AnMBR technologies (CSTR and UASB type) for winery wastewater treatment at low temperatures

N. Basset*, M.E. De Arana-Sarabia**, C. Vidal*, A. Coll*, I. Fernández*, J. Dosta* and J. Mata-Álvarez*

* Department of Chemical Engineering. University of Barcelona. Martí i Franquès, 1, 6th floor; 08028 Barcelona, Spain. (E-mail: nuria.basset@ub.edu; jdosta@ub.edu; jmata@ub.edu)
** Department of Engineering and Architecture, University of Trieste, via A. Valerio 6, Trieste, Italy (E-mail: MARIAEUGENIA.DEARANASARABIA@phd.units.it)

Abstract
The AnMBR technology has shown good performance for winery wastewater treatment, removing organic matter with low nutrient requirement. Winery wastewater was treated at low temperatures and low organic load simulating winter season conditions.The operation at 25˚C resulted in slightly better COD removal than the operation at 15˚C. By using UASB-MBR configuration higher removal rates and biogas production could be reached mainly due to the capacity to retain more biomass in the reactor compared with the CSTR-MBR, reaching a COD removal efficiency of 92±4% with an effluent COD of 0.11±0.06 mg L^{-1} and free of suspended solids. Due to the operation at low temperatures, up to a 10% of methane was dissolved in the permeate and low biogas production was obtained. Moreover, higher degree of fouling was observed despite the amount of suspended solids was lower. Frequent cleanings were necessary, although they were carried out without chemicals since the main resistance was due to the cake layer on the surface, thus a high crossflow velocity was enough to recover the initial flux.

Keywords: Winery wastewater; anaerobic membrane bioreactor; organic matter removal.

INTRODUCTION

Winery wastewater contains high amount of biodegradable matter that can be valorised into biomethane by means of anaerobic digestion. It is well-known that the kinetics of anaerobic digestion carried out at low temperature, and especially the hydrolysis of particulate organic matter, are slower (Lettinga et al., 2001). An acceptable efficiency of COD removal has been observed at psychrophilic temperatures (about 25°C) when solids are retained in the digester by using technologies as the upflow anaerobic sludge blanket (UASB) or the anaerobic membrane bioreactor (AnMBR) (Bandara et al., 2012). The AnMBR configured as a continuous stirred tank reactor (CSTR) presents the advantage of well mixing that promotes a high hydrolysis rate thus increasing biogas production. However, the mixed liquor with high solid concentration is directly in contact with the membrane, favouring the solid attachment on its surface. With the growing application of AnMBR for urban and industrial wastewater treatment, the conventional UASB has gained interest especially for wastewater treatment at low temperature (Ozgun et al., 2013). Despite AnMBR has several advantages in comparison with UASB, as the shorter start-up periods and the higher effluent quality, factors such as membrane fouling and high capital and operational costs may limit the application of the AnMBR.

The interest in the evaluation and comparison of the CSTR-MBR configuration with other types of anaerobic digestion technologies lies on its limitation of amount of biomass retained. If higher biomass could be retain, the treatment capacity would improve. For this reason, the combination of UASB-MBR appears as an interesting option due to the retention of biomass by means of good settling properties so that a higher amount of biomass could be reached and the mixed liquor in contact with the membrane would contain much less solid concentration (An et al., 2009). Although the most of the UASB-MBR examples found are applied to urban wastewater treatment (Cerón-Vivas et al., 2012; Salazar-Peláez et al., 2011), its application to winery wastewater treatment at low temperature is of interest particularly in winter season when the organic load is low.

Another aspect to bear in mind is the origin of the inoculum that may have a significant impact on the start-up of the AnMBR at low temperature. According to Smith et al. (2012) after 275 days of operation at 15°C the microbial communities were similar comparing a mesophilic and a psychrophilic inoculum, and it was concluded that the communities were mainly formed by psychrotolerant mesophilic microorganisms. Even though the AnMBR worked properly with a psychrophilic inoculum, its efficiency at higher temperatures should be considered since the temperature of wastewater may vary up to 20°C throughout the year (Smith et al., 2013). For this reason, a combination of both communities (psychrophilic and mesophilic) would be worth considering in order to deal with the seasonal variations typical of winery wastewater.

