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– MASTER OF ENVIRONMENTAL ENGINEERING –

MASTER FINAL PROJECT

Operation of an Anaerobic Membrane Bioreactor at low temperatures

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"No todo lo que cuenta puede ser cuantificado,

y no todo lo que puede ser cuantificado cuenta."

Albert Einstein

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Summary

The growing concern in the development of new intensive technologies, is due to increasingly stringent regulations regarding waste disposal and to reduce energy needs. During vintage, the high organic load of wastewater from the production of wine, favors the application of mesophilic anaerobic processes to convert organic matter into biogas. However, when loads are low during the winter season, anaerobic digestion has been carried out at room temperature.

The main objective of this project is to run an anaerobic membrane bioreactor (AnMBR) fed with synthetic wastewater wine at low temperatures (15ºC and 25ºC) and assess the activity of methanogenic biomass.

During the experiment conducted, the AnMBR shows a good performance for the treatment of these waters, getting a good elimination of organic matter with a low requirement of nutrients. COD removal efficiency was not sufficient to meet the current regulations established. The operation at 25ºC had a better removal of COD than at 15 ºC, 80% and 71%, respectively. A higher acid value was detected when operated at 15ºC. It was obtained of the VFA accumulation on average 132±135 mg VFA/L and 183±135 mg VFA/L at 25ºC and 15ºC, respectively. The flux decline was 2.14 and 3.63 LMH d^{-1} at 25°C and 15°C, respectively, this coincides with the increased removal of organic matter at 25°C. The biogas production at 25ºC was 0.007±0.002 m^3 biogas/$m^3_{reactor}$d and at 15ºC was not possible to determinate experimentally. At 25ºC were favored the methanosaetas spp and methanosarcinas. Instead, only the methanosarcinas were developed at 15ºC.

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1. Introduction

Winery wastewater is an industrial wastewater characterized by its high content in biodegradable organic matter and by a strong seasonal variability. During summer, the production of winery wastewater is high and contains elevated organic matter, but in winter, production decreases and organic matter in the wastewater is low.

Due to the high organic load, anaerobic digestion can be an interesting option to recover energy (biogas) from the wastewater. It is well known that the activity of the methanogenic microorganisms is higher at 35ºC (mesophilic conditions). However, only if winery wastewater has a high organic load (COD over 3 g/L) can be treated at high temperature because the biogas obtained would cover the heating expenses (Basset et al. (2014)). The problem is in the season when winery wastewater contains less COD. The biogas produced is not enough to maintain the bioreactor and it is necessary to add supply external energy.

In recent years, membrane bioreactor (MBR) technology has experienced a huge growth due to its numerous advantages compared to conventional treatments, and it is considered a successful technology for urban and industrial wastewater treatment (B.Lew et al., 2009; Ho and Sung., 2010).

The Membrane BioReactor (MBR) combines the biological degradation of wastewater and membrane filtration. This system has many advantages: it ensures an effluent without suspended solids and colloidal matter; provides effective retention of biomass in the reactor, avoids problems related to filamentous biomass and accomplishes legal requirements with a reduced footprint. Hence, it is obtained a higher quality effluent compared with a conventional activated sludge system.

Despite aerobic MBRs represent the vast majority of the total MBRs installed at full-scale (M. Krawme, et al., 2010), the interest in the AnMBR is increasing because of the advantages of an anaerobic digester combined with a membrane filtration. Conventional anaerobic processes are well-known to achieve high organic matter removal efficiencies without oxygen requirement, low biomass production and energy generation from biogas. However, AnMBR technology enables a wider range of anaerobic digestion possibilities. It has been introduced for industrial application since 1990s for the

treatment of organic waste and industrial wastewater with high organic content from distilleries, septic tanks, food and paper industries, etc. (G. Skouteris et al., 2012).

However, membrane fouling, which causes a reduction in the flux throughout the operation, remains an unavoidable drawback of the anaerobic membrane bioreactor (AnMBR) and limits its widespread application in water treatment (Wang et al., 2008). Another drawback to working with this system are the high costs due membrane replacement and energy consumption.

2. Objectives

The main objective of this work is to test the operation of an AnMBR to treat winery wastewater at 25ºC (psychrophilic temperature) and at 15ºC, simulating winter season. To reach this global objective, the following goals are proposed

- To carry out several anaerobic digestion batch tests to assess the activity of biomass at different temperatures (25ºC and 15ºC).
- To study the quality of the effluent in order to assess its potential to be reused.
- Measure the production of biogas at 25ºC and 15ºC.
- To determine the microorganism population at each temperature.
- To observe the membrane surface after few months of operation.

3. Materials and methods

3.1. AnMBR configuration

As it is shown in *figure 3.1*, a membrane external unit (Orelis, Rayflow Module) with 100 cm^2 of surface was coupled to an anaerobic digester. The digester was a jacketed vessel mechanically stirred at 100 rpm and cooling at 25 and 15ºC by recirculating water from a cooling water bath (HUBER 118A-E). Influent wastewater was feed from a 10-L tank with a winery wastewater. Digester feeding was performed by pressure equilibrium keeping the digester in contact with a 500 mL cylinder at a constant volume of wastewater. Due to the early degradation of COD content (900 – 1700 mg/L)

wastewater was prepared every day. The reactor volume was maintained between 5-6 L. The biogas was quantified with an on-line measuring device (Ritter MGC-1) connected to the headspace of the digester.

