Universitat de Barcelona

Faculty of Chemistry

Department of Chemical Engineering

OPTIMIZATION OF AN ANAEROBIC MEMBRANE BIOREACTOR (AnMBR) TREATING WINERY WASTEWATER

Master's Final Project

Master in Environmental Engineering

Eric Santos Clotas

Directors:

Núria Basset Olivé

Dr. Joan Dosta Parras

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A la meva família i amics, pel suport incondicional que demostren sempre.

Abstract

The interest in anaerobic membrane bioreactors (An-MBR) has increased in the last years due to the several advantages of combining the anaerobic digestion with filtering membrane systems. Moreover, the necessity to reduce energy consumption and space by means of new technologies development in water treatment is of big concern nowadays. The main aim of the present project is to operate an An-MBR with real winery wastewater, with special emphasis on how its COD fluctuations disturb biogas production.

The experimental development of the current study was carried out with real winery wastewater generated during the vintage period, observing COD concentrations between 0.5 and 7.6 g COD L^{-1}, and OLRs from 0.4 up to 3.7 kg COD m^{-3} d^{-1}. Effluent COD concentrations were detected to be low, achieving an average organic matter removal efficiency of 97 % during the 132 days of study.

Biogas production increased progressively according to the organic load applied in each period. Its production varied from 0.04 to 0.3 m^3 CH_4 $m^{-3}_{digester}$ d^{-1}, for COD concentrations from 1 to 5.2 g L^{-1}, respectively. Energy demands were estimated to be 7.5 kWh m^{-3}, and biogas production was able to cover the energy costs when inlet COD was 3,200 mg L^{-1}.

The co-digestion of winery wastewater with reject water from a municipal WWTP was developed, aiming to provide the former with nutrients avoiding external chemicals. An average COD removal of 89 % was achieved treating an OLR of 1.8 kg COD m^{-3} d^{-1}. However, economic data showed that its viability would be fulfilled at distances shorter than 10 km.

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1. Introduction

Wine industry is one of the most important industries that represent the economy of Spain. According to the International Organisation of Vine and Wine, in 2012, Spain appeared to have the 13.4% of the world's total vineyard cultivated extension, making it the third world's producer country with 29.7 million of hectolitres (11.8 % worldwide and 21 % of the EU). It is known that vineyards produce huge volumes of wastewater, mainly from washing operations. This water consume is reported in the range 1–4 m^3/m^3 of wine produced (Andreottola *et al.*, 2002).

1.1 Winery wastewaters characterization

Winery wastewaters (WW) appear to have the particularity that their organic load fluctuates considerably along the year, basically due to the harvesting of wine grapes season (vintage). The organic matter content of WW has been reported to be up to 12.8 kg m^{-3} of Chemical Oxygen Demand (COD) by Petruccioli *et al.*, (2002), in which the main components are sugars and carbohydrates, thereby being very biodegradable (Colin *et al.*, 2005). On the contrary, WW are poor in nutrient content.

This last lack of nutrients makes the biological treatment more complex, since nutrients are indispensable for microorganisms' growth. In many cases nutrients are added externally, increasing the operational costs. Nevertheless, this problem might be corrected by applying a combination of winery wastewater with reject water (co-digestion), so that the addition of synthetic solution of nutrients is not necessary and biomass growth is not limited at the same time. Reject waters from the dewatering of digested sludge in a Wastewater Treatment Plant (WWTP) are frequently found with low COD and high nutrient content, in contrast with WW.

1.2 Winery wastewater management

In order to reduce the costs associated to conventional WWTPs as well as to accomplish the increasingly stringent regulations regarding waste disposal, and reduce energy demands, there has been a growing interest in developing new intensive and compact technologies in wastewater treatment. Several types of technologies have been studied in the winery industry.

Since winery wastewaters have a particular composition and differs seasonally, their direct discharge to the environment without previous treatment might have negative impacts on the consequent ecosystem. This is the case of Portugal, for instance, where many wineries release their wastewaters generated into water bodies (Oliveira *et al.*, 2009).

In Europe, winery wastewaters are commonly discharged in sewers and treated with municipal wastewaters in centralized WWTP (Beck *et al.*, 2005). However, most of the large wineries treat these wastewaters by means of conventional activated sludge (CAS) in aerobic reactors, and then release them to the sewerage system (Torrijos *et al.*, 2006). Although CAS treatments are well known and studied, they present some drawbacks that need to be considered: oxygen consumption, production of sludge, greenhouse effect gases emissions. Moreover, the variability in flow and characteristics, the high organic load during short periods (vintage and harvesting) and the imbalance of nutrients (COD:N ratios) determine problems for the operation of CAS that need to be highlighted (Bolzonella *et al.*, 2010). Hence, the necessity to study other possible treatments to overcome these problems increases.

1.2.1 Aerobic biological processes

Aerobic treatments are more commonly used in wastewater treatment due to its simplicity of the process as well as the quicker growth of the aerobic biomass, and also simpler reactor configurations than anaerobic processes, for instance. On the other hand, the release of greenhouse effect gases, the high sludge production and the oxygen requirements are the main disadvantages of this sort of processes. Power costs associated with the aeration process in typical secondary treatment may run from 30 up to 60 % of the total electrical power used by an ordinary WWTP (Ferrer *et al*., 1998).

1.2.1.1 Sequencing Batch Reactors (SBR)

The SBR system is a modified design of the CAS process and it has been widely used in municipal and industrial wastewater treatment (Mace & Mata-Alvarez 2002). The operation of this reactor is based on a sequence of fill and draw cycles, which generally includes a biological nutrient removal process, and combines aeration and sedimentation-clarification steps.

In the last few years, two different studies have been published studying WW treatment by SBR. Firstly, López-Palau *et al*., (2009) successfully treated up to 15 kg COD m^{-3} d^{-1} with 6 g VSS L^{-1} of aerobic granular sludge in an SBR. Secondly, McIlroy *et al*., (2011), treated daily flows from 30 to 550 kL with averaged COD from 1 (non-vintage) to 16 kg L^{-1} (vintage) within three different full scale plants.