The main goal of this study was to operate an AnMBR (CSTR type coupled with an external membrane unit) fed with synthetic winery wastewater at low temperatures (15 and 25°C) evaluating its acclimation capacity, removal efficiency and membrane performance. The operation of an UASB that was further coupled to an external membrane unit was also carried out, discussing their advantages and drawbacks. It should be taken into account that at low temperatures, biogas tends to dissolve in the liquid phase, thus the loss of methane should be quantified in order to evaluate its impact on the overall biogas production. The evolution of the microbial population was also of interest, especially because the inoculums of the CSTR and the UASB had mesophilic and psychrophilic origins, respectively.

MATERIAL AND METHODS

Experimental set-up

Continuous stirred tank reactor set-up. The CSTR-MBR was set-up as a conventional stirred anaerobic digester of 5 L coupled with an external membrane unit (Orelis, Rayflow Module) of 100 cm^2 of membrane area. The digester was a jacketed vessel mechanically stirred at 100 rpm and heated at 35°C by recirculating water from a heated water bath (HUBER 118A-E). Influent wastewater was fed from a 10 L tank with a cooling system to avoid early degradation. However, significant oscillations in COD concentration were observed, therefore wastewater was prepared every 1-2 days. Digester feeding was performed by pressure equilibrium keeping the digester in contact with a 500 mL cylinder at a constant volume of wastewater. Thus, the working volume inside the digester was kept at 4.5 L. Since the membrane unit was placed outside the digester, biogas was quantified with an on-line measuring device (Ritter MGC-1) connected to the headspace of the digester.

Upflow anaerobic sludge blanket set-up. The UASB was set up as a glass tubular reactor of 1.5 L fed continuously by a peristaltic pump with a HRT of 16h. The height to diameter ratio was H/D=3.5, which favoured the washout of the biomass with poor settling properties and granular biomass was kept inside the reactor. The UASB was inoculated with granular anaerobic biomass filling about the 50% of the volume. The biogas was collected in the upper part. The gas collector was connected to an on-line measuring device (Ritter MGC-1). The UASB was coupled to the external membrane unit previously described. The UASB effluent was collected in an intermediate tank, from where it was pumped through the membrane unit and the retentate was directly returned to the UASB.

Substrate and inoculum

Synthetic wastewater was used to feed the system controlling the amount of the influent COD content. This synthetic wastewater was prepared with diluted white wine (Artiga et al., 2005) and NH_4Cl and K_2HPO_3 that coped the lack of nutrients in accordance to the ratio COD/N/P of 800/5/1 (Moletta, 2005). In addition, alkalinity was added (around 1,000 $mgCaCO_3\ L^{-1}$) to keep the pH at neutral values.The inoculum of the CSTR-MBR came from the mesophilic treatment of real winery wastewater, previously carried out in the same digester. The methanogenic archaea found in the suspended biomass were mainly *Methanosaeta* spp. In contrast, the inoculum of the UASB, which came from a full-scale UASB treating food waste at ambient temperature, was composed by *Methanosaeta* spp. and *Methanomicrobiales.*

Analytical methods

Analyses of COD, TS, VS, TSS, VSS, pH and alkalinity were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Individual volatile fatty acids (VFAs) (acetic, propionic, butyric, valeric, hexanoic and heptanoic acids) were analysed by a Shimadzu GC-2010+ gas chromatograph equipped with a capillary column Nukol (0.53 mm ID; 15 m length) and a flame ionization detector (FID). Biogas composition 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). Biomethane potential (BMP) tests were carried out at mesophilic temperature (35°C) following the procedure defined in VDI 4630 and Angelidaki et al. (2009). Biological population was determined by fluorescence in situ hybridization (FISH) following the procedure of Amann et al. (1990). In anaerobic digestion processes many bacteria species coexist, although the limiting step is usually the methanisation, driven by archaea. For this reason the specific oligonucleotide probes used were: ARC915 for *Archaea* (Cy3); MX825 for *Methanosaeta* spp. (6-fam); MS821 for *Methanosarcina* spp. (Cy3); MG1200b for *Methanomicrobiales* (6-fam); and MB311 for *Methanobacteriales* (minus *Methanothermus*) (Cy3). Fluorescent signal were recorded with TCS-SP2 confocal laser scanning microscope (Leica, Germany) equipped with a DPSS 561nm laser for the detection of Cy3 fluorochrome (red) and a Ar ion laser for 6-fam fluorochrome (green). Two probes were applied in each sample, always combining a Cy3 probe with a 6-fam probe.