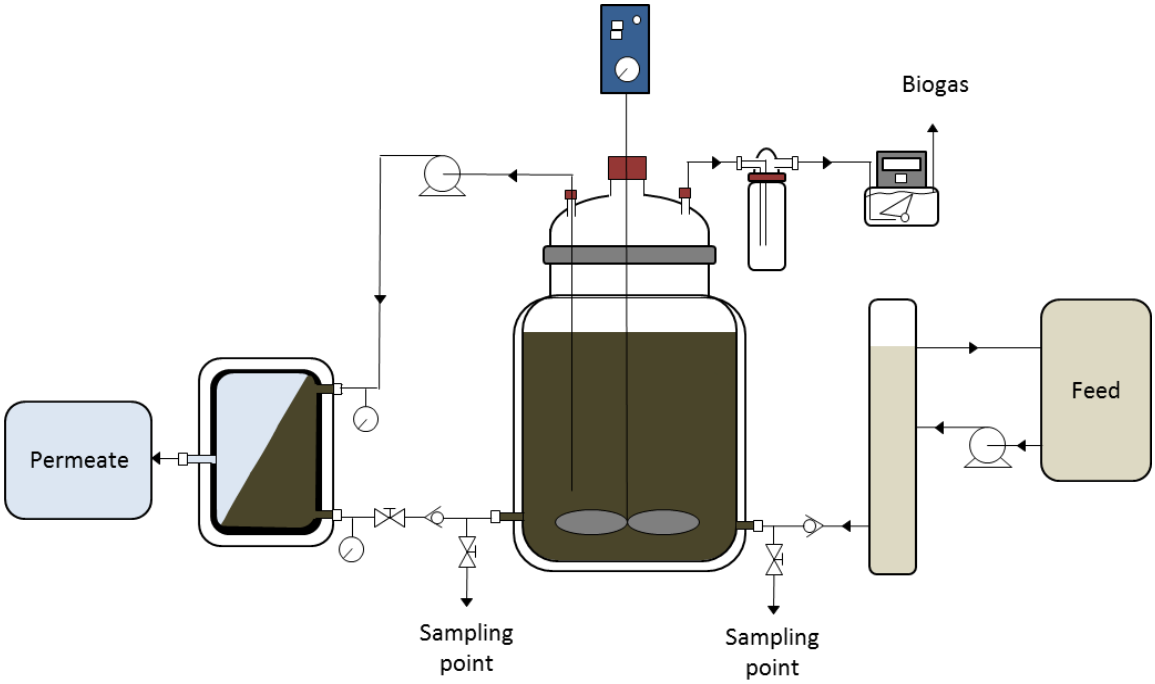


Figure 3.1: *Experimental anaerobic membrane bioreacor (AnMBR)*

The AnMBR system was fed with winery wastewater by a peristaltic pump through pressure equilibrium and connected to an anaerobic digester. The digester's effluent was pumped every 45 minutes for 30 minutes to the microfiltration membrane. The permeate flowed into an effluent tank, and the solids retained were recirculated to the reactor. The reactor was agitated every 15 minutes. *Figure 3.2* shows the bioreactor with communicating vessel (a) and membrane module used (b).

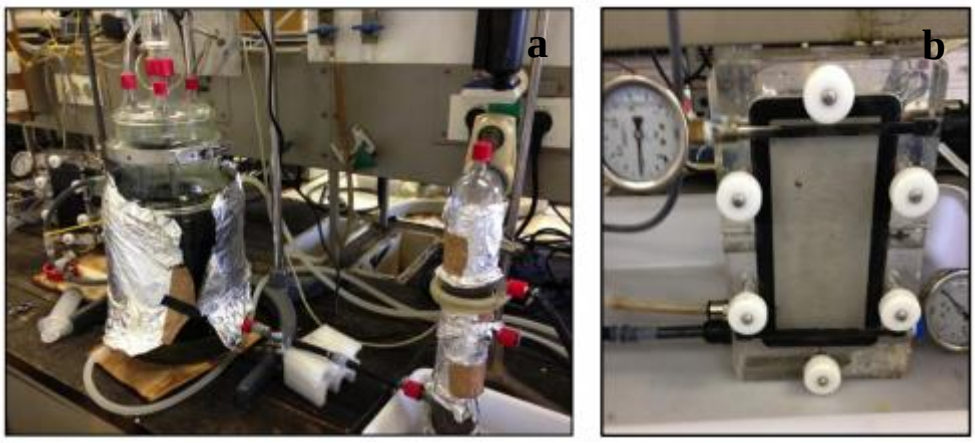


Figure 3.2: *Reactor with communicating vessel (a) and membrane module (b).*

3.2. Synthetic winery wastewater

In the season that the wine production was low, the wastewater contained between 500-800 mg L^{-1} to COD. In accordance with the ratio COD:N:P of 800:5:1 wastewater was prepared with white wine and NH_4Cl and K_2HPO_4 to provide nutrients. Moreover, alkalinity ($NaHCO_3$) was added to keep the pH a neutral values.

3.3. Analytical methods

The analytical methods used in this work were performed according to the *Standard Methods for the examination of Water and Wastewater* (APHA, 2005).

