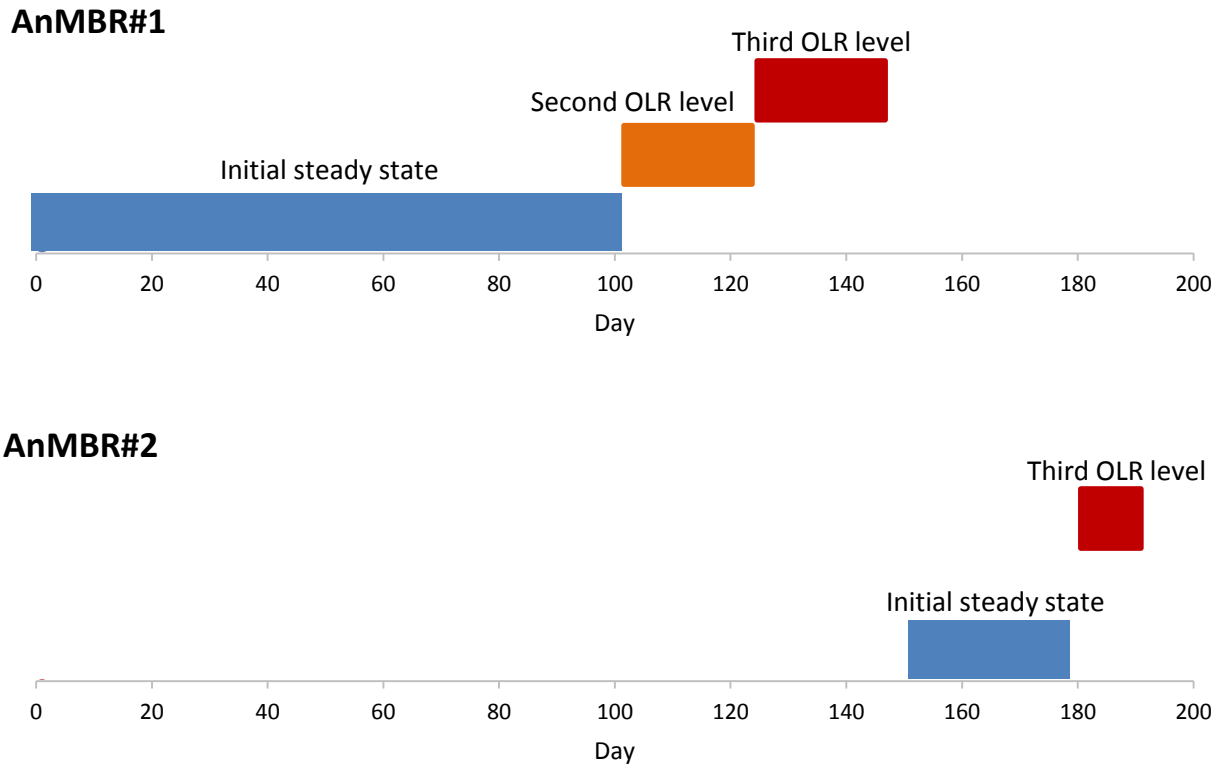


Figure 6: Timeline of operations for the AnMBR#1 and AnMBR#2

4 Results and discussions

In order to evaluate the stability of the biological processes in the reactor while increasing the organic loading rate (OLR), the stability indicators mentioned in the method section were used. In order to ease the understanding for the reader the results for OLR increase is presented first in the results section, followed by results for the stability indicators and finally the secondary results are presented. Stability indicators concerning biological processes consisted of the pH of the reactor, the specific biogas production rate, the concentration of volatile fatty acids (VFA) and the alkalinity ratio. Moreover, a non-biological stability indicator was used, the membrane fouling, to attest for the fouling occurring faster at higher OLR.

4.1 Increase of the organic loading rate

AnMBR#1

The different levels of organic loads applied to the reactor AnMBR#1 during the experiment are presented in Figure 8. After reaching steady state, the OLR was increased by raising the concentration of the substrate. It consists of adding the initial OLR level to the previous level (increases are represented by vertical lines in the figure).

As seen on Figure 8, an initial organic loading rate was fed to the reactor from experiment day 1 until day 103, where the first increase occurred. The second increase to reach the third level of OLR occurred 23 days later. Daily OLR is represented by orange line while blue triangles correspond to the week average values.

Practically, the weekly OLR didn't reach the desired values (aimed OLR) exactly in AnMBR#1 as it can be seen in Table 7, especially for the third level of OLR beginning at day 126. It can also be noticed that the variation is high according to the standard deviation (SD) with an emphasis on the second increase. That lower OLR and its variation can be explained by some malfunctions (shutdowns) concerning automatic apparatus and pumps, but also because of the fouling of the membrane, which reduces the outflow of the reactor and thus decrease the feeding (because the reactor level must be constant). One other parameter to take into account is the fact that several persons are working on the reactors and thus have to swap during the weekend and working days, which can explain some difference in the monitoring and thus variations of the reactor level. All of these reasons explain the difficulty to obtain a stable OLR during the different phases of the increase.

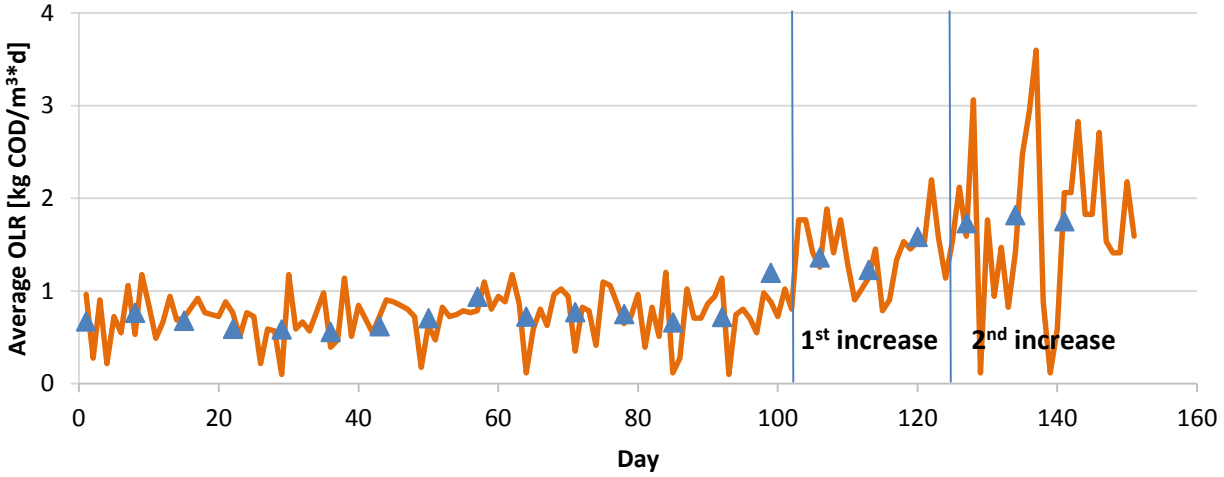


*Figure 8: Increase of the OLR [kg COD/m^3*d] in AnMBR#1. The two increases of OLR (vertical lines) separates the three different level of feeding. Line is the daily OLR while triangles are average OLR per week.*

*Table 7: Aimed and corrected OLR [kg COD/m^3*d] during the OLR increases of AnMBR#1*

OLR Phase	Experiment day	Aimed OLR (kg COD/m^3*d)	Observed OLR (kg COD/m^3*d)	SD (%)
1 (Steady state)	1 (Feeding start)	0.9	0.730	35
2	103	1.8	1.41	24
3	126	2.6	1.74	50

AnMBR#2

The two levels of organic loadings applied to the reactor AnMBR#2 during the experiment are presented in Figure 9 and Table 8. After a short first steady state, the increase of OLR of the AnMBR#2 was directly initiated from the steady state until the third level of OLR (see vertical line in figure 9), last level reached by the AnMBR#1. Just as the feeding of AnMBR#2, the OLR didn't reach the desired values (aimed OLR), once again especially for the third level of OLR which has a substantial SD. The same explanations than AnMBR#1 can be given as the feeding of both methods were identical.