RESULTS AND DISCUSSION

The main operational parameters and results obtained in the different anaerobic digestion configurations (CSTR-MBR, UASB and UASB-MBR) at low temperature are summarised in Table 1. Many aspects should be highlighted when anaerobic digestion is carried out at low temperature. Since the kinetics are slower, the risk of acidification is higher (Lettinga et al., 2001). As determined in Basset et al. (2016), to keep a ratio IA/TA below 0.3 would assure a neutral pH, thus the synthetic feed was prepared with a minimum alkalinity of 1,000 mgCaCO$_3$ L^{-1}. In addition, the slower kinetics affected the process efficiency, while at higher temperatures COD removal was over 95% (Basset et al., 2016), at 15°C efficiency decreased to 70%. This effect would not be so negative in a full-scale winey industry, because it presents the advantage that during winter the wastewater flow rate is lower as well as the organic load, thus the operational conditions could be adjusted to achieve the desired efficiency. In the case of the CSTR-MBR, the effluent contained a notable amount of volatile fatty acids (VFA) that provided COD over the discharge limits (0.125 g/L). Only with the combination of UASB-MBR, effluent COD was below the limits, mainly due to a higher amount of biomass in the reactor that maintained the specific organic loading rate (sOLR) lower than the other configurations studied.

The observed biogas production was very low in the case of the CSTR-MBR. Similar results were obtained by Giménez et al. (2011) treating urban wastewater, and they attributed the lack of biogas to the presence of sulphate reducing bacteria that can consume 1 mg of COD to reduce 1.5 mg of sulphate (Gerardi, 2003). In the case of the synthetic winery wastewater, sulphate concentration was very low (ratio COD/SO$_4^{-2}$=166), thus as the influent COD was 1,500 mg COD L^{-1}, only 6 mg COD L^{-1} could be fated to this purpose. Considering that the maximum methane production is 0.35 Nm^{3}CH$_4$ kg$^-$

[1]$COD_{removed}$, which corresponds to the theoretical COD of methane, the specific methane production (SMP) was expected to be closer to these values. However, the loss of methane dissolved in the permeate can play a significant role, especially at low temperatures (McKeown et al., 2012). Considering Henry's law, dissolved methane can be calculated by means of Equation 1, where the pressure in the gas phase is related with the molar concentration in the liquid phase by Henry's constant. The temperature dependence of Henry's constant for methane dissolved in water can be calculated based on Van't Hoff equation, simplified in Equation 2 (Sander, 2015), where $H^*=1.4\cdot10^{-5}$ mol m^{-3} Pa^{-1}; $T^*=298.15$ K and -$\Delta H/R=1{,}600$ K.

$$X_{CH_4}(mol\ m^{-3}) = P_{CH_4}(Pa)\cdot H\ (mol\ m^{-3}Pa^{-1}) \quad (1)$$

$$H(T) = H^*\cdot \exp\left(\frac{-\Delta H}{R}\cdot\left(\frac{1}{T}-\frac{1}{T^*}\right)\right) \quad (2)$$

Table 1. Operational parameters at different reactor configurations and working temperatures