3.3.1. Gas chromatography

Individual VFAs (acetic, propionic, butyric, valeric, hexanoic and heptanoic acids) were analysed by a Shimadzu GC-2010+ gas chromatograph (*Figure 3.3*) equipped with a capillary column Nukol (0.53 mm ID; 15 m length) and a flame ionization detector (FID). Specifically, the chromatograph oven temperature program was as follows: increase from 85ºC to 110ºC at 10ºC min^{-1}; increase to 145ºC at 15ºC min^{-1}; increase to 190ºC at 20ºC min^{-1}, and hold 0.10 min. Injector and detector temperature was set at 280ºC and 300ºC, respectively. Carrier gas was helium at a rate of 36.9 mL min^{-1} and 17.6 kPa. Biogas composition as percentage of methane and carbon dioxide was determined by a Shimadzu GC-2010+ gas chromatograph equipped with a capillary column Carboxen 1010 Plot (0.53 mm ID; 30 m length) and a thermal conductivity detector (TCD). The analysis program was as follows: hold 6 min at 40ºC; increase to 230ºC at a rate of 25ºC min^{-1} and hold 2 min at this temperature. Injector and detector temperature was set at 200ºC and 230ºC, respectively. Helium was the carrier gas at 47 mL min^{-1} and 20.4 kPa.

Figure 3.3. Shimadzu GC 2010+

3.3.2. Suspended solids content

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined following the reference methods 2540D and 2540E, respectively. A known volume of sample (V) was filtered through a 1.2 µm Millipore standard filter, previously weighted (W_1). Then, the filter with the TSS was placed at 105°C during 4h, afterwards in a desiccator for 10 minutes and it was weighted (W_2). TSS concentration was calculated according to *Equation 3.1*. Finally, the filter with TSS was introduced at 550°C for 15 minutes, after that in a desiccator for 10 minutes and was weighted (W_3). The VSS were calculated as shown in *Equation 3.2*.

$$TSS\ (g/L) = \frac{W_2(g) - W_1(g)}{V\ (L)} \quad (3.1)$$

$$VSS\ (g/L) = \frac{W_2(g) - W_3(g)}{V\ (L)} \quad (3.2)$$

3.3.3. Chemical oxygen demand (COD)

The COD indicates the quantity of matter present in a wastewater sample that is susceptible to be oxidised. This parameter is expressed as $mgO_2\ L^{-1}$, so that the COD is defined as the quantity of oxygen used in biological and non-biological oxidation of materials in water. The reference method 5220C was the standard method utilized to perform the COD measurement. It consisted on the complete oxidation of the matter in a liquid sample with a strong oxidising agent under acidic conditions, by means of potassium dichromate and sulphuric acid (with silver and mercuric sulphate). *Equation 3.3* shows the reaction of potassium dichromate with organic compounds. Silver sulphate was used to catalyse the reaction and mercuric sulphate to avoid the interference of chloride (*Equation 3.4*).

$$C_nH_aO_bN_c + \left(\frac{2n}{3} + \frac{a}{6} - \frac{b}{3} - \frac{c}{2}\right) Cr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \left(\frac{a + 8d - 3c}{2}\right) H_2O + cNH_4^+ + 2dCr^{3+} \quad (3.3)$$

$$6Cl^- + Cr_2O_7^{2-} + 14H^+ \rightarrow 3Cl_2 + 2Cr^{3+} + 7H_2O \quad (3.4)$$

Following the guidelines of the reference method, 2.5 mL of the wastewater sample were mixed with 1.5 mL of sodium dichromate 0.04 mol/L (with 80 g/L of mercuric sulphate) and 3.5 mL of sulphuric acid (with 10 g/L of silver sulphate). Together with the samples, 5 standards of potassium biphtalate with 0, 50, 250, 500 and 1000 mg COD/L were analysed to determine the calibration curve. The samples were maintained at 150ºC during 2h in a digester (*Figure 3.4a*) to ensure the complete reaction. After the digestion, the samples were removed from the digester to cool down and to allow the solids formed to settle at room temperature. Finally, the absorbance at λ = 620 nm of the COD samples was measured by means of a spectrophotometer Shimadzu UV-1203 (*Figure 3.4b*).

(a) (b)

Figure 3.4: *COD digester (a) and spectrophotometer Shimadzu UV-1203 (b)*

The results of absorbance from the standards were used to obtain a correlation between concentration of COD and absorbance, therefore the COD of the samples was calculated as shown in *Equation 3.5*.

$$COD\ (mg\ O_2/L) = a \times ABS + b \tag{3.5}$$

3.3.4. Alkalinity

Alkalinity is a measure of the buffering capacity of a water or wastewater to neutralise acids. The buffering capacity of a wastewater is mainly related to the bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) content. However, the presence of other buffering substances such as hydroxide (OH^-), borates, silicates, phosphates, ammonium, sulphides and organic ligands can also provide alkalinity to the wastewater.

The alkalinity was measured using an automatic titration device (CRISON pH Burette 24) equipped with a pH meter (CRISON Basic 20) (*Figure 3.5*). The method consists in a titration into a 25 mL of sample with standard acid (HCl 0.1) to desired end point.

Figure 3.5 : *Automatic titration device (CRISON pH Burette 24)*

The alkalinity, expressed as mg $CaCO_3$ L^{-1}, is calculated with the *Equation 3.7.*

$$Alkalinity\ (mg\ CaCO_3/L) = \frac{mL_{HCl} \times 0.1N \times 50.000}{mL_{sample}} \qquad (3.7)$$

3.4. Biomethane potential test

The Biomethane potential (BMP) test can be used as an index of the anaerobic biodegradation potential as it is the experimental value of the maximum quantity of methane produced per gram of COD added. The BMP is measured with the BMP test, which consists in measuring the bio-methane or biogas produced by a known quantity of waste in batch and anaerobic conditions. An organic substrate is mixed with an anaerobic inoculum at psychrophilic temperature (25ºC and 15ºC) following the procedure defined in VDI 4630 and Angelidaki et al., 2009, and the methane in biogas is quantified by a gas chromatography.