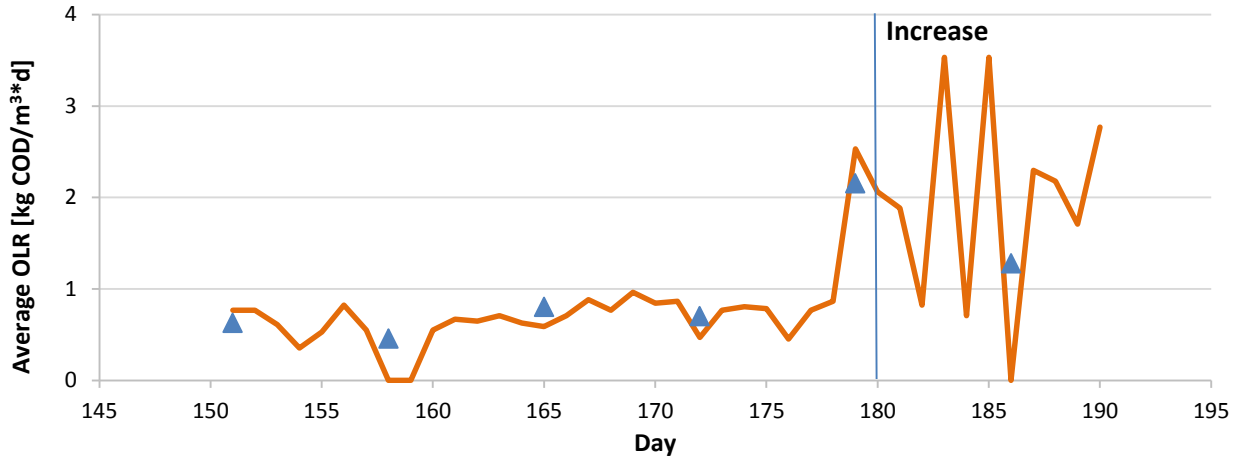


*Figure 9: Increase of the OLR [kg COD/m^3*d] in AnMBR#2. The increase of OLR (vertical line) separates the two different level of feeding. Line is the daily OLR while triangles are average OLR per week.*

*Table 8: Aimed and corrected OLR [kg COD/m^3*d] during the OLR increases of AnMBR#2*

OLR Phase	Experiment day	Aimed OLR (kg COD/m^3*d)	Observed OLR (kg COD/m^3*d)	SD (%)
1 (Steady state)	151 (Feeding start)	0.9	0.640	36
2	179	2.6	2.00	54

4.2 Stability indicators

4.2.1 pH

AnMBR#1

One of the most important parameters for good stability of the anaerobic digestion process is the pH of the reactor. Its recommended range is generally 6.5 – 7.5 to allow a good process (Bolzonella, 2011), especially in order to keep methanogenic archea in their active pH range (Khanal, 2008). According to Figure 10, pH of AnMBR#1 was almost constantly remaining between 6.3 – 6.5 during the experiment. It is thus surprising that the anaerobic process goes on with that low pH, as the other parameters like gas production will show. In conclusion, it seems that a pH above 6.3 is enough in order to perform methanization. The pH tolerance would then be lower than stated in the literature (Khanal, 2008).

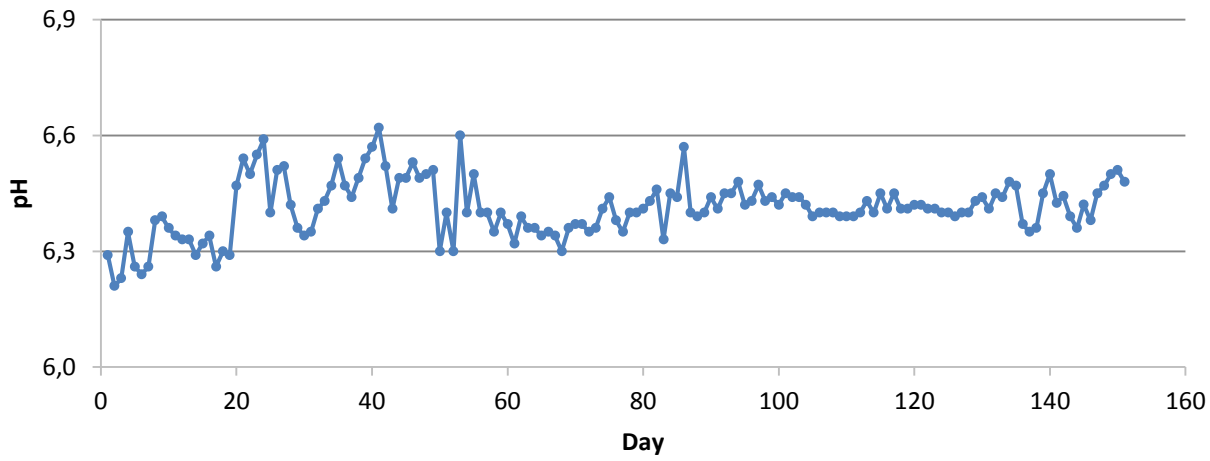


Figure 10: pH of AnMBR#1 during the experiment. The pH was almost constantly remaining between 6.3 – 6.5.

AnMBR#2

After a short period without any feeding (less than 1 week), initial OLR feeding start at day 151. The steady state lasts 28 days. During that time pH remained constant at a value slightly above 6.5. At day 179, the feeding level was dramatically increased in order to reach the third OLR. Subsequently a critical pH drop can be seen from day 179, indicating the process failure of AnMBR#2.

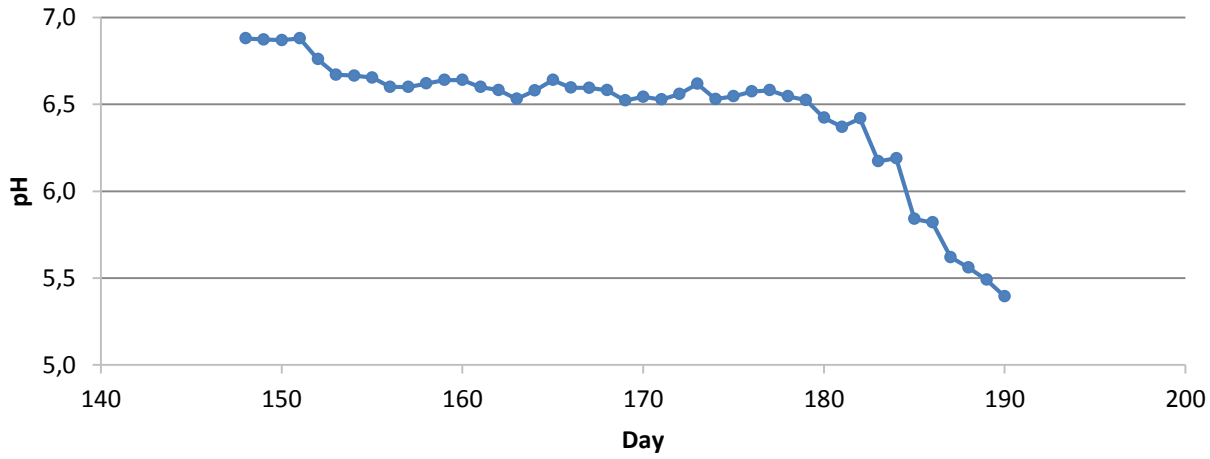


Figure 11: pH of AnMBR#2 during the experiment. After a stable period above 6.5, a critical pH drop can be seen at day 179.

Conclusion

pH of AnMBR#1 was stable during the whole experiment, even if pH was below the recommended value. As the AD of AnMBR#1 was effective, the recommended pH range could be extended to a lower limit than 6.5. Concerning AnMBR#2, its pH remains constant until the dramatic OLR increase, indicating the reactor failure.

4.2.2 Specific biogas production

AnMBR#1

The methanation process is a key factor to account for the stability of the anaerobic digestion process. In Figure 12 the accumulated CH_4 production accounted in COD is plotted against the COD load, where each red dot represents one day. This ratio allows visualizing the yield of the biomethanation process, according to the linear curve produced. The observed ratio has a slope of 0.75, which is above the 0.5 benchmark recommended by Sanchez et al. (2005), which means that 75% of the incoming COD substrate was transformed into methane. The ratio is remarkably linear with a coefficient of correlation of 0.9978, meaning that there was no variation in the specific biogas production; thus the biological process was stable during the whole experiment.