	CSTR-MBR	**CSTR-MBR**	**UASB**	**UASB-MBR**
Temperature	15±2°C	25±2°C	20±6°C	22±5°C
Type of wastewater	Synthetic	Synthetic	Synthetic	Synthetic
pH	7.5±0.2	7.4±0.2	7.4±0.6	8.1±0.3
Alkalinity (mg$CaCO_3$ L^{-1})	915±71	898±179	730±300	954±126
MLSS (g L^{-1})	2.74±0.34	2.69±1.16	36.5±1.0	31.7±0.2
HRT (d)	4.2±2.0	4.4±1.4	0.6±0.2	0.8±0.1
SRT (d)	565	435	790	790
COD influent (g L^{-1})	1.10±0.30	1.41±0.39	3.19±0.40	1.4±0.2
COD effluent (g L^{-1})	0.39±0.15	0.28±0.14	0.49±0.30	0.11±0.06
VFA effluent (mg L^{-1})	183±135	132±105	0.26±0.22	15.5±8.8
%COD removal	71±9	80±9	84±9	92±4
OLR (kgCOD m$^{-3}_{digester}$ d^{-1})	0.29±0.21	0.35±0.19	5.5±1.2	1.6±0.5
sOLR (kgCOD kg^{-1}MLSS d^{-1})	0.11±0.07	0.13±0.09	0.15±0.03	0.052±0.016
Biogas production				
P_B (Nm$^3_{biogas}$ m$^{-3}_{digester}$ d^{-1})	-	0.007±0.002	0.79±0.16	0.24±0.1
%CH_4 in biogas	81±1%	83±3%	94±1%	95±1%
SMP (Nm^3CH_4 kg^{-1}COD)	-	0.03±0.01	0.17±0.03	0.17±0.06
Membrane performance				
Flux (LMH)	13.8±6.8	12.2±4.4	-	7.5±2.3
Flux decline (LMH d^{-1})	3.36±1.03	2.14±1.62	-	1.3±0.8
TMP (bar)	0.2	0.2	-	0.3
Crossflow velocity (m s^{-1})	0.64	0.64	-	0.05

Hence, the amount of methane lost in the permeate of the CSTR-MBR was 18.8 mgCH_4 L^{-1} (75.4 mgCOD L^{-1}) at 25°C, and 22.15 mgCH_4 L^{-1} (88.6 mgCOD L^{-1}) at 15°C. Smith et al. (2013) observed a loss of the 40-50% of methane production operating at 16h of HRT and 15°C. Since the HRT was relatively high (4 d), the rate of dissolved methane was 0.017 and 0.022 kg COD m$^{-3}_{digester}$ d^{-1}, for 25°C and 15°C, thus the amount of methane lost in the liquid phase corresponded to a 6.7% and 10.2%,

respectively. Nevertheless, this fact does not explain the lack of biogas production observed. Another possibility could be the overdesign of the headspace of the digester (being a 30% of the total volume), where the biogas produced could be accumulated in due to the pressure applied by the gas counter. Therefore, when biogas production was high as in Basset et al. (2016), 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 manually increasing the working volume of the digester, 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%.
In contrast, the UASB did not present this lack of biogas. A SMP of 0.17 m^3CH_4 $kg^{-1}COD$ was obtained in the UASB and the UASB-MBR. Nevertheless, higher SMP were expected in the case of the UASB-MBR because the biodegradation efficiency was over 90% and the percentage of methane in biogas was 95%. Once more, the low temperature of operation promoted the sorption of methane in the liquid phase. By applying Henry's law considering the operational parameters (HRT, OLR, COD removal, methane percentage), it can be calculated that an 8% of the methane production in terms of kgCOD $m^{-3}_{digester}$ d^{-1} was lost in the permeate of the UASB-MBR.

CSTR-MBR operation at low temperature

The temperature of the CSTR-MBR was reduced from 35°C (Basset et al., 2016) to 25°C and then to 15°C, thus the inoculum was not acclimatised at these low temperatures. In fact, this would be the procedure in a real winery. In summer, the temperature of operation could be 35°C to cope with the high organic load and recover a significant amount of biogas from it, as determined in Basset et al. (2014). However, in winter, since the organic load is much lower, temperature would decrease progressively, because the biogas produced would not cover the heating requirements. In order to reach acceptable removal efficiencies, the HRT was relatively long, of 4.5 days. However, average effluent COD was higher than the standard limits due to the VFA accumulated, obtaining 183±135 mg L^{-1} and 132±105 mg L^{-1} at 15°C and 25°C, respectively.