1.2.1.2 Membrane Biological Reactors (MBR)

In the last years, the use of membrane bioreactors has increased as a promising technology, cost efficient and effective in removing a wide range of pollutants. A large number of papers have been published on wastewater treatment through MBR systems at laboratory, pilot plant and full-scale. According to the global market for MBR, the growth rate has been reported to be over 10 % annually due to MBRs effectiveness (Bérubé 2010). Aerobic MBRs combine CAS systems and membrane treatments to remove wastewaters contaminants.

Many publications may be found in the literature that have reported to fulfill effluent requirements with proper performances of MBRs at all scales (Guglielmi *et al.*, 2009; Valderrama *et al.*, 2012), and combining it with advanced oxidation processes (Ioannou *et al.*, 2013).

1.2.2 Anaerobic biological processes

Anaerobic conventional processes are known to highly remove organic matter, reduce sludge generation, recover energy, although they are more complex and generally need further treatments. However, conventional stirred anaerobic reactors are more commonly used for solid waste rather than wastewater.

On the other hand, several anaerobic configurations have been studied for wastewater treatments, highlighting winery wastewater in the present project.

1.2.2.1 Moving Bed Biofilm Reactors (MBBR)

Moving bed biofilm reactor (MBBR) is an effective biological treatment process that incorporates the benefits of both the activated sludge process and a biofilm reactor by the moving carrier element, which provides a large surface for biomass attachment. The movement within the reactor may be carried out by aeration under aerobic conditions or by a mechanical stirrer under anaerobic conditions.

MBBR system has been extensively used in wastewater treatments, yet only few case studies may be found in the literature of this configuration treating winery wastewater. The performance of an anaerobic MBBR with loads up to 18 g COD L^{-1} d^{-1} of WW has been studied by Chai *et al.*, (2013). By removing 40–95 % of organic content in two different reactors, peak conversions from COD to methane over 85 % ware achieved.

1.2.2.2 Up-flow Anaerobic Sludge Blanket (UASB)

The Up-Flow Anaerobic Sludge Blanket is the most widely used high rate anaerobic system for anaerobic sewage treatment worldwide (Karthikeyaan & Kandasamy 2009). The success of this system relies on the establishment of a dense sludge bed in the bottom of the reactor, in which all biological processes take place. Positive and negative aspects of this configuration are quite similar to other anaerobic configurations (Seghezzo *et al.,* 1998).

Winery effluents have been studied in UASB by different authors. Kalyuzhnyi *et al.,* (2000) worked with two laboratory UASB reactors treating diluted vinasse from 0.3 to 7.5 g COD L^{-1} d^{-1} achieving high concentrations of biomass (>30 g VSS L^{-1}), where COD removals were in the range 21-92 %.

1.2.2.3 Anaerobic Membrane Bioreactor (An-MBR)

The interest in Anaerobic Membrane Bioreactors (An-MBR) is increasing due to its advantages of an anaerobic digester combined with a membrane filtration system. Anaerobic digesters are able to remove high percentages of organic matter without oxygen; a low sludge generation and they also obtain energy from the biogas produced (Metcalf & Eddy, 2003). Furthermore, the membrane system allows the retention of the biomass, enabling to decouple the Hydraulic Retention Time (HRT) from the Solids Retention Time (SRT), which would make possible to treat waters with high COD content, up to 25 kg COD m^{-3} d^{-1} (Skouteris *et al*., 2012).

The implementation of an An-MBR with an external membrane would mean no oxygen consumption, which would lead to economical savings. It would also lead to a decrease in sludge production, since the biomass is retained in the membrane and returned to the reactor. And finally, electric energy would be generated from the biogas produced through the anaerobic reactions taking place.

The present project aims to evaluate the efficiency of an An-MBR treating winery wastewater, as well as to co-digest this wastewater with reject water in order to avoid the use of external chemicals to provide nutrients and to test the synergistic effect of combining these two wastewaters. Finally, the viability of co-digesting these wastewaters will also be studied.

2. Justification and objectives

The main objectives of this present project are the operation of a lab-scale anaerobic membrane bioreactor with real winery wastewater and its co-digestion with reject water aiming to supply the lack of nutrients and reduce costs.

In order to achieve the objectives, the steps below are proposed:

1. The **operation of the anaerobic membrane bioreactor**, which is controlled by means of analytical parameters such as pH, temperature, total and soluble COD, ammonium phosphates, total and volatile suspended solids, and volatile fatty acids.
    - Achieve high percentages of organic removal in the effluent, as well as high biogas production.
    - Estimate the energy demands of this configuration according to heat and electrical requirements.
2. The **acclimation of the biomass** in the bioreactor aiming to remove the organic matter of a real winery wastewater. Besides removing the COD, study the daily biogas production according to the load applied, and the methane content.
    - The characterization of the biomass responsible for the methane production by means of microbiological methods.
3. The **performance of an external flat sheet membrane** within the configuration.
    - Determine the flux decline, thereby observing the degree of fouling of the membrane, which would be helpful information so as to decide when the membrane cleaning is required.
    - Characterize the membrane fouling in detail to determine its origin (organic/inorganic).
4. The **co-digestion of winery wastewater and reject water** with the purpose of saving the external chemicals added to balance the nutrients.

3. Materials and methods

3.1 WW feed characterization

The real winery wastewater was collected from a viticulture industry in Vilafranca del Penedès (Barcelona, Spain) in 25 L plastic containers and stored at 4 ºC. Same procedure was carried out for reject water, which was collected from a municipal WWTP close to Barcelona.

Its treatment was divided in several dilutions (5, 4, 3, 2, 1.5) until not diluting the WW at all. These different periods were tested in order to achieve a proper acclimation of the biomass in the reactor and also to study different scenarios concerning to the organic loading rate. This way, periods with low organic content might be simulated as well.

External synthetic nutrients for N (NH_4Cl), and P (K_2HPO_3) were added according to the ratio COD:N:P of 800:5:1 (Moletta, 2005). Alkalinity ($NaHCO_3$) was supplied as well due to the wastewater low pH.

3.2 Bioreactor configuration

The anaerobic MBR system consisted of a communicating vessel of 500 mL (maintained at constant volume) fed by a peristaltic pump through pressure equilibrium and connected to an anaerobic digester of 5 L coupled to an external membrane. The bioreactor was also connected to a biogas counter (Milligas Counter). The digester's effluent was pumped at 15 L h^{-1} to the 100 cm^2 microfiltration membrane (Orelis, Rayflow Module) by a peristaltic pump, where the permeate flowed into an effluent tank, and the solids retained were recirculated to the reactor. Feed was located in a 10 L tank placed in a cool box in order to avoid early degradation.