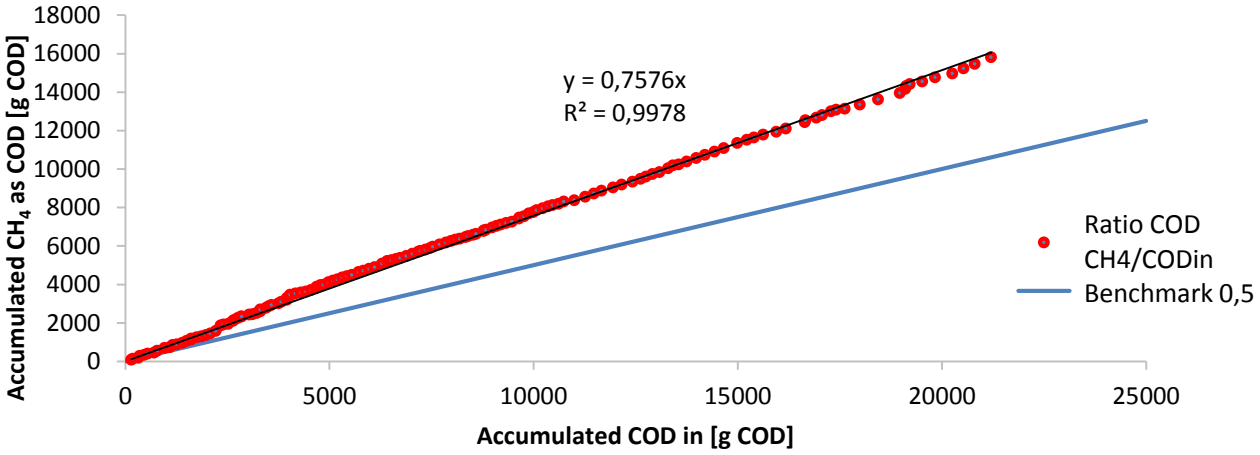


Figure 12: Accumulated COD from the OLR versus accumulated gas COD [g COD] in AnMBR#1. The benchmark ratio of 0.5 was met and remarkably linear, meaning that there was no variation in the specific biogas production; the biological process was stable.

AnMBR#2

Figure 13 shows the specific gas production of AnMBR#2. The gas production was lower than the first reactor as the ratio barely reach the 0.5 benchmark until the last 11 days where an inhibition of the process can be seen. The decrease of the gas production corresponds to the failure of the system occurring at day 179, corroborating the pH drop occurring the same day.

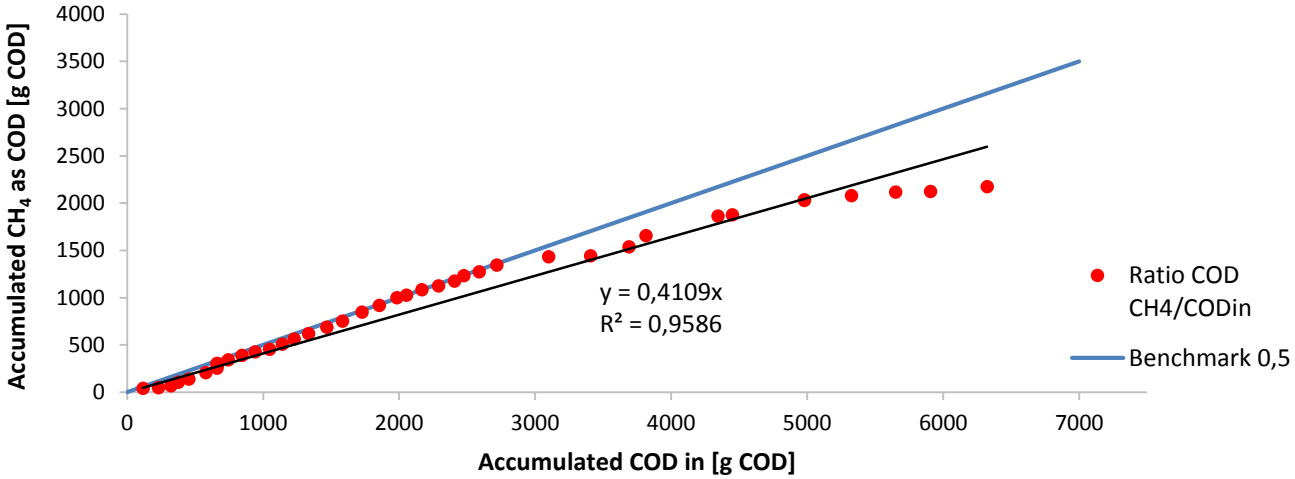


Figure 13: Accumulated COD from the OLR versus accumulated gas COD [g COD] in AnMBR#2. The benchmark ratio was barely reached until an inhibition was observed the 11 last experimental days.

Conclusion

The gas production of AnMBR#1 was stable during the whole experiment according to the benchmark, which means that the stability indicator is met for the first reactor. In contrary, the failure of AnMBR#2 is demonstrated again by the sudden decrease of the gas production after the OLR increase.

4.2.3 VFA

AnMBR#1

VFA are intermediate products in the anaerobic digestion process and are transformed into methane by methanogens archea. Their accumulation are an indication to the unbalance of the process, which can lead to an acidification of the reactor and thus the failure of the methanization process due to a low pH. Figure 14 is the combined graph of the VFA concentration (acetate and propionate) and the OLR fed in. As it can be seen, the level of the feeding and the VFA concentration are linked, although the peaks of VFA follow those of the OLR with a short delay. The delay might be the time needed by acetogenic and methanogens micro-organisms to hydrolyze the substrate. VFA concentration remained low during the first period of the OLR steady state, with an average concentration around 100 $mg*L^{-1}$. During the first increase, where sampling of VFA began to be daily analysis, the VFA's concentration remained in the same range except at the end where a 255 $mg*L^{-1}$ peak can be observed, following a high peak in the feeding. Concerning the second increase, variations are much more important, and the VFA's concentration exceeded twice the benchmark limit of 316 $mg*L^{-1}$ (at days 139 and 144, red rounds in the figure), which means that AnMBR#1 wasn't stable these two days. Nevertheless, except those two outliers, all the values met the benchmark. Thus AnMBR#1 can be assumed stable globally during the third OLR level and the whole experiment.

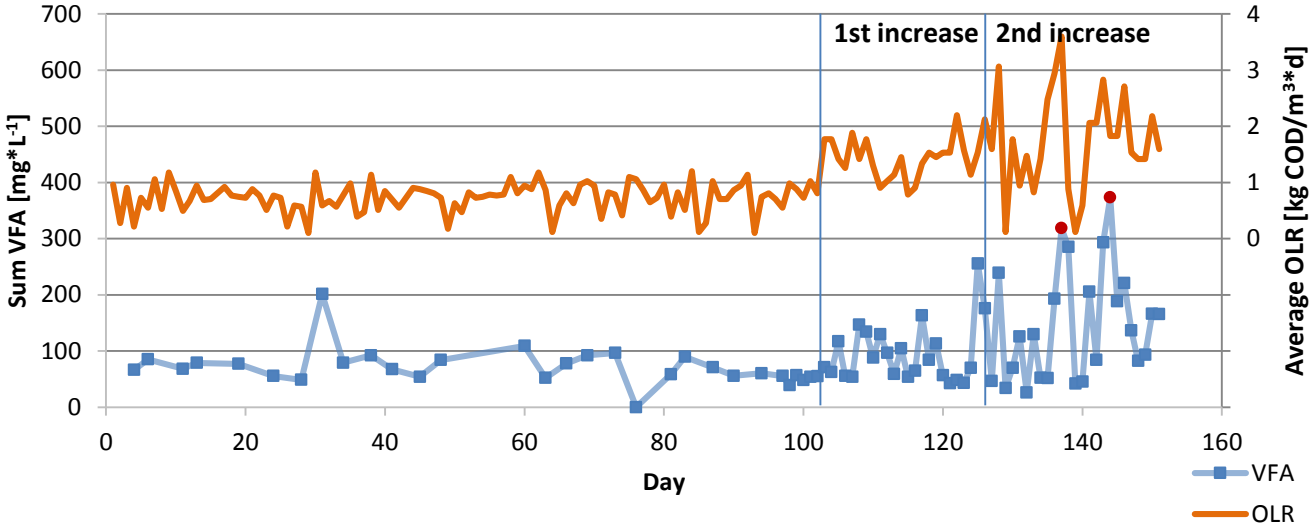


*Figure 14: VFA concentration (acetate and propionate) [mg/L] and OLR [kg COD/m^3*d] in AnMBR#1 during the experiment. Level of the feeding and the VFA concentration are linked, although a short delay can be observed. AnMBR#1 can be assumed stable during the experiment.*

AnMBR#2

Concerning the AnMBR#2, VFA concentration reaches globally the 316 mg*L^{-1} benchmark during the initial steady state (except day 153, 161-3, red squares in the figure). However, after the subsequent increase of the OLR, VFA's concentration reached values exceeding the limit since day 182 until the end of the feeding (see the red rounds in the figure). VFA's accumulated to reach a concentration of around 2500 mg*L^{-1}, leading to the acidification of the reactor and the failure of the anaerobic digestion process.