CSTR-MBR operation at 25°C. The AnMBR was operated during 45 days at 25°C. The COD removal efficiency was on average 80±9%. Since winery wastewater contained easily biodegradable COD, the removal efficiency decreased due to occasional VFA accumulation, mainly composed by acetic acid, being on average 132±105 mgVFA L^{-1}. The alkalinity added to the system was enough to maintain a stable pH when high amount of VFA were produced. By keeping a ratio between intermediate and total alkalinity (IA/TA) below 0.3, the neutral conditions were assured. However, as shown in Figure 1, on day 30 VFA were 416 mg L^{-1}, thus the IA/TA ratio increased to 0.4 and the removal efficiency decreased to 59%. The specific organic loading rate (sOLR) suffered huge variations because of the influent COD and the decrease in flux caused by the cake layer on the membrane surface. Hence, after a cleaning, the flux increased significantly and so the sOLR. For this reason, the sOLR slope presented a similar shape as the influent COD, although more abrupt oscillations were obtained as a result of the flux influence.

CSTR-MBR operation at 15°C. After the period at 25°C, the temperature was decreased to 15°C. In Figure 1, it can be observed that an acclimation period of around 15 days was required to achieve acceptable removal percentages (from day 45 to 60). Since VFA were accumulated easily during this acclimation period, the influent COD was decreased to 500 mgCOD L^{-1}, and progressively increased to 1,500 mgCOD L^{-1} from day 60 to 65. The average COD removal efficiency from day 65 on was 71±9%. The lower efficiency compared with the previous period was mainly due to a higher amount of VFA accumulated. Since the kinetics of methanogenic archaea decreases significantly at lower temperatures, VFA tended to accumulate faster. Which indicated that the bacteria responsible for VFA production were not as affected by the low temperature as the methanogenic archaea. Despite VFA were present in the mixed liquor, pH was maintained at neutral values and the ratio IA/TA below 0.3 by keeping an alkalinity concentration in the AnMBR of 915±71 $mgCaCO_3\ L^{-1}$. Only during the first 15 days, the ratio IA/TA was between 0.35 and 0.4, because VFA reached 515 mg L^{-1}, fact that warned about the possible acidification and for this reason influent COD was decreased during those days. In addition, on day 76, a peak of 386 mgVFA L^{-1} was observed because the membrane cleaning caused a huge increase in the flux up to 30 LMH and thus the sOLR reached 0.32 kgCOD kg^{-1}MLSS d^{-1}. However, the flux rapidly decreased due to the cake layer formed as well as VFA concentration, recovering a more stable operation.