The bioreactor was maintained at 37 ºC by recirculating water from a heated water bath (HUBER 118A-E). Alkalinity dosages were provided to achieve the acclimation of the biomass, since it is known that methanogenic bacteria have their optimal growth under mesophilic conditions. Moreover, gases solubility increase at low temperatures, and according to Henry's law, CO_2 is more soluble than CH_4, which would help the methane to be released easily, and the CO_2 to stay in the liquid phase.

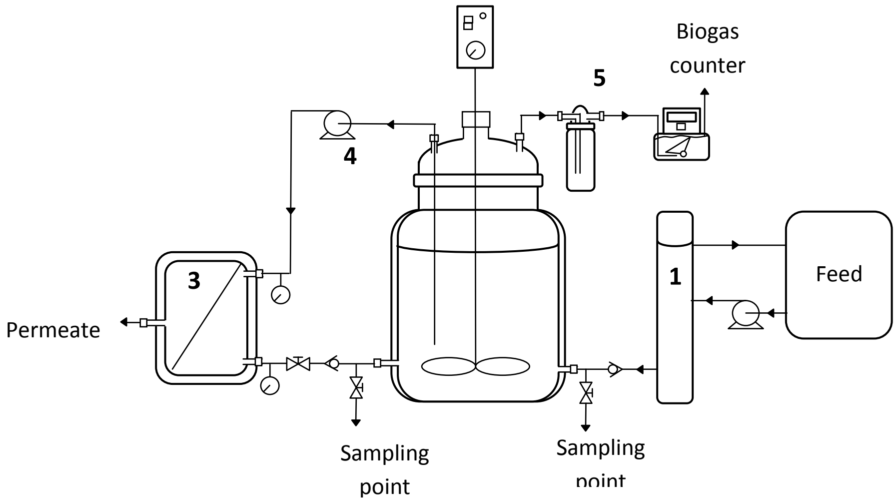

***Figure 1**. Schematic diagram of the lab-scale AnMBR. (1) Feed by pressure equilibrium, (2) AnMBR chamber, (3) Membrane filtration system, (4) Recirculation of retained biomass and (5) Biogas outlet.*

The reactor was also mechanically stirred at 100 rpm during periods of 30 minutes every hour. 15 minutes without agitation were provided before permeation. Permeation period, then, was set to work 15 minutes every hour.

The biomass' acclimation was observed through pH testing, methane production and COD degradation in the effluent, among other parameters.

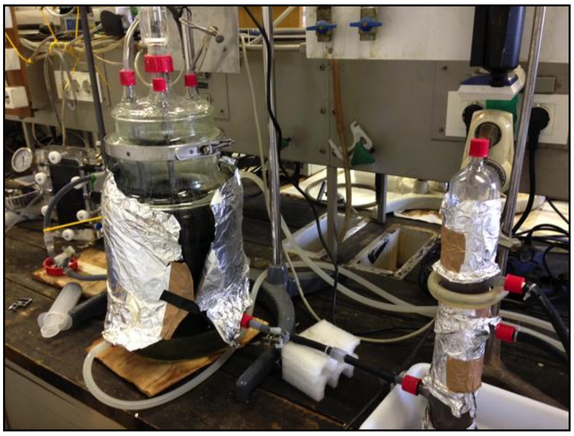

***Figure 2.** Reactor and communicating vessel*

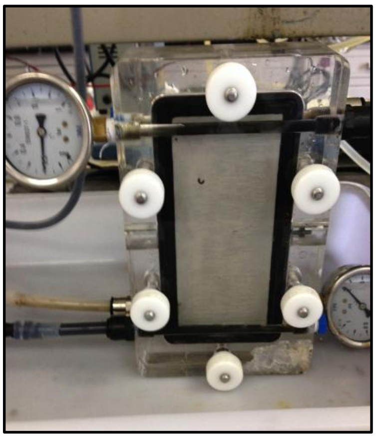

***Figure 3.** Membrane module*

3.3 Analytical methods

Analyses carried out during this project were performed according to the *Standard Methods for the examination of Water and Wastewater* (APHA, 2005) in the laboratories of the University of Barcelona, and in the scientific-technical services of the University of Barcelona.

3.3.1 Chemical Oxygen Demand (COD)

The determination of the total oxygen requirement for both biological and non-biological oxidation of materials was carried out according to the method 5220D of the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). The complete oxidation of the matter in the sample was achieved by the addition of a strong oxidizing agent (potassium dichromate) under acidic conditions (with sulphuric acid). After mixing the samples with all the required agents, and digested at 150 ^oC for 2 hours, the absorbance of the samples was measured by a Spectrophotometer (Shimadzu UV-1203) at a λ=620 nm.

On the other hand, the soluble COD was determined using the same methodology explained above, but first the sample was filtered at 0.45 µm.

3.3.2 Suspended solids content

Total (TSS) and Volatile Suspended Solids (VSS) were determined following the methods 2540D and 2540E of the *Standard Methods* (APHA, 2005), respectively. The procedure was to filter a known volume of wastewater (V) through a 0.45 µm standard filter, previously weighted (W_1). The filter with the TSS was maintained at 105 ^oC for 2 hours, and then weighted (W_2). The weight difference between the filter itself and the filter with the solids ($W_2 – W_1$), and divided by the volume added gives the TSS. For the VSS, the filter with the solids was maintained at 550 ^oC for 20 minutes, and weighted (W_3). The calculation for VSS was the $W_2 – W_3$ divided by the volume (V).

3.3.3 Alkalinity

The buffering capacity of a water or wastewater to neutralize acids, also known as alkalinity, and expressed as mg $CaCO_3$ L^{-1}, was measured in a titrator (pH-Burette 24) that automatically performed the addition of HCl 0.1 N to the wastewater sample.

3.3.4 Volatile Fatty Acids (VFA)

Volatile fatty acids (acetate, propionate, butyrate and valerate) were analysed by a HP 5890-Serie II gas chromatograph

3.3.5 Biogas

Biogas composition was analysed by a Shimadzu GC-2010+ gas chromatograph.

Biogas and methane productions were normalised and reported at normal temperature and pressure conditions (i.e. 0°C and 1 atm).