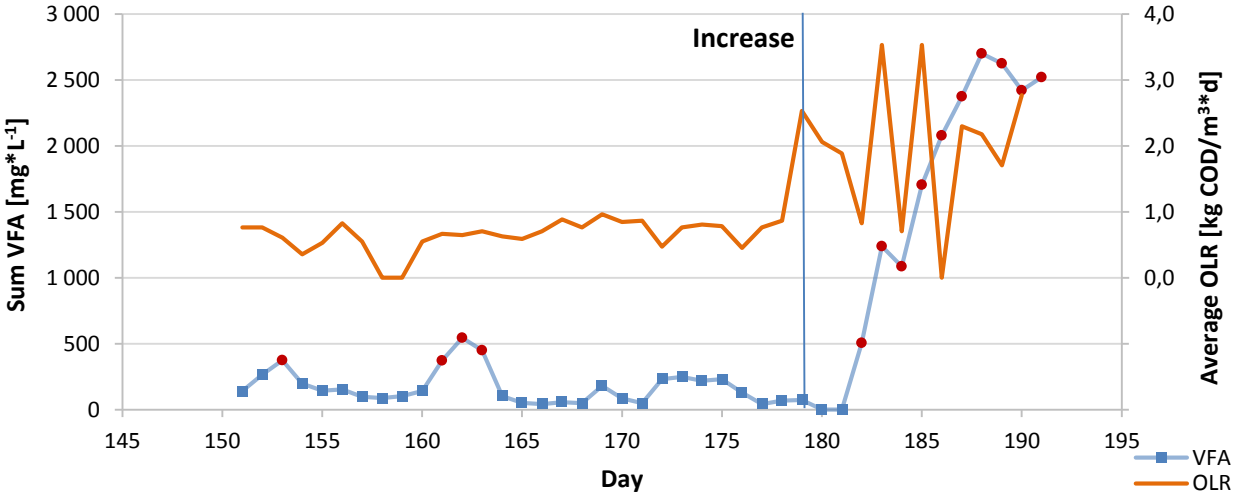


*Figure 15: VFA concentration (acetate and propionate) [mg/L] and OLR [kg COD/m^3*d] in AnMBR#2 during the experiment. Three days after the OLR increase (vertical line), VFA concentration exceeded the benchmark, leading to the failure of the reactor.*

Conclusion

AnMBR#1 can be considered stable during the experiment, as the stability indicators are most of the time met (except two values slightly above the benchmark). In contrary, the failure of AnMBR#2 is demonstrated again by the dramatic accumulation of VFA's after the OLR increase.

4.2.4 Alkalinity ratio

AnMBR#1

The alkalinity ratio represents the buffering capacity of the system and how it can neutralize the acidification of the VFA's. This stability indicator is interesting because it is more sensitive to process changes than the pH. According to Carballa et al. (2011), the alkalinity ratio should be below 0.5 to allow a good performance and avoid the risk of acidification. All along the experiment, AnMBR#1 was fulfilling the 0.5 benchmark (see Figure 16), nevertheless we can observe a low increase as shown with the linear curve. The linear increase of alkalinity ratio can be explained by the OLR increases that have occurred during the experiment, which has released VFA and thus stressed the system. Nevertheless we can state that AnMBR#1 was operating in stable conditions according to the alkalinity ratio indicator.

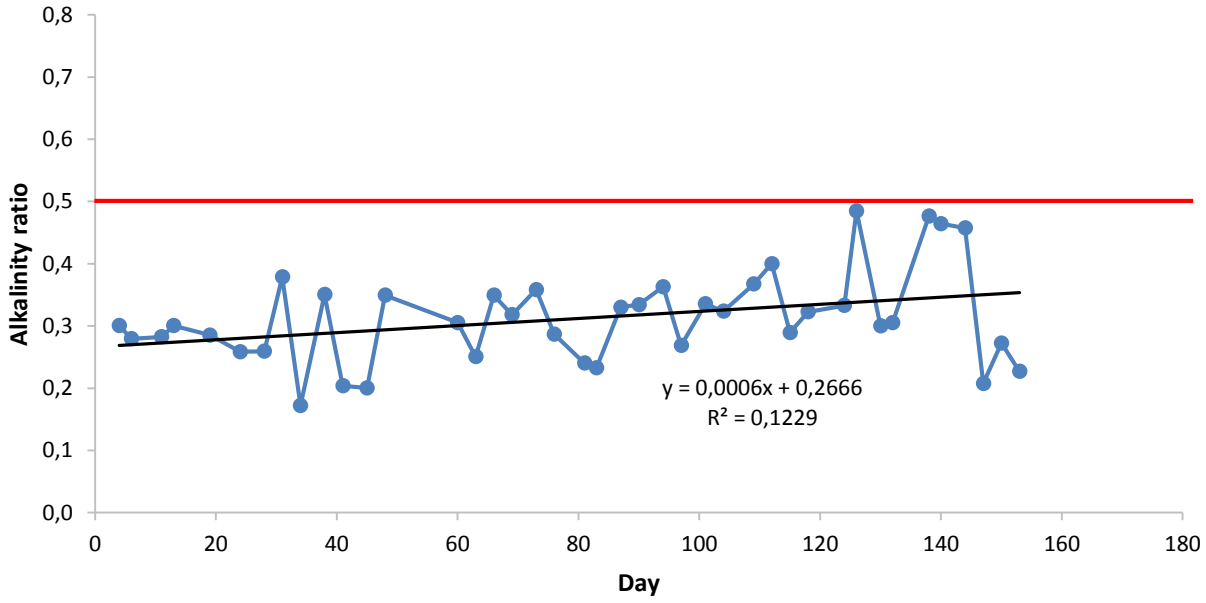


Figure 16: Alkalinity ratio (intermediate alkalinity on partial alkalinity) in AnMBR#1. Although a linear increase can be observed, the 0.5 benchmark was met during the whole experiment.

AnMBR#2

Concerning AnMBR#2, the alkalinity ratio benchmark was roughly met during the initial steady state of 3 weeks (Figure 17). After the subsequent increase of the OLR (occurring at day 179), alkalinity ratio values exceeded dramatically the 0.5 limit from day 186 until the end of the feeding. The reactor process was then unbalanced and led quickly to the failure of the system. This is another indicator demonstrating the acidification of AnMBR#2, which began around day 180.

AnMBR#2

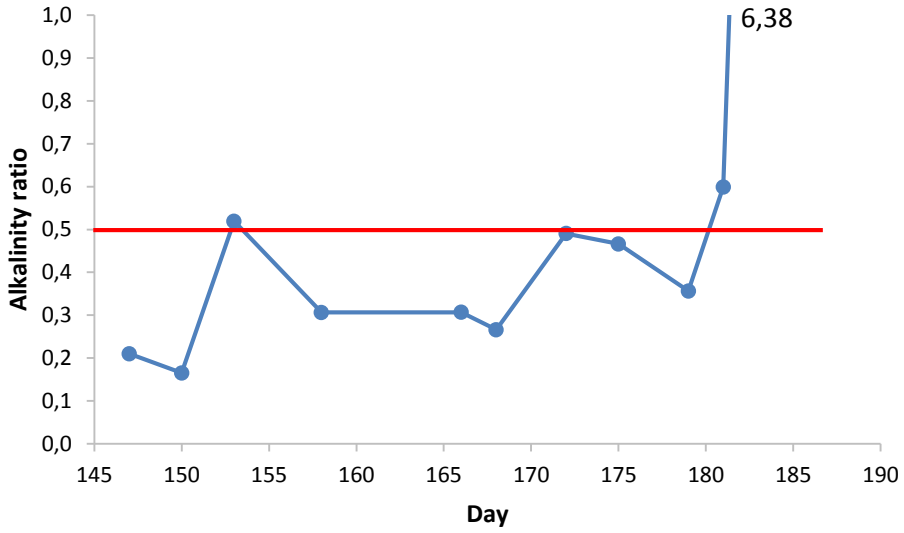


Figure 17: Alkalinity ratio (intermediate alkalinity on partial alkalinity) in AnMBR#2. The alkalinity ratio exceeded the stability benchmark after day 180.

Conclusion

AnMBR#1 can be considered stable according to the alkalinity ratio as its measured ratios during the experiment were always below the benchmark limit. In contrary, the failure of AnMBR#2 is demonstrated once again by the dramatic increase of the alkalinity ratio after the dramatic OLR increase.