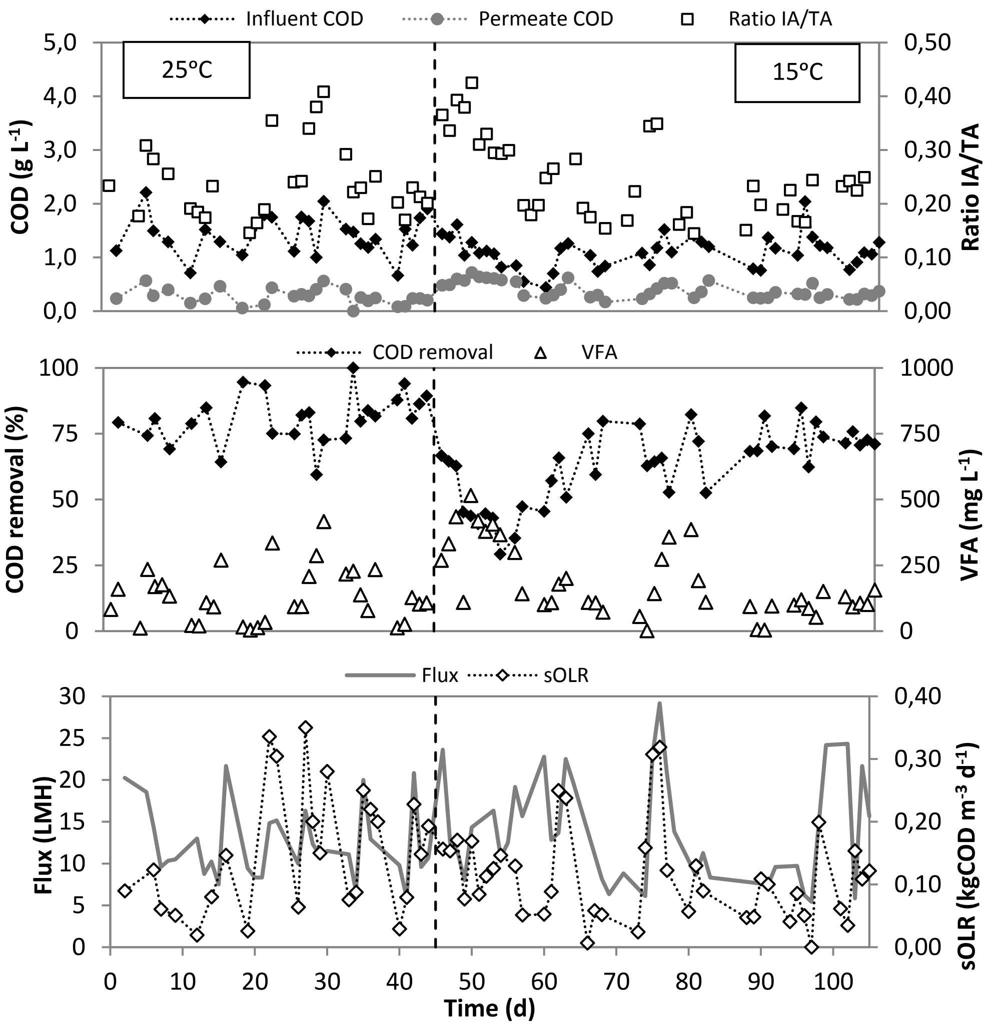


Figure 1. Evolution of the COD, ratio IA/TA, flux and sOLR of the CSTR-MBR at 25°C and 15°C

Membrane performance in the CSTR-MBR. Regarding the membrane performance, a higher degree of fouling was detected when lowering the temperature that caused the rapid decrease of flux. In order to maintain a similar flux, around 15 LMH, cleanings were often required once per week. The flux decline, calculated as the slope of flux between cleanings, was 3.63 and 2.14 LMH d^{-1} at 15°C and 25°C, much higher than the 0.10 LMH d^{-1} measured at 35°C (Basset et al., 2016). Although the manufacturer procedure recommended chemical cleanings, in this case they were performed with distilled water at a high crossflow velocity of around 3 m s^{-1}. By applying only clear water to remove the cake layer, the flux afterwards increased significantly as shown in Figure 1, and chemicals were not required at least at short term operation. The mixed liquor suspended solids (MLSS) were relatively lower, around 2.7 gSS L^{-1}, compared with Basset et al. (2016) that were up to 8.5 gSS L^{-1}. However, the reduction in the MLSS concentration did not improve the filtration efficiency. Ng et al. (2006) determined that fouling was not controlled directly by MLSS but the EPS. Although EPS were not determined in this study, many references can be found stating that these polymeric substances contribute to fouling phenomenon (Lin et al., 2014; Meng et al., 2009; Robles et al., 2013). Despite these studies revealed that at low temperatures the amount of EPS should be lower due to the reduced biomass activity, Stuckey (2012) stated that under any type of stress occasioned in an anaerobic digester can dramatically increase the soluble EPS production that results in increasing fouling. Therefore, apart from the mechanical stress caused by pumping the mixed liquor through the membrane module, the oscillating OLR that suffered the influent wastewater may have an important contribution to the EPS release. Moreover, Wang et al. (2010) determined that seasonal changes in temperature led to a deterioration of settling and dewatering properties.