3.3.6 Fluorescence in-situ hybridization (FISH)

FISH is a cytogenetic technique used to detect the presence of DNA sequences in chromosomes. It consists of chemically preparing a short strand of a specific sequence of nucleic acids, an oligonucleotide, and appending a coloured fluorescent marker at its end. Cells are then made porous to the marked oligonucleotide, which binds to its complementary strand of RNA. After removing the unbound markers, bacteria containing the target genetic material emit light that can be observed under a fluorescent microscope (Henze, 2008).

The development of this technique was carried out according to López-Palau 2012, and fluorescent signals were recorded with a TCS-SP2 confocal laser scanning microscope (Leica, Germany), equipped with a DPSS 561 nm laser for the detection of Cy3 and one Argon ion laser for the detection of 6-fam. The probes applied were MX825 and ARC915.

3.3.7 Membrane fouling determination

The membrane fouling composition was determined by Scanning electron microscopy (SEM), which uses a focused beam of electrons to generate a variety of signals at the surface of solids specimens. The electrons interact with the atoms in the sample and the signals produced reveal information about the morphology and chemical composition in the sample's surface. The examination of the samples, after sputter-coating them with carbon, was developed by an ESEM Quanta 200 FEI, XTE 325/D8395, operating at 20 kV. Moreover, a blank sample was also analysed, which consisted of a piece of the membrane before being used.

3.4 Energy requirements

One of the main and most interesting advantages that present anaerobic digestions is the energy conversion from methane recovery. However, the energy obtained from COD degradation should cover the costs of energy demands from the rector operation (stirrer, pumps, membrane unit, etc). Nevertheless, An-MBR technology is considered to have low energy demands (Lin *et al.,* 2013), compared to CAS and aerobic MBR.

In order to estimate the energy demand of the An-MBR, equations (1) and (2) were used to calculate it by energy balance, considering the net energy production of a combined heat and power (CHP) unit, pumping and stirring requirements, influent heating and heat losses (Astals *et al*. 2012). Biogas production obtained and reactor's operating HRT were considered to calculate the energy production. Moreover, it was also considered the energy consumption of a membrane unit was included in the balance. Energy balance equations are:

$$E_{electricity}(kWh \cdot m^{-3}) = E_{CHPunit} - E_{pumping} - E_{stirring} - E_{membrane\ unit}$$

$$E_{electricity}(kWh \cdot m^{-3}) = [P_B \cdot HRT \cdot \xi \cdot \pi] - \theta - [HRT \cdot \omega] - E_{membrane\ unit} \quad (1)$$

$$E_{heat}(kWh \cdot m^{-3}) = E_{CHPunit} - E_{sludge\ heating}$$

$$E_{heat}(kWh \cdot m^{-3}) = [P_B \cdot HRT \cdot \xi \cdot \psi] - [\rho\gamma(T_d - T_{SS})(1-\varphi)(1+\eta)] \quad (2)$$

Where P_B is the specific biogas production ($m^3\ m^{-3}_{digester}\ d^{-1}$); HRT is the hydraulic retention time (4.5 d); ξ is the biogas heat capacity (4.18×10^4 kJ m^{-3}); π is the CHP efficiency for electricity (0.35); θ is the electrical requirement for pumping wastewater (250 kJ m^{-3}); ω is the electrical requirement for stirring (300 kJ $m^{-3}_{digester}\ d^{-1}$); ψ is the CHP efficiency for heating (0.55); ρ is the influent density (10^3 kg m^{-3}); γ is the influent specific heat (4.18 kJ $kg^{-1}\ {}^{o}C^{-1}$); T_d is the digester temperature (35 ºC); T_{SS} is the influent temperature (15 ºC); φ is the energy recovered from the effluent (0.85); η is the heat loss (0.08).

4. Results and discussion

4.1 Bioreactor operation

The system was operated for 132 days at a temperature of 37 ºC throughout the entire study. The average values for pH in both influent and effluent were 7.2 and 7.4, respectively. Alkalinity was also controlled and maintained at around 700 mg $CaCO_3$ L^{-1}, although it appeared to be increased when raising the organic content. The HRT average value was 4 days, and although it depended on the membrane fouling, no significant variations were observed.

The characterization of the winery wastewater is presented on *Table 1*. Considering these results; the winery wastewater was diluted several times with distilled water in order to acclimate the biomass.

Table 1. *Winery wastewater characterization*

Parameter	Winery wastewater
pH	4.3
Total COD (mg O_2 L^{-1})	6283.4
Soluble COD (mg O_2 L^{-1})	4529.8
Total N (mg N L^{-1})	3.8
Total P (mg P L^{-1})	26.1
COD/N	1684.8
COD/P	240.7

4.1.1 COD removal

The An-MBR operation with the winery wastewater started with a COD concentration of 1,005 mg O_2 L^{-1} during 50 days, which corresponded to a 1:5 dilution with deionized water. The load was increased up to 1,161 mg O_2 L^{-1} for 15 days, 1,360 mg O_2 L^{-1} for one week, 2,814 mg O_2 L^{-1} for 20 days, to 3,280 mg O_2 L^{-1} for 7 days, and finally 20 days for the wastewater without diluting. The purposes of diluting the wastewater were firstly to acclimate the biomass and secondly to study the methane production at low OLR operation conditions. By increasing faster the organic content, a proper acclimation of the microorganisms might not have been achieved and therefore they would not have been able to eliminate the COD.

As shown in *Figure 4,* it can be observed that COD in the influent (feed) increased along time as expected, due to the raise of winery wastewater content. On the other hand, COD in the effluent remained below 29 mg L^{-1} in most cases, which proves a proper degradation by the biomass in the reactor. The highest effluent CODs are found at day 110 (263 mg L^{-1}) and 121

(220 mg L^{-1}), possibly due to a sharp increase in OLR. On the overall, the average COD removal during the 132 days of operation was 97%.