3.5. Fluorescence in situ hybridization (FISH)

The different populations of microorganisms present in the sludge samples of the reactors were studied by means of fluorescent in situ hybridization (FISH). It is a powerful molecular tool for rapid, reliable and cultivation-independent monitoring of

phylogenetically defined bacterial populations in environmental samples. With this technique, specific regions in 23S or 16S rRNA are detected with fluorescently labelled probes. If the corresponding domain, phylum, genus or species is present, the probe hybridizes to the targeted sequence and can later be detected microscopically. The procedure described by Amann (1995) includes the fixation and permeabilization of the sample, hybridization of the targeted sequence to the probe, washing steps to remove unbound probe and finally, the detection of labelled cells by microscopy.

3.6. Scanning Electron Microscope (SEM)

The membrane surface (new and after months of operation) was observed in more detail using scanning electron microscopy (SEM). This technique uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition or and crystalline structure.

4. Results and discussion

4.1. AnMBR performance at 25 and 15ºC

The AnMBR was operated during two experimental periods, to 25ºC and 15ºC. In *table 4.I* the main parameters and results obtained at different temperatures are summarised. During 50 days, the AnMBR was operated at 25 ºC, and at 15ºC during 60 days. More COD removal efficiency was obtained at 25ºC (80% vs 71% at 15ºC). However, since the kinetics are slower (Lettinga et al., 2001), the risk of acidification is higher, being the VFA accumulation on average 132±135 mg VFA/L and 183±135 mg VFA/L at 25ºC and 15ºC, respectively. In spite of the accumulated acids, the pH was maintained neutral at both temperatures. The alkalinity added to the system should be enough to maintain a stable pH when high amount of VFA were accumulated. With the reactor operation at 15ºC, the alkalinity was on average 915±71 $mgCaCO_3$ /L, a little more than at 25ºC. The HRT is similar in both temperatures, around 4 days.

The flux was on average 12.2 and 13.8 LMH at 25ºC and 15ºC, respectively. And the flux decline was 2.14 and 3.63 LMH/d at 25°C and 15°C, respectively. These values was much more higher than the 0.10 LMH d^{-1} observed at 35°C (Basset et al. 2014).

Operational parameters		
Tempearture	**25°C**	**15°C**
Type of wastewater	Synthetic	Synthetic
pH	7.4±0.2	7.5±0.2
Alkalinity ($mgCaCO_3\ L^{-1}$)	898±179	915±71
MLSS ($g\ L^{-1}$)	2.69±1.16	2.74±0.34
HRT (d)	4.4±1.4	4.2±2.0
SRT (d)	435	565
COD influent ($g\ L^{-1}$)	1.41±0.39	1.10±0.30
COD effluent ($g\ L^{-1}$)	0.28±0.14	0.39±0.15
VFA effluent ($mg\ L^{-1}$)	132±105	183±135
%COD removal	80±9	71±9
OLR ($kgCOD\ m^{-3}_{digester}\ d^{-1}$)	0.32±0.18	0.29±0.21
sOLR ($kgCOD\ kg^{-1}MLSS\ d^{-1}$)	0.13±0.09	0.11±0.07
Membrane performance		
Flux (LMH)	12.2±4.4	13.8±6.8
Flux decline ($LMH\ d^{-1}$)	2.14±1.62	3.36±1.03
Biogas production		
P_B ($m^3_{biogas}\ m^{-3}_{digester}\ d^{-1}$)	0.007±0.002	-
$\%CH_4$ in biogás	83±3	81±1
SMP ($m^3CH_4\ kg^{-1}COD$)	0.03±0.01	-

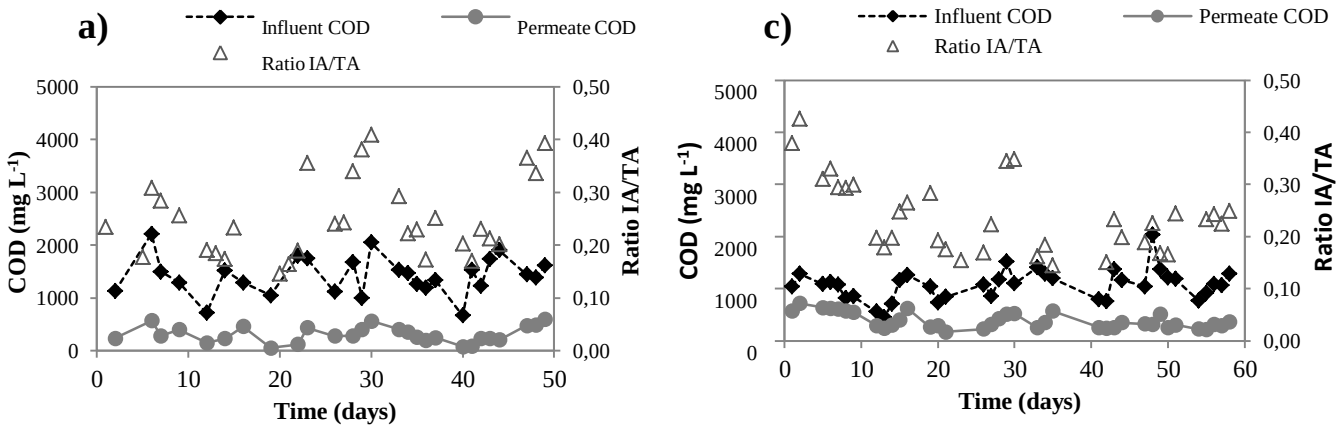
Table 4.I: *Operational parameters to AnMBR, membrane performance and biogás production at 25ºC and 15ºC.*