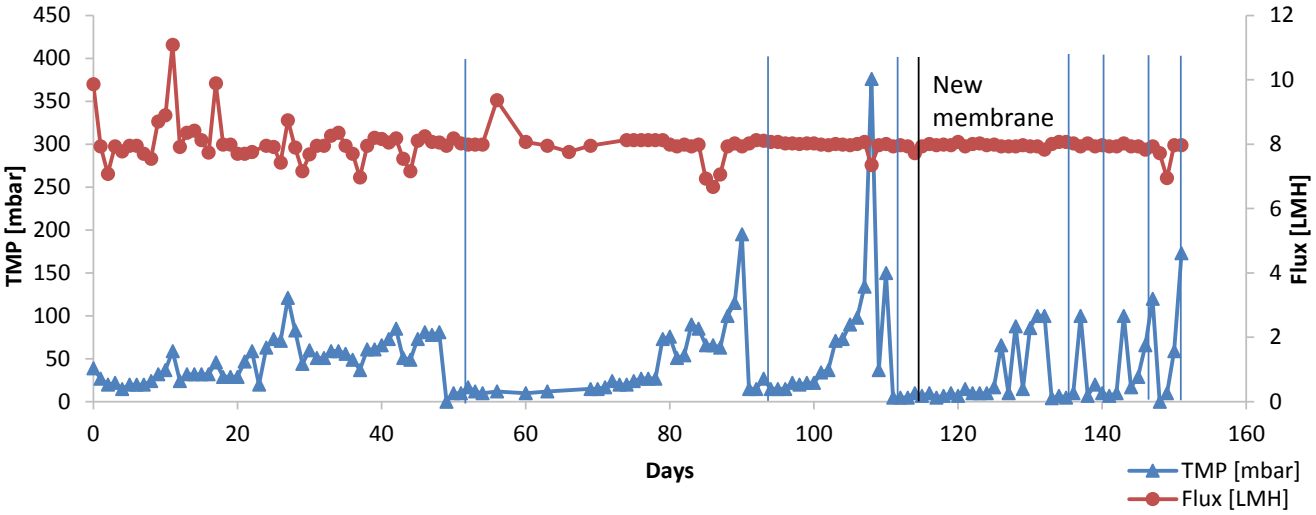
4.2.5 Membrane performance

AnMBR#1

According to Christian et al. (2011), fouling is one of the major drawbacks of the membrane bioreactor, especially in anaerobic conditions. Practically the benchmark for the membrane performance has been fixed at 7 days without cleaning. The pressure limit was set to 100 mbars after which the membranes were cleaned (vertical lines), to recover a low functional TMP. If the TMP reached a higher pressure than the 100 mbars limit before 7 days without cleaning then the membrane performance benchmark wasn't reached, and thus the reactor wasn't in a stable operational state. This choice is justified by the excessive overload of workload needed in order to clean the reactor and also by the disturbance for the running of the reactor, since each cleaning needs a purge of the filtration tank (taking at least 5 hours).

Figure 18 is the combined graph of the flux with the transmembrane pressure (TMP). As it can be seen flux was almost constant at the aimed flux of 8 LMH (L/m^2*h) throughout the experiment while the TMP [mbar] is slightly increasing through the running of the reactor showing the fouling of the membrane. From day 1 to day 108, the membrane fouling was slow though its rate was slowly increasing. At day 109 however, a change of the membrane (black vertical line) was carried out in order to solve the unexpected increase of the TMP just after the membrane cleaning, which was the first time that the membrane performance wasn't met. The new membrane was then firstly cleaned after running 22 days. However, at day 138, fouling became again the limiting stability indicator as its rate increased and did not meet anymore the benchmark (at least 7 days between each membrane cleaning), especially since the second OLR increase was performed (at day 126). Membrane cleanings were then performed at day 143, 148 and 151. Fouling was problematic as it decreased the feeding and thus the OLR, in addition to have added an extra load of work

with the cleanings. Finally, it was decided to pause the increases of OLR on AnMBR#1 and to



investigate the reason of this quick membrane fouling (see further, section 4.4).

*Figure 18: Flux [L/m^2*h] and transmembrane pressure (TMP) [mbar] of system 1 during experiment. While the flux remained stable at 8 LMH, an increase of the cleaning (vertical lines) can be seen through the experiment, as the TMP increased more rapidly.*

AnMBR#2

Concerning AnMBR#2, the flux remained almost constant at 8 LMH during the experiment, except at day 158 where the reactor was shut down for one day before the membrane cleaning for practical reason (Figure 19). The first membrane cleaning occurred after only 8 days of feeding, which is a low performance comparing to the first hundred days of AnMBR#1, where only two cleanings were performed. At day 171, membrane fouling became the limiting stability indicator as its rate increased. AnMBR#2 didn't met the benchmark, which is an interval of at least 7 days between each membrane cleaning) for the rest of the experiment. Moreover, the fouling began to be stronger after day 179 (day of the increase of the OLR from the initial steady state to the third level) as it can be seen with high values of TMP (420 mbars). AnMBR#2 didn't fulfill the membrane performance during the experiment.

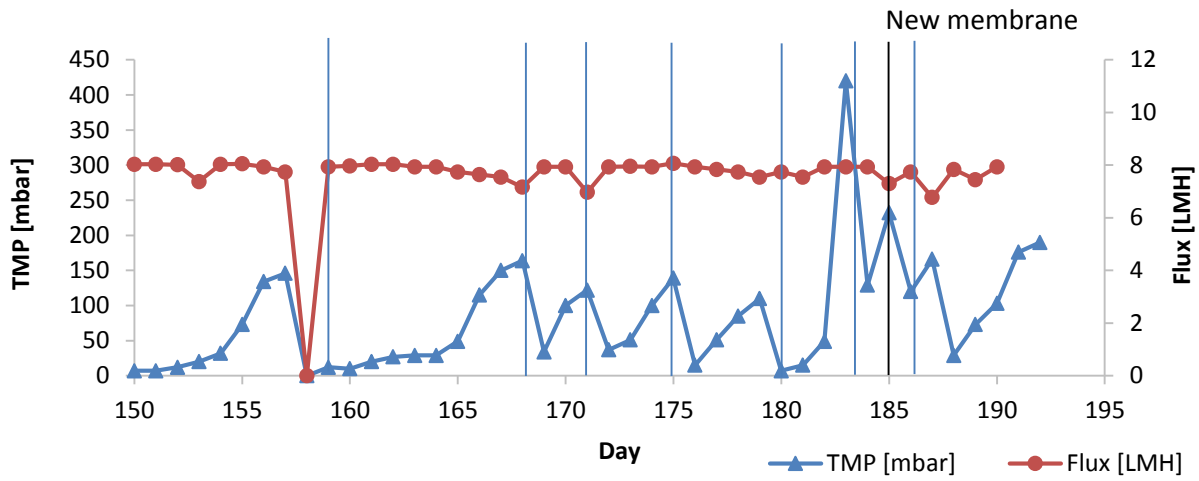


*Figure 19: Flux [L/m²*h] and transmembrane pressure (TMP) [mbar] of system 1 during experiment. While the flux remained stable at 8 LMH, an increase of the cleaning (vertical lines) can be seen through the experiment, as the TMP increased more rapidly.*

Conclusion

None of the reactors met the membrane performance benchmark as they both needed a membrane cleaning cycle shorter than 7 days.

4.2.6 Summary of the stability indicators

As seen on Table 9, AnMBR#1 has fulfilled all the biological benchmarks during its running. Nevertheless the membrane performance wasn't met, which led to the pause of the OLR step-increases in order to investigate the membrane fouling.

AnMBR#2 didn't meet any of the biological benchmarks as well as the membrane performance parameter. The anaerobic digestion process failed due to the intense feeding. One reason can be the need for a step-increase of the OLR (like in AnMBR#1) instead of the direct dramatic OLR increase from the steady state that has occurred in AnMBR#2. Another reasons to explain this failure could be that the initial steady state can have been too short, and thus the micro-organisms were not enough adapted and ready to handle the intense feed.