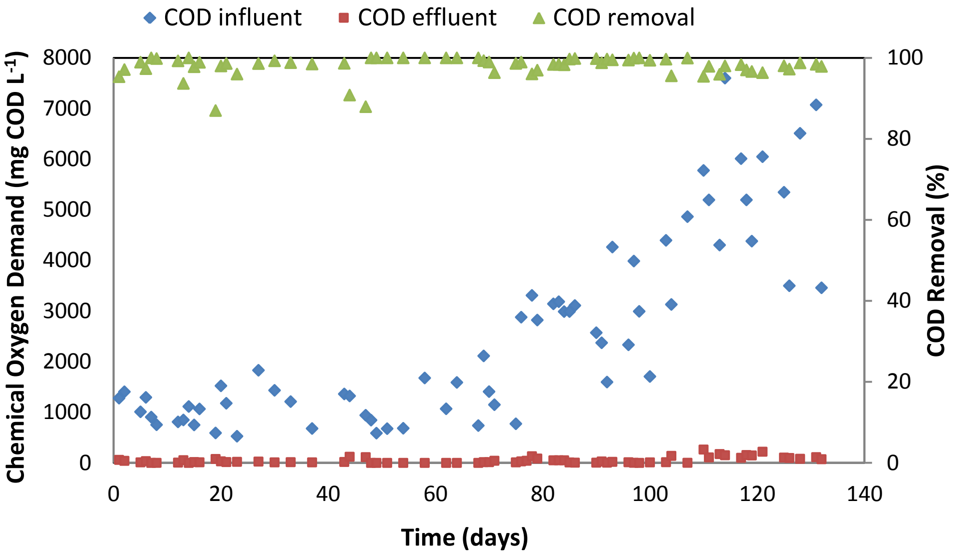

Figure 4. *Performance of the AnMBR during the start-up stage for COD.*

It has also been observed a light decrease in the pH of the influent when increasing the WW content, which is basically due to the acidity of the wastewater. The pH in the first dilution was of 7.67 (SD 0.48), and of 5.83 (SD 0.9) within the no dilution period. On the other side, the pH was 7.60 (SD 0.31) and 7.63 (SD 0.2), respectively, in the effluent stream, which demonstrates an accurate activity from the biomass.

A similar study has been carried out by Wang *et al.,* (2013) in China treating Bamboo industry Wastewater, which has also high COD concentrations, with an An-MBR. Although their configuration was relatively different, they also obtained high removals of the COD (>90%) treating OLR up to 4.4 kg COD m^{-3} d^{-1}.

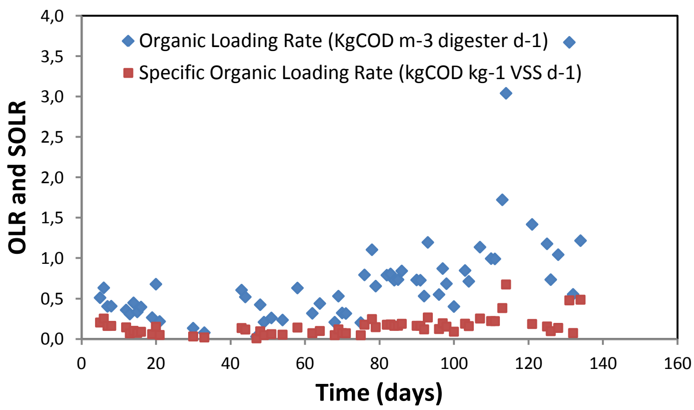

Figure 5*. Variation of OLR and SLR with time.*

The organic loading rate (kg COD m^{-3} digester d^{-1}) and the specific organic loading rate (kg COD kg^{-1} VSS d^{-1}) were also studied throughout this project, as shown in *Figure 5*. A slight increase of both parameters was observed according to the wastewater content applied, being more notorious for the OLR, since VSS remained fairly constant.

OLR values within this project were low compared to results in other studies, as in the case of Torres *et al*., (2011), who treated up to 12 kg COD m^{-3} d^{-1} of brewery wastewater (from beer industry) with an An-MBR. Nonetheless, their specific OLR is low due to a much higher VSS concentration in the reactor (25 g L^{-1}), making an SOLR of 0.48 kg COD kg^{-1}_{VSS} d^{-1}. Similar case was obtained by Saddaud *et al*., (2007) treating 4 kg COD m^{-3} d^{-1} of cheese whey in an An-MBR as well, but with 6.5 g VSS L^{-1}, thereby treating an SOLR of 0.6 kg COD kg^{-1}_{VSS} d^{-1}. In contrast, with the present project, OLR values were found to be low and SOLR, compared to the former, were higher than other studies because of a low concentration of biomass in the reactor. Most of the studies conducted that may seem to the present, treat wastewaters much more concentrated, with influent CODs up to 10 times higher than in the present case.

4.1.2 Methane recovery

The production of biogas was studied throughout the entire operation of the An-MBR, having a production of 300 mL CH_4 g^{-1} COD with an 85% of methane content.

The biogas generation increased as expected with the raise of influent COD content, as it can be observed in both *Figure 6* and 7 below. The different periods were differentiated concerning the dilution of winery wastewater.

***Table 2**. Methane production according to the dilutions established concerning the COD*

Period	Methane daily production		Methane yield	Average COD		Average OLR	
days	mL CH_4 d^{-1}	m^3 CH_4 d^{-1} m^{-3} digester	L CH_4 g^{-1} COD	mg COD L^{-1}	SD	kg COD m^{-3} d^{-1}	SD
0 – 47	64.91	0.01	0.04	1008	686	0.4	0.2
48 – 73	94.35	0.02	0.06	1360	565	0.4	0.2
74 – 91	619.20	0.16	0.21	2814	487	0.8	0.1
92 – 130	1312.68	0.33	0.30	5173	1294	1.2	1

A clear increase of methane production is observed in both figures presented. The daily production of biogas was affected by the oscillations of influent COD, as seen in *Figure 6*. However, considering the accumulated methane production, in *Figure 7*, the differences among periods is clearer. It is observed how the load applied, being more pronounced in the last periods, influences the slope of the profile.