The following graphics show the relation between COD content in the influent with the accumulation of VFAs in the reactor, and how this affect to the elimination COD. Ratio IA/TA indicates the risk of acidification reactor. In the same study at 35ºC, it was found that when de ratio was greater than 0.3, there was risk of acidification (Basset et al. 2014). As it shown the graphics a and c, high ratios IA/TA values correspond with high influent COD. This means that the reactor had accumulation of acids. Acid accumulation occurred because the acetogenic microorganisms degrade faster the organic matter, but methanogenic microorganisms cannot convert acids into methane at the same rate. This was related with the removal of COD. In b and d graphics, removal of COD versus acids accumulation is represented. When the VFA values were high, the COD removal was low. The picks of removal of COD coincide with low VFA values (<250 mg/L) and with a low ratio IA/TA (<0.3).

When the AnMBR was operated at 25ºC, during the first month, the removal efficiency was very variable due to the accumulation of VFAs that led to an increase in the effluent COD. One of the main reasons for the VFAs accumulation was the increase in the influent COD. Since the synthetic winery wastewater was easily degraded in the feeding tank, every time that it was refilled with new wastewater, its COD increased and VFAs were accumulated in the digester. For this reason, synthetic winery wastewater was then prepared daily, achieving a more constant COD removal (from day 35 on) shown in *figure 4.1a*.

The alkalinity added to the system was enough to maintain a stable pH when high amount of VFA were accumulated. By keeping a ratio between intermediate and total alkalinity (IA/TA) below 0.3 the stable operation was assured. However, as shown in *figure 4.1a and 4.1c*, the day 30 VFA were accumulated up to 400 mg/L, thus the IA/TA ratio increased to 0.4 and the removal efficiency decreased to 68%.

After the period at 25ºC, the temperature was decreased to 15ºC. In *figure 4.1b and 4.1d*, it can be clearly observed that an acclimation period of around 15 days was required to achieve acceptable removal percentages. Since VFA were accumulated easily during this acclimation period, the influent COD was decreased to 500 mgCOD L^{-1}, and progressively increased to 1500 mgCOD L^{-1} from day 15 to 20. As shown in *figure 4.1b and 4.1d*, the day 30 VFA were accumulated up to 350 mg/L, thus the IA/TA ratio increased to 0.35 and the removal efficiency decreased to 55%. After this, it can be observed a stabilization process.



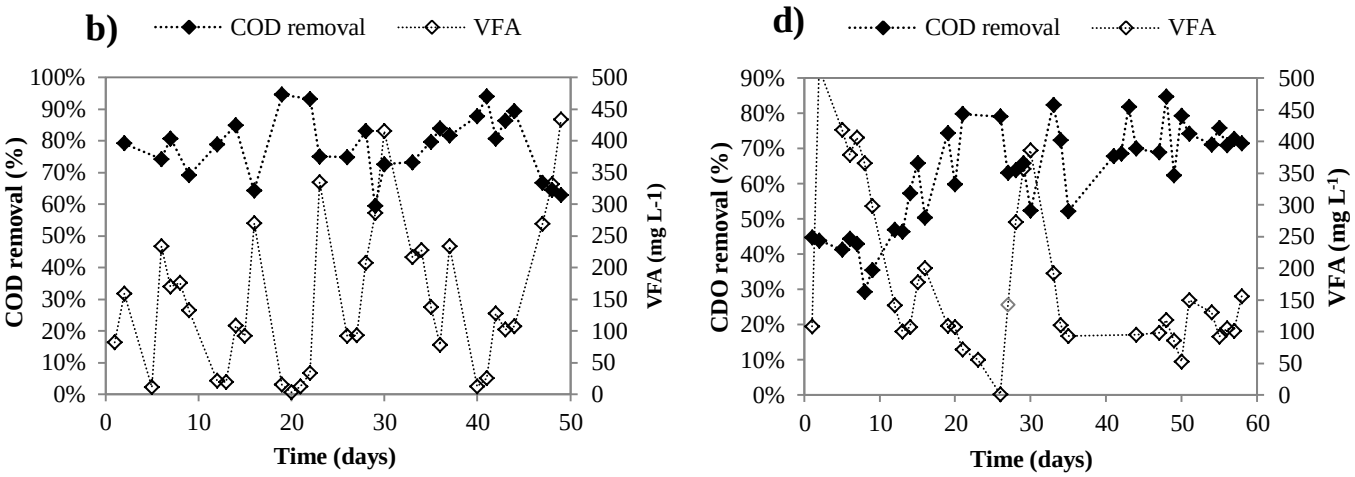


Figure 4.1: *Influent and Permeate COD vs Ratio IA/TA at 25ºC (a) and at 15ºC (c), and COD removal vs VFA at 25ºC (b) and at 15ºC (d).*

Regarding the membrane performance, the following graphics show that the flux and organic load have a directly proportional variation. The permeate flux decreased due to the membrane fouling. *Figure 4.2* shows the variation of the flux due to the cleaning of the membrane. In order to maintain a similar flux, around 15LMH, cleanings were required more often because the flux decline was 3.63 and 2.14 LMH d^{-1} at 15°C and 25°C, respectively. They were carried out when the flow observed was below 10LMH. Cleanings were performed with distilled water at a high crossflow velocity. By applying only clear water to remove the cake layer, the flux afterwards increased significantly as shown in *figure 4.2* promoting VFA accumulation.