Table 9: Results of the stability indicators during the running of AnMBR#1 and AnMBR#2 (✓: stability indicator met during the experiment; X: stability indicator not met)

Stability indicators	AnMBR#1	AnMBR#2
pH	✓	X
Specific gas production	✓	X
VFA	✓	X
Alkalinity ratio	✓	X
Membrane performance	X	X

4.3 Others parameters

4.3.1 Conductivity

Conductivity measurement can be used as early warning indicators of process failure due to the acidification (Aceves-Lara et al., 2012; Lei et al., 2014). According to De Vrieze et al. (2014) it is recommended to stay below a conductivity of $20*10^3$ µS.cm^{-1}. The failure after these limits could actually be explained by severe VFA accumulations. As seen in Figure 20, both reactors met this benchmark during the whole experiment. However, distinctive increases can be observed during the increase of the OLR (day 103 and 126 for AnMBR#1, and day 179 for AnMBR#2). Nevertheless, the failure that occurred in AnMBR#2 at day 179 is not linked with any exceeding of the conductivity benchmark. Therefore it can be concluded that monitoring the process only by conductivity measurements is not sufficient in order to prevent any failure of the anaerobic digestion.

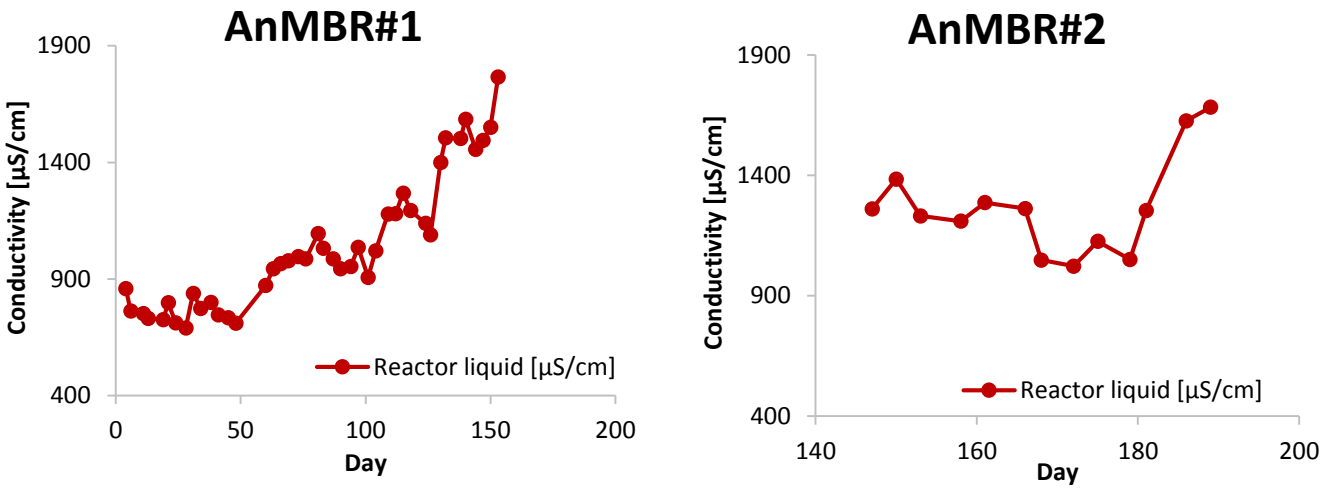


Figure 20: Conductivity in AnMBR#1 and AnMBR#2 during the experiment.

4.4 Fouling of the membrane

According to the stability indicators, it wasn't the biological process that was limiting but the membrane performance. It is surprising considering the first 100 experimental days of AnMBR#1 where only two membrane cleanings were needed. For both reactors, sudden increase of the cleaning rate occured, leading to unsustainable operation processes. Two hypotheses could explain this unexpected increase of membrane fouling. First, the suspended solid content of the reactor is known to be factor of membrane fouling (Rosenberger et al., 2005). TSS (total suspended solids) content of the reactor could have been too high (and then sludge would have been too thick).

A second hypothesis could be the accumulation of fat in the reactor. The accumulation of fat would have led to the fouling as the membrane is hydrophobic and thus have a high affinity with fatty matters. Fat was poured into the reactors as reactors were fed with synthetic wastewater constituted with "Nido" milk powder (Nestlé), which contains 3% of fat. All the following investigations in order to figure out the reason of the unsustainable membrane fouling were performed in AnMBR#1.

The following experiments were carried out to investigate the membrane fouling:

- Decrease of the TSS content was carried out (TSS is a factor of membrane fouling according to Rosenberger et al. (2005)).
- An eventual accumulation of fat in the reactor was investigated by sedimentation. The presence of a top layer from a sample taken during the intense feeding could be undigested fatty matters.
- Fat analysis was performed.
- Feeding with skim milk was tested for few days, to figure out if the absence of fat would decrease the fouling rate.
- A prototype detergent (with protease and lipase activity) provided by Alfa Laval were used during one membrane cleaning in order to remove possible accumulation of fat into the membrane.

4.4.1 Decrease of the total suspended solids (TSS)

According to Rosenberger et al. (2005), TSS content of the reactor is a factor of membrane fouling above the concentration of 15g/L of MLSS (mixed liquor suspended solids). As seen in the Figure 21, TSS reached 19 g/L at day 126. Thus decreasing the TSS could be a method in order to decrease the membrane fouling rate and thus reach an operational state in the reactor. 30L of sludge (out of 150L, the complete volume of the reactor) were removed (progressively, 5L per day from day 131 to day 136 as seen in the Figure 21) in order to reduce 20% of the TSS content. This measure was set during the last OLR increase of AnMBR#1. No effect on the membrane fouling was observed as the rate of cleaning still increased dramatically during the next days.

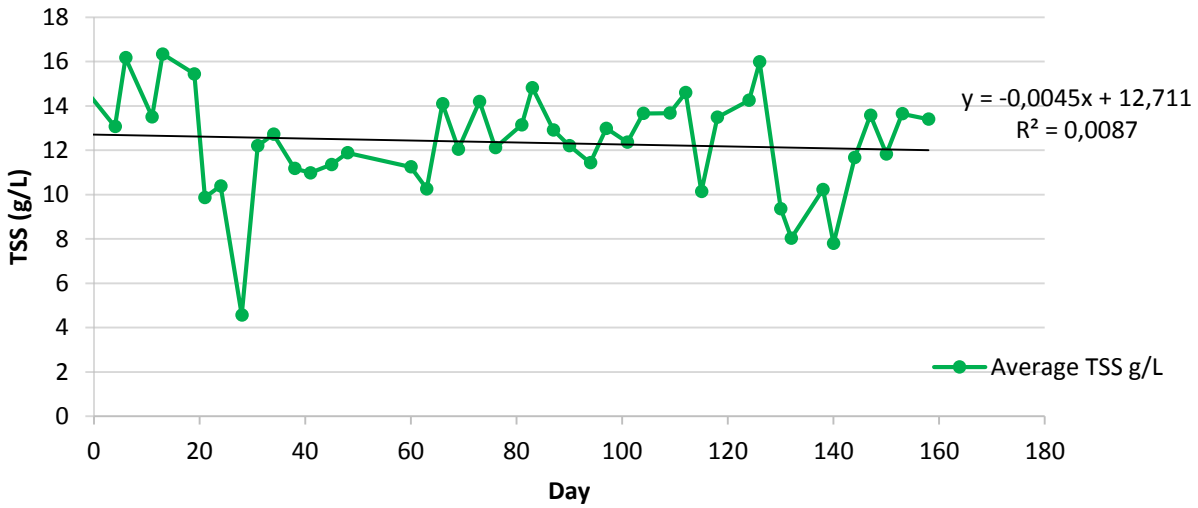
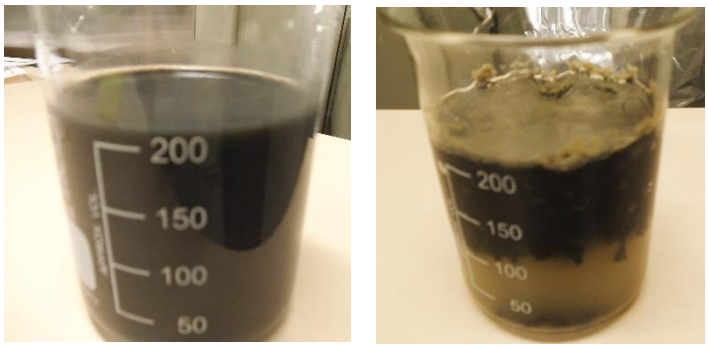


Figure 21: TSS content (g/L) of AnMBR#1

4.4.2 Sludge sedimentation test

In order to bring out a possible accumulation of fat matter inside the reactor, a sludge sedimentation test was performed. Sludge from different phases (during intense feeding with milk powder containing fat, and during phase with no feeding) was poured into a beaker, and the sedimentation was carried out during few days. The objective of the test is to show a possible difference in the way the sludge sediment depending the substrate feeding; a possible accumulation of fat would be observed during the intense feeding, in contrary of the no feeding period. Accumulation of fat would be seen as a top layer as the density of fat is smaller than the water.