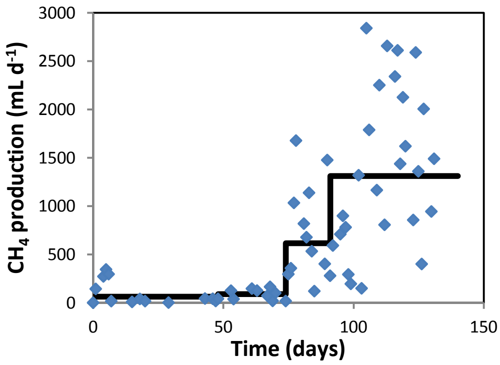

Figure 6. *Daily production of methane*

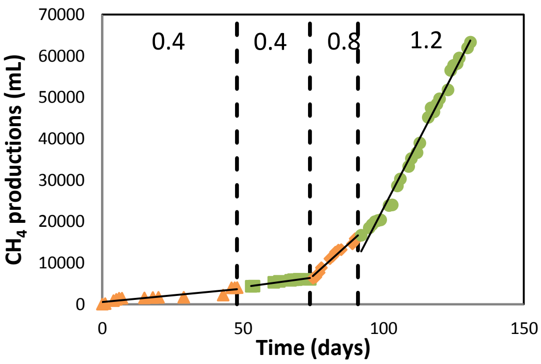

Figure 7. *Accumulated methane production according to the organic loading rate.*

The results shown as daily production in mL CH_4 d^{-1} (*Table 2*) are the slopes between the different profiles in *Figure* 7. This figure demonstrates a significant increase of methane production as the organic content of the wastewater treated was raised throughout the study. At the beginning of the experiment, when the WW was highly diluted, no important differences were noticed in the biogas generated. Nevertheless, in the last stage, when OLR values were as high as 1.2 kg COD m^{-3} d^{-1} being treated, the methane generation reached its peak up to 2840 mL CH_4 d^{-1} (day 105) as the maximum output.

The methane yield expressed as the volume of methane per mass of COD removed (*Table 2*) appeared to increase according to the OLR. In the last period studied the methane yield achieved an average value of 0.3 L CH_4 g^{-1}. Similar results for methane yield have been observed in the literature with comparable configurations (Saddaud *et al*., 2007; Lin *et al.*, 2011), which is generally lower than the theoretical yield (0.382 L CH_4 g^{-1} $COD_{removal}$). This lower observed methane yield would be attributed to high methane solubility reported by Brown *et al*., (2006) or other inhibitors observed by Chen *et al*., (2008). Furthermore, a study carried out by Lettinga *et al*., (1993) attributed more than 50% methane escape in treated effluent to dilute nature of the sewage.

The estimation of the energy costs of this technology has been calculated considering electric demand in the range of 0.3-3.7 kWh m^{-3} from pilot An-MBRs (Martín-Garcia *et al*., 2011), since laboratory scale demands might be unrealistic. The studied configuration presented an energy demand of 2.2 kWh kg^{-1} $COD_{removed}$, for treating an influent COD of 3.2 kg m^{-3}, from which the energy balance became positive. Similar costs were estimated by Basset *et al*., (2014) developing a similar study. Nonetheless, it was reported that 2.02 kWh kg^{-1} $COD_{removed}$ may be produced from an An-MBR treating synthetic wastewater (Van Zyl *et al*., 2008), which is more than the technology needs. It has been demonstrated, though, that immersed membranes have lower energy costs, around 0.3 kWh m^{-3} according to Martínez-García *et al*., (2011).

4.1.3 Membrane performance

As part of the reactor configuration, it was also studied the development of the membrane and the influence of the mixed liquor in its fouling during the 130 days of experimental period.

The chemical cleanings were carried out at days 21, 95 and 130, shown as dashed lines in *Figure 8*. The first period of study presented more process instabilities, which might explain the fact that the membrane's permeability decreased much faster than the other periods. The flux in the second period maintained above 10 L m^{-2} h^{-1} for 74 days. The TSS have been included in *Figure 8*, although its increase may not be related to a more rapid membrane fouling.

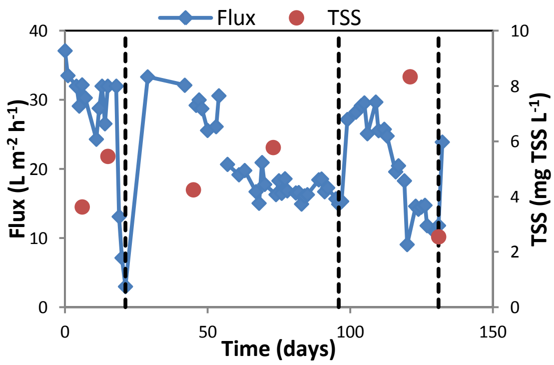

Figure 8. *Flux of the membrane in the operation of the An-MBR. Black dashed lines represent chemical cleanings and red points the TSS.*

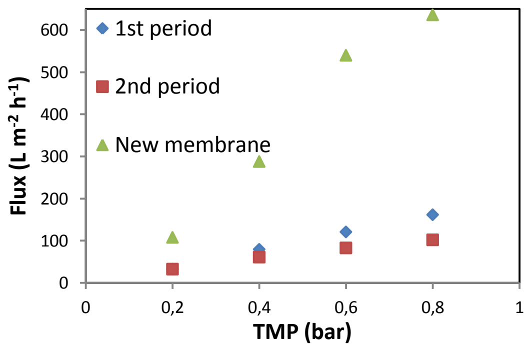

Figure 9. *Effect of the operation with WW on membrane's permeability.*

Apart from the operation with the An-MBR, the flux and permeability of the membrane module with distilled water was studied before and after filtering the mixed liquor according to the transmembrane pressure (TMP) applied. In *Figure 9* the values of flux obtained vs. TMP are depicted after chemical cleanings with NaOH and HNO_3 0.1 N. Green triangles show the flux of the membrane when it was new, and red squares and blue diamonds represent the flux after the first and second chemical cleanings, respectively. A huge difference in the flux was observed after its first use, but after a long time of operation, the permeability did not appear to decrease considerably. These permeability losses may be attributed to irreversible fouling, since chemical cleanings were applied and no improvement in flux was observed.

Being 0.2 bar the operating TMP of the membrane module during the entire study filtering the mixed liquor, it was observed a filtering efficiency drop of 70 % after the first cleaning, in reference to the new membrane after 21 days of study. However, no efficiency decrease was observed after the second chemical cleaning working at 0.2 bar, therefore, proving a total recovery of the permeability, which suggests that the chemical cleaning was effective. On the contrary, it was observed a permeability recovery of 77, 69 and 63 % at 0.4, 0.6 and 0.8 bar of TMP, respectively. Higher permeability recoveries, up to 91 %, were observed by Artiga *et al*., (2005) studying the treatment of winery wastewater with an MBR.