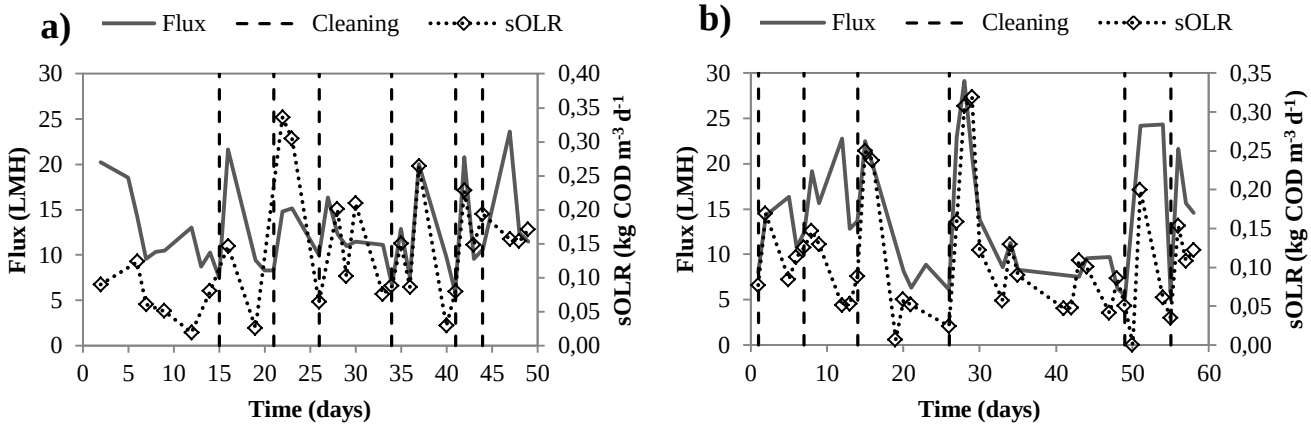


Figure 4.2: *Flux and sOLR of the AnMBR at 25ºC (a) and at 15ºC (b).*

The specific methane production (SMP) at 25°C was very low 0.03 m^3CH_4 kg^{-1}COD and at 15°C was not possible to determine. It is well known that is preferable to operate an anaerobic digester at high temperatures (i.e. 35˚C or 55˚C). The biogas production at 25ºC was 0.007±0.002 m3 biogas/m3 digestor d and at 15ºC was not possible to determinate too.

Therefore theoretical methane production at both temperatures was calculated. It is well known that per gram of COD degraded, it is obtained 0.35L of methane, and it was considered that the biogas consisted of 82% methane. Thus the amount of methane and biogas produced in one day per liter of reactor was calculated. The following graphics were obtained.

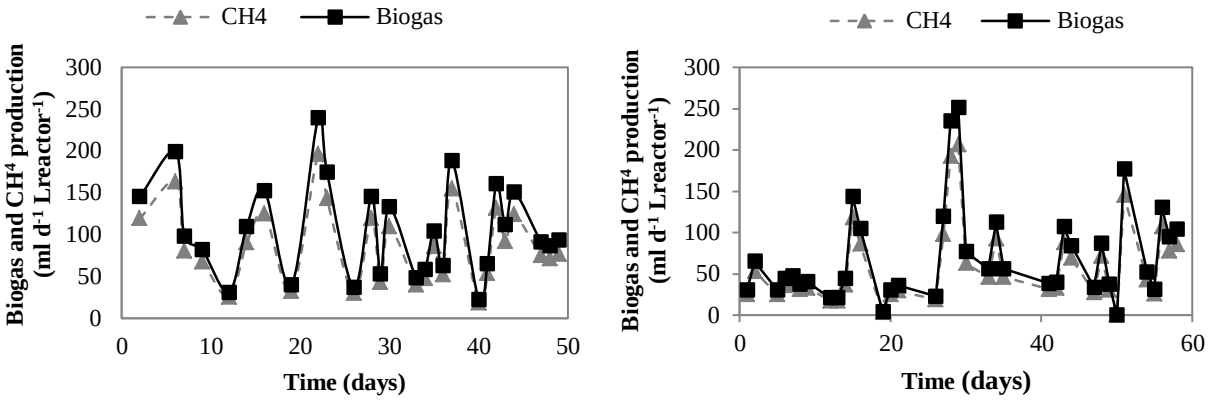


Figure 4.3: *Biogas and CH4 production at 25ºC (a) and at 15ºC (b).*

As a theoretical calculation, the graphics have the same shape as the graph that depicts the removal of organic matter, since the methane produced is calculated from the organic matter degraded.

It was observed that the biogas production was high at 25ºC. At beginning of this period, the production was more variable. From de first week, a production stabilitzation was observed. On day 22, it was obtained a very high production, 240 ml biogas/$L_{reactor}$ d. This data coincide with a important removal of COD. At 15°C, the biogas production was low in general, except on day 20 that 250 ml biogas/$L_{reactor}$ d were obtained.