Sludge from the reactor sampling point was poured into a beaker at day 143 (Figure 22 top pictures), during the third level of OLR and at day 164 (bottom pictures), after 11 days with no feeding, in order to figure out an eventual fat accumulation. Left pictures are the beakers at the first day, and the right ones five days later. A top layer can be noticed in the sludge of day 143, in contrary with the other one, which can lead to the hypothesis that there is a fat accumulation in the reactor during the feeding at high OLR.



Before sedimentation After 5 days of sedimentation
Day 143: Sludge sampled during intense feeding

Before sedimentation After 3 days of sedimentation
Day 164: Sludge sampled after 11 days without feeding

Figure 22: Pictures of sludge sampled during intense feeding and sludge sampled after 11 days without feeding.

4.4.3 Fat analysis

In order to determine the fat content of the reactor, a fat analysis was performed in the Alcontrol laboratory (Malmö, Sweden). Reactor samples were sent away and analyzed within 24h to avoid any degradation. Fat analysis was performed, giving a fat concentration of 11 $mg*L^{-1}$. This result is difficult to discuss because no references in literature were found for a similar case. Moreover, this result has to be looked carefully due to the difficulty of performing an analysis on sludge.

4.4.4 Feeding with skim milk powder

Feeding with skim milk powder was performed later on to investigate the membrane fouling, which might be caused by the fat content of the standard milk powder. If the membrane fouling came from the fat content of the substrate, feeding without any fat would allow better operational state. After 13 days without any feeding, in order to allow an eventual previous fat accumulation to be removed, AnMBR#2 was fed for 4 days at the third level of OLR (days 165-168, with an average OLR of 1.3 [kg COD/m^3*d]). Nevertheless, no relevant differences in the membrane fouling rate were observed with the use of that fat-free synthetic wastewater (membrane cleaning had to be performed in day 167, after 3 days of feeding).

4.4.5 Protein cleaning

A prototype detergent (with protease and lipase activity) provided by Alfa Laval was used during one membrane cleaning (day 148) in order to remove possible accumulation of fat or proteins into the membrane. Next membrane cleaning occurred 3 days after the use of the detergent, showing its inefficiency to decrease the membrane fouling.

4.4.6 Summary of the investigations on the membrane fouling

Membrane fouling was investigated in several ways.

- Decrease of the TSS content was carried out, but no change on the membrane fouling was observed.
- Accumulation of fat in the reactor was investigated by sedimentation, where the presence of a top layer from the sample taken during the intense feeding could be undigested fatty matters.
- Fat analysis was performed, giving a fat concentration of 11 $mg*L^{-1}$.
- Feeding with skim milk was tested for few days, without any relevant difference in the membrane fouling rate compared to the standard milk powder.
- A prototype detergent (with protease and lipase activity) provided by Alfa Laval were used during one membrane cleaning in order to remove possible accumulation of fat into the membrane. No change on the membrane fouling was observed.

4.5 COD balance

To attest the reliability of the results, the COD going out of the reactor must recover the major part of the incoming COD. In order to visualize the COD flows, a COD balance can be made summarizing the repartition of the COD substrate into the different COD output. The balance has to be closed, which means that the difference between the flows going in and out is nearly zero and thus the COD account of the reactor is correctly monitored. This can be important for example to spot any gas leakage. As seen on the scheme of the COD balance (Figure 19), the CODin consist of the influent, while CODout consists of the biogas production, the permeate flow and the sludge sample. In the experiment, CODin consists in the synthetic wastewater feeding, while CODout consists in the methane in the biogas, COD of effluent, the methane dissolved in the effluent, the waste of sludge (samples) and finally the accumulation of COD in the reactor. All of the fractions were determined with the laboratory analysis results, except the dissolved methane which was estimated using 100% of methane solubility in the water. COD balances were realized per week in order to lower the daily variations.

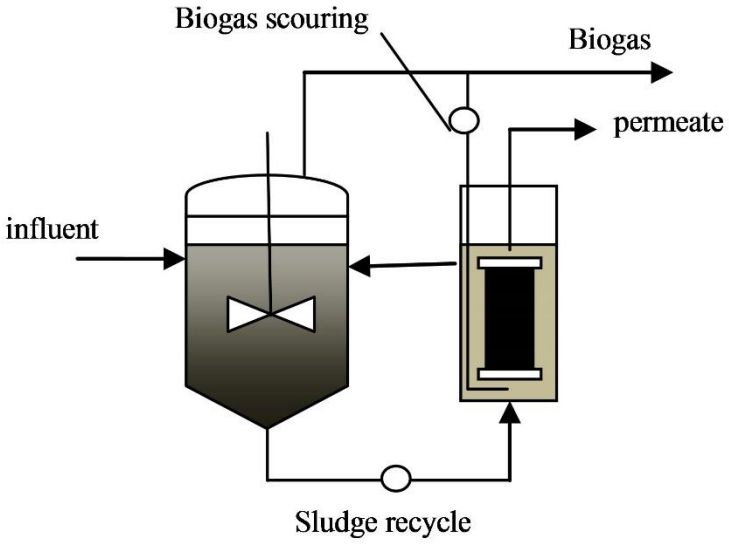


Figure 19: Scheme of the different COD flows crossing the reactor (Chang, 2014).

AnMBR#1

As seen in Figure 20, the ratio CODout/CODin remained constant around 80% during the whole experiment, which means that 20% of the COD going out doesn't have any explanation. The methane production was the most remarkable fraction of the CODout with 78% of incoming COD turning into CH_4. All the other fractions explained 3-4% of the balance.

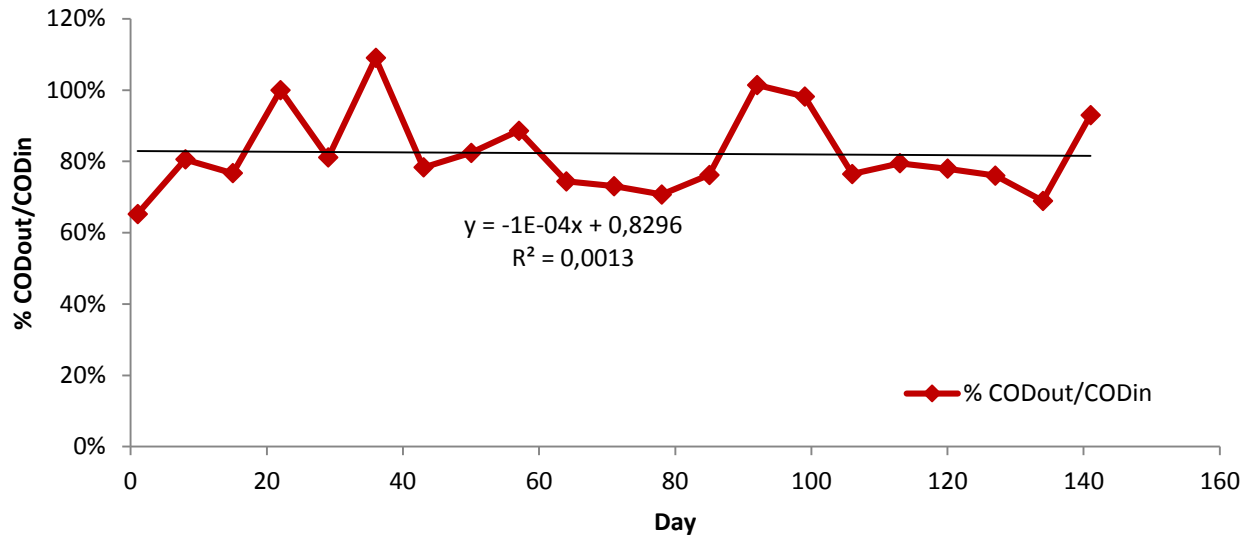


Figure 20: COD recovery (CODout/CODin) per week in AnMBR#1 during the experiment

AnMBR#2

Concerning AnMBR#2 (Figure 21), the ratio CODout/CODin was variable during the experiment as it reached 44% on day 151, then reached 80% the next two weeks before going down dramatically. The decrease from day 179 can be explained by the failure of the anaerobic process and thus the decrease of the gas production, which explains the most important part of the balance (in average 40% CODout). Interestingly the permeate COD explained in average 13% of the CODout. The membrane of AnMBR#2 is an output for a high amount of COD.