4.1.3.1 Anaerobic biomass and fouling characterization

Both anaerobic biomass suspended in the reactor and retained in the membrane were studied after the operation of the An-MBR by Fluorescence in-situ Hybridization (FISH).

Figure 10 shows that the population of *Methanosaeta spp.*, from the Methanosaetaceae family, dominated as main methane producers. In the left picture (*a*), the overlapping of two probes demonstrates that all Archaea match Methanosaeta, which suggests that no *Methanosarcina spp*. might be found. Nevertheless, a different probe for this latter species was carried out and proved that they were not present. These results seem to coincide with Buntener *et al*., (2013), who also found similar populations treating dairy wastewaters in a UASB and MBR system. A rather low organic load applied, compared to conventional digesters, might be the cause of this lack of Methanosarcina. On the other side, picture *b* shows the biomass retained in the membrane being part of the cake layer. As it was expected, same populations as in the reactor were observed.

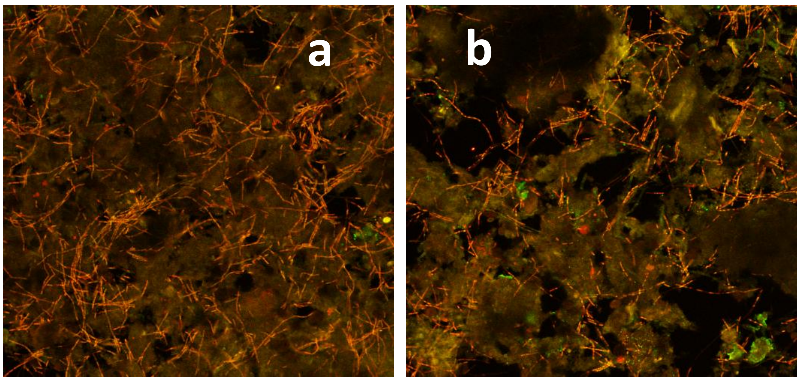

Figure 10. *Fluorescence in-situ hybridization of the anaerobic Archaea with probes MX825 (red) and ARC915 (green) in the reactor (a) and retained in the membrane (b). Orange color represents Methanosaeta as the result of overlapping both probes.*

Furthermore, the fouling of the membrane was studied by Scanning Electron Microscopy (SEM) with a new membrane and after its use filtering the digested winery wastewater and retaining the anaerobic biomass.

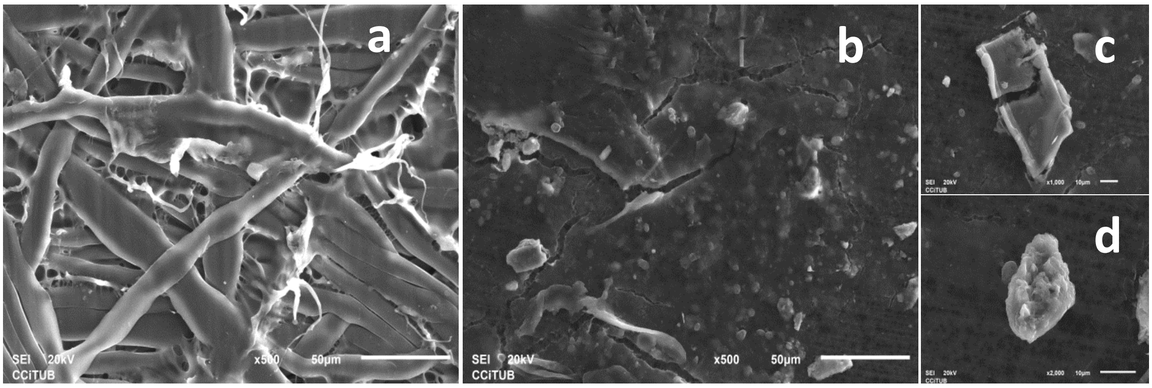

Figure 11. *Scanning electron micrographs of the new membrane (a) and the fouled membrane after the filtration of WW (b, c and d).*

As it can be seen in *Figure 11*, picture *a* shows how the new membrane looked like, where basically organic fibres were observed, the composition of which was C, H and O. Next to it, in picture *b* no fibres were observed anymore, since organic fouling covers it all. Besides, in pictures *c* and *d* inorganic materials were detected, being salts most of them. Some of the elements detected by SEM were Si, Al, Mg, S and K. In order to study and differentiate reversible from irreversible fouling, performing another scanning from the membrane after a chemical cleaning should be considered for further studies.

4.2 Co-digestion

Winery wastewater is found to be low in nutrient content, which may hinder anaerobic biomass activity. Hence, synthetic nutrients must be applied in order to enhance organic matter degradation, and thus biogas generation. In order to save these synthetic solutions, which raise the costs of the plant operation, it has been suggested to study the co-digestion of the WW with any other rich-nutrient water

4.2.1 Characterization of reject water

The supernatant of the centrifuge of a WWTP, also known as the reject water, commonly have high concentrations of nutrients. Its ammonia concentrations would be as high as to provide the WW without diluting it significantly, since decreasing the OLR is not desired. The characterization of both wastewaters and the result of co-digesting them are shown at *Table 3*.

Table 3. *Winery and reject wastewater characterization*

Parameter	Winery wastewater	Reject water	Co-digestion water
pH	4.3	7.7	5.1
Total COD (mg O_2 L^{-1})	6283.4	1163.8	6079.5
Soluble COD (mg O_2 L^{-1})	4529.8	327.8	3143.3
Total N (mg N L^{-1})	3.8	964.5	61.4
Total P (mg P L^{-1})	26.1	20.9	81.3
COD/N	1684.8	1.5	127.4
COD/P	240.7	170.8	229.1

Pertinent calculations were performed and it was estimated that the required ratios, for achieving a COD/N/P of 800/5/1, were 160 for COD/N and 800 for COD/P. The volume ratio required for the co-digestion process was estimated to be 0.04 L_{reject} L^{-1}_{winery}, which would not dilute the organic content (shown in *Table 3*), and also, such low volume of reject water would not have relevant transport costs.

As a result of mixing both waters, an ammonia concentration was successfully achieved to provide the biomass with N. On the contrary, P appeared to be found in excess.