One hypothesis to explain why not experimentally obtained biogas production can be the overdesign of the headspace of the digester (being a 30% of the total volume), where the biogas production could be accumulated in by the pressure applied by the gas

counter. Therefore, when biogas production was high, the gas counter worked properly. However, when the biogas production was low because the OLR decreased, the pressure inside the headspace was not enough to overcome the liquid column of the gas counter. Samples from the inside of the headspace could be taken by increasing the volume of the digester significantly, thus the gas accumulated in the headspace passed through the gas counter. In this way, the concentration of methane could be determined in both cases being 81% and 83%.

4.2. Biomethane potential test at 25 and 15ºC

Biomethane potential (BMP) tests were performed in order to evaluate biomass activity at different temperatures. In each case, the inoculum used was taken from the AnMBR after operating at the selected temperature for at least 30 days. The specific methanogenic activity (SMA) and the specific methane production (SMP) obtained in the batch tests are presented in *table 4.II*. The SMA and SMP were higher at 25ºC. At 15ºC, the SMA and the SMP were notably lower, as expected due to the low temperature that promoted a poor methane production. The percentages of methane in the biogas obtained decreased from 77% at 25ºC to 66% at 15ºC.

Inoculum	Wastewater	SMA (gCH_4-COD $gVSS^{-1}d^{-1}$)	SMP ($m^3 CH_4\ kg^{-1}COD$)	CH_4 in biogas
AnMBR at 25ºC	Synthetic WW	0.35	0.26	77%
AnMBR at 15ºC	Synthetic WW	0.14	0.09	66%

Tabla 4.II: *SMA and SMP obtained in the AnMBR at low temperatures.*

As it is shown the *figure 4.4*, the specific methanogenic activity was higher at 25ºC. The SMA was calculated from the slope of the first 2 days of SMP vs time, per amount of biomass (gVSS) added as inoculum.

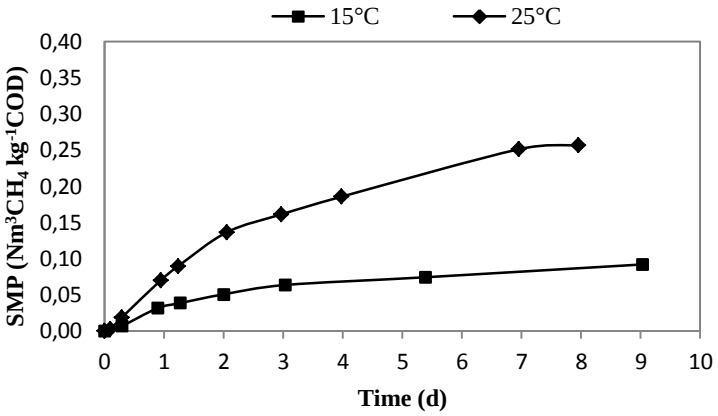


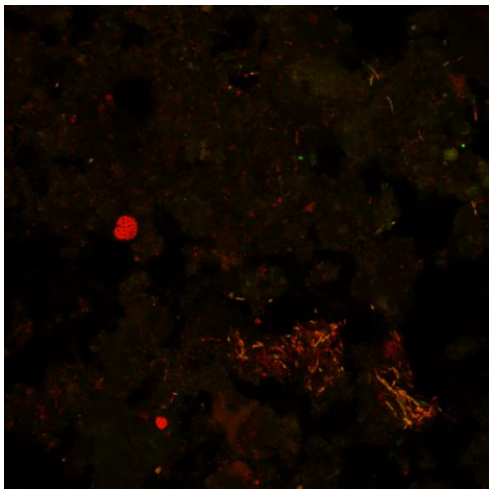
Figure 4.4: *BMP test at 25ºC and 15ºC.*

4.3. Microorganisme population at 25 and 15ºC

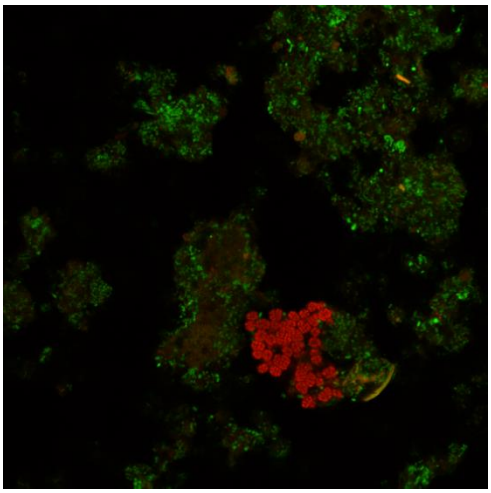
Biological population was determined by fluorescence in situ hybridization (FISH). The specific oligonucleotide probes used were: EUB338 for Bacteria (6-fam); ARC915 for Archaea (Cy3); MX825 for Methanosaeta spp. (6-fam); MS821 for Methanosarcina (Cy3); MG1200b for Methanomicrobiales spp. (6-fam); and MB311 for Methanobacteriales (minus Methanothermus) (Cy3).

Samples at each temperature were taken to determine the changes on the microbial population. At 25ºC, Methanosaeta spp and Methanosarcina were observed (*figure 4.5a and 4.5b*). At 15ºC only Methanosarcina were detected (*figure 4.5c and 4.5d*). The probes of Methanomicrobiales spp. and Methanobacteriales resulted negative in both tempertures.