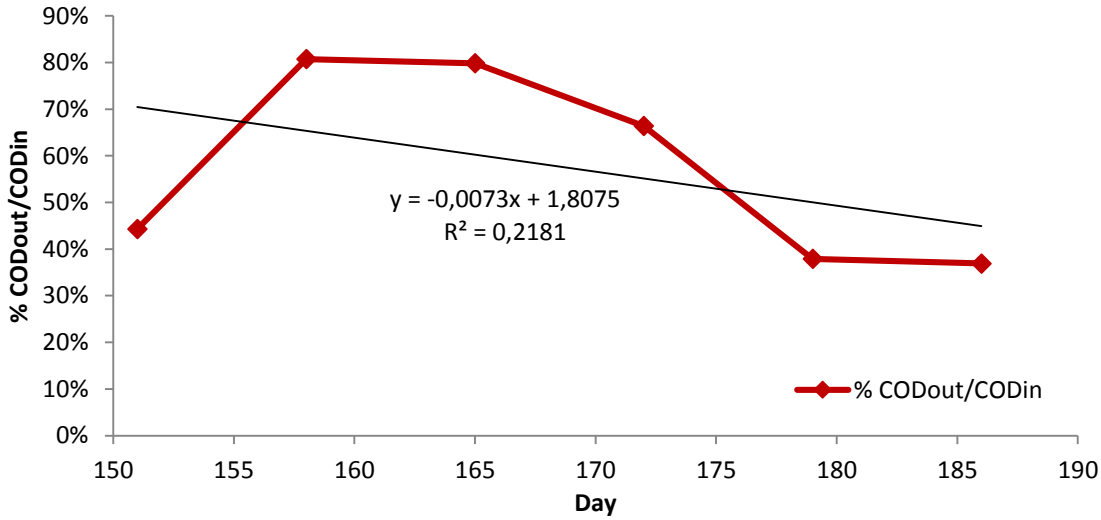


Figure 21: COD recovery (CODout/CODin) per week in AnMBR#1 during the experiment

Conclusion about COD balance

The COD balances of both reactors aren't closed and thus are perfectible. Several ways to improve it exists. First the substrate degradation occurring in the substrate tank wasn't taken in account in the balance. As the synthetic milk powder stays for 24h in open air, some micro-organisms can degrade it and thus lower its COD value. The incoming COD would have been over-estimated. Some substrate degradation test has been performed to account of the COD decrease occurring every day between each feeding preparation. Among the results obtained, 6 values account for 5% of COD degradation in 24h, while 2 other values account for an average of 10% of COD degradation in 24h. It is likely that some missing COD percents can be explained by the micro-organisms degradation of the substrate. The H_2S production, which wasn't taken in count, can also explain some of the missing COD, but in a very little scale (less than 1%).

Finally, gas leakages could also explain several of the missing percents. Even if gas leakage tests were done with the help of sniffers and special foam, no leakages were found in the reactors.

Considering the COD permeate, the two membranes retained COD in a very different way. Membrane of AnMBR#2 didn't retain COD as expected. This can be explained by the difference of pH of the two reactors as the filtration conditions are important for the efficiency of the membrane. In AnMBR#2, the pH drop at day 179 led to an increase of the COD in permeate.

5 Conclusions

In order to find a stable way to increase the organic loading rates (OLR) during the start-up of an anaerobic process two different ambient AnMBR's were monitored by stability indicators. The reactors were given two modes of operation; a step wise increase of OLR after a long adaptation period (AnMBR#1) or a direct increase to the maximum OLR after a short adaptation period (AnMBR#2).

AnMBR#1 fulfilled all the biological benchmarks during its operation. The reactor handled the increase of the OLR without any failure in the anaerobic process. Nevertheless the membrane performance wasn't met since the membrane was fouled too often.

AnMBR#2 didn't meet any of the biological benchmarks or the benchmarks for the membrane performance. The anaerobic digestion process failed due to the intense feeding. One reason can be the need of a step-increase of the OLR (like in AnMBR#1) instead of the direct dramatic OLR increase from the steady state that occurred in AnMBR#2. Another reason to explain this failure could be that the initial steady state can have been too short, and thus the micro-organisms not well enough adapted to handle the intense feed. Thus it can be concluded that a slower step-increase is needed to assure the stability of the biological process and avoid failure.

Nevertheless, the membrane performance turned out to be limiting for both conditions as the membrane fouling was too important to maintain sustainable operations.

The use of conductivity as stability indicators still needs some improvement. A reliable benchmark need to be found in order to prevent the failure of the system.

Following conclusions can be made concerning the use of ambient anaerobic membrane bioreactor:

- A successful increase of OLR was achieved using step-increases and stability indicators as monitoring tools.
- The optimal OLR increase rate for AnMBR working at ambient temperature (25°C) should lay between the two experiments that has been done in this thesis.
- Membrane fouling and not microbiological aspects are limiting for a full scale implementation, at least for dairy wastewater and at ambient temperature.
- Stable anaerobic digestion in AnMBR´s can be achieved at a pH of 6.3 at ambient temperature, despite being lower than reported threshold values in literature (6.5 as low limit, Bolzonella, (2011), at least at low OLR).
- Selected stability indicators found out to be powerful monitoring tools to control the anaerobic digestion process.

6 Future studies

Operation of ambient AnMBR is quite a recent field and lots of potential has still to be discovered. Establishing the optimal start-up by continuing the experimental work would be interesting in order to be able to propose full-scale implementation in industry.

Membrane performance should be enhanced before going further with the experimentation. Membrane fouling investigation should continue in order to figure out the reason of the intense membrane fouling and to find solution to decrease it at a sustainable level. Feeding with another substrate, like for example real wastewater, should be tried in order to determine the impact of the type of wastewater in the fouling.

Further investigation should be made at lower temperature in order to reveal operational differences with standard mesophilic anaerobic bioreactors.

Carry out OLR increases with other types of wastewater would also be interesting to collect results leading to the design and operation of full-scale digesters.

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Popular science article

How to recover energy from wastewater treatment? Or how to turn wastewater into gold? Well you have to know that every year, a lot of industries (like slaughterhouse or dairies and beverage industries producers) release a lot of wastewaters into the environment without any valuable reuse. It's a big waste, because wastewater can be valorise. One good way to do it is to produce biogas from it, thanks to the anaerobic digestion (fermentation in a closed environment, without any oxygen). That process allows to recover energy (with the biogas released) from the wastewater treatment, which can also decrease the energy cost!

However anaerobic digesters can't handle large volume of feeding due to the washing out of the micro-organisms producing methane into the outlet. One solution is to use an anaerobic membrane bioreactor, which is a reactor coupled with a membrane that maintain particles and micro-organisms into the reactor, allowing to treat larger volumes of wastewater. Moreover, the use of an anaerobic membrane bioreactor working at ambient temperature (25°C) instead of higher temperatures (37°C) is interesting as it would decrease the energy consumption of the reactor.

Nevertheless, how to start that kind of reactors while keeping the process stable is still unknown. In one hand, a too quick start-up would lead to the failure of the reactor, as the anaerobic digestion would be saturated .In the other hand, a too slow start-up wouldn't be cost-effective. Then a suitable speed of start-up for the wastewater feeding has to be determined.

The aim of the work was to evaluate the stability of the reactor during two different start-ups, using stability indicators found in the scientific literature. Two anaerobic membrane bioreactors were used (see the photo), fed with synthetic wastewater (prepared with milk powder, in order to mimic a real wastewater). One reactor was first used to perform a slow start-up (with several step-increases of the feeding) after a long adaption time for the micro-organisms. The second reactor was used to perform a quick start-up from a short adaption time, in order to figure out if both long adaptation time and slow start-up are really needed in order to avoid the failure of the reactor.

The first reactor fulfilled all the stability indicators during its operation, which means that the reactor has handled the start-up without any failure in the anaerobic process. However, the second reactor didn't meet any of the biological benchmarks; it failed. Finally, for both reactors, fouling of the membrane was found to be limiting in order to continue the start-up. The membrane fouling rate was too high to properly operate the reactors. Reasons for the membrane fouling were investigated (accumulation of fat in the reactor, thickness of the sludge, use of fat-free milk powder), without any relevant results… Investigation must continue! In conclusion, a step-increase start-up of the feeding was successfully carried out while monitored by stability indicators, which found out to be powerful monitoring tools to control the anaerobic digestion process.