4.2.2 COD removal of the co-digestion

The An-MBR was operated with the combination of WW and reject water for 22 days. Operational conditions were the same as detailed earlier. Alkalinity concentrations were provided as well, since winery water pH is low.

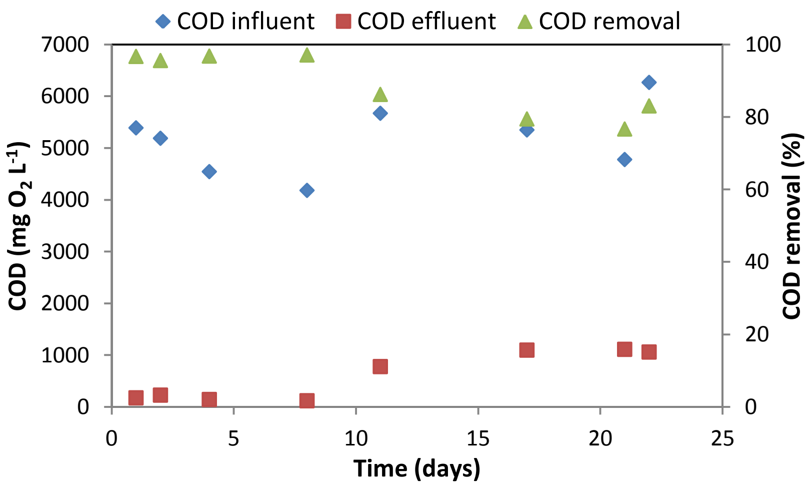

Figure 12. *Performance of the An-MBR for COD removal in co-digestion*

As shown in *Figure 12*, an averaged COD removal of 89 % was achieved by co-digesting both wastewaters with an OLR of 1.8 kg COD m^{-3} d^{-1}. Higher eliminations of organic matter were observed from the beginning of the study until day 11, when biomass concentration decreased down to 1 g VSS L^{-1}. OLR at that moment was of 1.6 kg COD m^{-3} d^{-1}, therefore the specific OLR equaled OLR, which may be considered really high. Moreover, an accumulation of VFA was detected, although pH in the effluent maintained neutral due to high alkalinity.

All these instabilities suggest that the biomass concentration in the reactor was so low that it was unable to remove such high loads of COD. Although this period is still ongoing and the viability of the process cannot be guaranteed, early results obtained suggest a proper degradation of the organic matter pointing to a positive viability of the process.

4.2.3 Viability of the co-digestion

The costs for synthetic nutrient solutions required for the winery wastewater were studied according to COD/N/P ratios for aerobic conventional treatments and anaerobic digestion in the An-MBR. Nutrient costs data come from the winery which water has been studied, and include their transport to the plant. Reject water transportation costs were also estimated according to a certified transportation company in Sant Cugat Sesgarrigues (Barcelona, Spain).

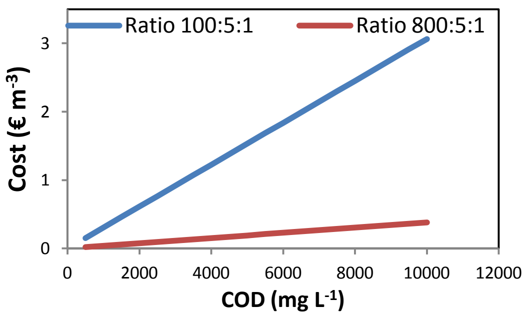

Figure 13. *Nutrient costs according to the influent COD.*

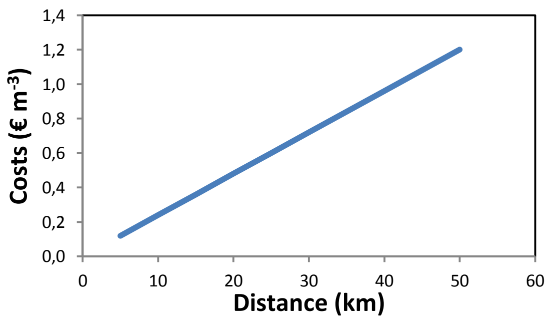

Figure 14. *Reject water transport costs vs. distance*

It may be observed in *Figure 13* how the increase of costs, associated to nutrients, is much more significant for aerobic treatments than for the AnMBR. A difference of almost 2 € m^{-3} to treat an influent COD of 5,500 mg L^{-1}, which is the average COD treated in the experimental stage.

Furthermore, transport costs for reject water were estimated to be 0.024 € m^{-3}_{Winery} km^{-1}, and is represented at *Figure 14* how costs vary depending on the distance (from WWTP to winery). These results show that treating winery wastewater by means of anaerobic MBR is, economically, more viable than aerobic processes. However, they also show that co-digesting the WW with reject water would be equally viable, compared to synthetic solutions, with short distances (<10 km).

5. Conclusions

The operation of an An-MBR treating winery wastewater was investigated as well as its co-digestion with reject water, leading this project to draw the following conclusions.

Operation of the lab-scale An-MBR

- The total COD removal was maintained above 97 % almost during the entire study, regardless the influent COD fluctuations.
- The daily biogas production increased according to the OLR applied, although no difference in methane recovery was observed with low loads.
- Energy demands were estimated to be significantly high (7.5 kWh m^{-3}), comparing to the literature. However, biogas production was able to cover the energy demands when inlet COD was 3,200 mg L^{-1}.

Acclimation of the biomass

- Biomass in the reactor was acclimated properly to the operational conditions established, demonstrating not only an organic matter degradation, but a high methane production as well.
- COD influent fluctuations did not affect biomass activity, because there was enough alkalinity (1,000 mg L^{-1}) to buffer the system.
- Species from Methanosaetaceae family were found to be the predominant microorganisms responsible for methane production, due to low OLR applied.

Membrane performance

- The flux of the membrane decreased with time after filtering the mixed liquor, although chemical cleanings appeared to be successful in order to maintain membrane's permeability.
- Most of the membrane fouling was determined to come from organic sources. However, some inorganic materials were also detected.

Effect of the co-digestion with reject water

- Further studies are needed in order to guarantee the viability of co-digesting winery wastewater with reject water. Nevertheless, early results in the present project point to positive expectations regarding COD removal, although synthetic nutrient solutions appear to be a more economical alternative if distances are longer than 10 km between the winery and the WWTP.

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