(a) Archea and Methanosaeta spp. (25°C)



(b) Bacteria and Methanosarcina (25°C)



(c) Archea and Methanosaeta spp. (15°C) (d) Bacteria and Methanosarcina (15°C)

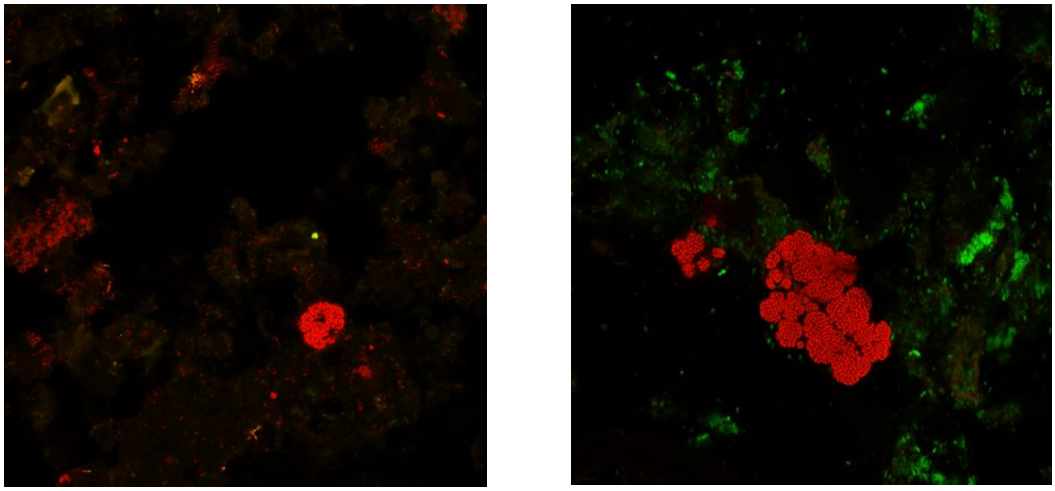


Figura 4.5: *FISH image of overlapping of Archaea (ARC915) and Methanosaeta spp. (MX825) (a) and overlapping of Bacteria (EUB338) and Methanosarcina (MS821) at 25ºC (b); and overlapping of Archaea (ARC915) and Methanosaeta spp. (MX825) (c) and overlapping of Bacteria (EUB338) and Methanosarcina (MS821) at 15ºC (d)*

4.4. Membrane fouling characterisation by SEM

The fouling on the membrane surface was analysed by scanning electron microscopy (SEM). *Figure 4.6* shows the surface of the new membrane (a) and after 3 months of operation at 15ºC (b). It is noted that the surface was completely covered by a cake layer (*Figure 4.6b*), which was the main responsible for flux decline.

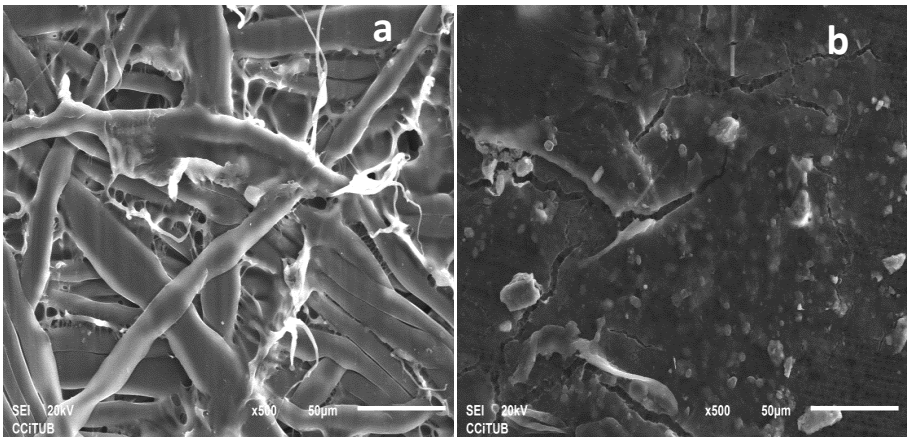


Figure 4.6: *Scanning electron microscopy (SEM) of the new membrane (a) and the membrane after 3 months (b).*

5. Conclusions

In this study, winery wastewater was anaerobically treated with a lab-scale membrane bioreactor at 25ºC and 15ºC. The following conclusions were based on observations during the study:

- At 25ºC and 15ºC was obtained 80% COD removal and without suspended solids and 71%, respectively. Although the effluent quality was expected to be very high due to the membrane filtration, the accumulation of VFA promoted that the effluent COD exceeded the discharge limits.
- AnMBR is able to produce 0.007 m^3 biogas/(m^3 reactor d) containing 83% of methane at 25ºC, and containing 81% of methane at 15ºC. This is a very low production but contain a high concentration of methane. The biogas production in the AnMBR was very low because the biogas was accumulated in the headspace wich was confirmed with BMP test.
- AnMBR can cope with variable influent COD because a ratio IA/TA of 0.3 was reached.
- Frequent membrane cleanings were necessary to maintain a flow around 15LMH, both operating at 25°C as at 15°C.
- The methanogenic activity decreased at low temperatures as expected. SMA obtained at 25º C was higher than SMA obtained at 15ºC. 0.35 gCH_4-COD/(gVSS d) and 0.14 gCH_4-COD/(gVSS d) respectively.
- At 25ºC were favored the methanosaetas spp and methanosarcinas. Instead, only the methanosarcinas were developed at 15ºC. Methanosarcina have the capacity to grow in environments with higher level of VFA.

6. Recommendations

- To test the same experiment at low tempertures but with granular biomass because it is another way to retain the biomass in the reactor.
- Study desorption processes to recover the dissolved methane and evaluate its feasibility.
- To carry out an economical study to determine the feasibility of an AnMBR for winery wastewater treatment.
- To try different membrane configurations to reduce the operational costs.

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