UASB operation at low temperature

The OLR applied was on average 5.5±1.2 kgCOD m^{-3} d^{-1}. It was observed that the granules were losing its aggregation capacity increasing the SS in the effluent significantly, up to 420 mgSS L^{-1}. Since the HRT was relatively short, the disaggregated biomass was washed-out in few days, reducing the SS in the effluent to 34 mgSS L^{-1}. As shown in Table 1, the average COD removal was 84±9%, whereas the COD removal in terms of sCOD was 92±5%. However, effluent sCOD was on average 0.26±0.22 gCOD L^{-1}, which was over the required limit. Similar results were obtained by Keyser et al. (2003) treating winery wastewater in an UASB reactor with an OLR of 5 kgCOD $m^{-3}_{digester}$ d^{-1} and a HRT of 30 h. They suggested that the use of a granular sludge reduced considerably the start-up for the UASB, reaching high removal rates in few days. However, they never reached a COD removal efficiency over 86% and the biogas production was low; probably because the granules were not selective enough to treat a substrate as winery wastewater.

The removal rates achieved in the UASB were high and the OLR applied was much higher than the OLR reached in the CSTR-MBR. However, at high OLRs the discharge limits in terms of COD and SS were not accomplished and the amount of biomass in the USAB was decreasing due to the washout of the non-aggregated microorganisms. The variability of the winery wastewater is a clear drawback for anaerobic processes that are sensitive to OLR shocks (Dereli et al., 2012). Therefore, when the substrate characteristics negatively affect sludge granulation, a solid/liquid separation may favour the full biomass retention and so the slow growing organisms. For this reason, it was considered for this study the coupling of a membrane module to the UASB reactor, becoming an UASB-MBR.

The removal rate achieved in the UASB-MBR was higher than in the CSTR-MBR, 92±4%, as well as the OLR applied 1.6±0.5 kgCOD $m^{-3}_{digester}$ d^{-1}, as presented in Table 1. The increase in the removal efficiency was mainly due to a higher amount of biomass in the digester. Since the UASB retained 31.7±0.2 gMLSS L^{-1}, the sOLR was kept at very low values improving COD removal rate.

Synthetic winery wastewater was fed to the UASB-MBR prepared with an influent average COD of 1.4±0.2 g L^{-1} as for the CSTR-MBR. In Figure 2 the evolution of influent and permeate COD as well as the ratio IA/TA and VFA are shown. It should be noted that in the first 10 days COD removal was between 85% and 90% and the ratio IA/TA also slightly increased to 0.25, due to the presence of VFA in the reactor. VFA accumulated were not so important, keeping a ratio IA/TA always below 0.30. The behaviour of the permeate COD, which was on average 0.11±0.06 gCOD L^{-1}, was closely related to the influent COD. Especially when influent COD was over 1.5 gCOD L^{-1}, the permeate COD slightly exceeded the required limit of 0.125 gCOD L^{-1}. The average COD removal efficiency was 92±4%, as expected from the previous experiments with the UASB without membrane, where sCOD removal was over 90%. Regarding VFA accumulation in Figure 2, during the first 10 days and then in particular on day 38, the highest peaks of VFA were obtained in the permeate. However, the amount of these VFAs was not so significant, being as maximum around 100 mg L^{-1}. For this reason, the ratio IA/TA was always around 0.2 indicating enough buffer capacity to cope the acids in the reactor. In this occasion, it was not observed any instability during the experimental period caused by VFA accumulated. Operating at a low sOLR of 0.052±0.016 kgCOD kg^{-1}MLSS d^{-1}, VFA were kept at 15.5±8.8 mg L^{-1} of acetic acid. The alkalinity supplied in the feed was the same as in the CSTR-MBR. However, much less VFA were produced so that the alkalinity in the reactor was 954±126 $mgCaCO_3$ L^{-1} and the pH was 8.1±0.3.

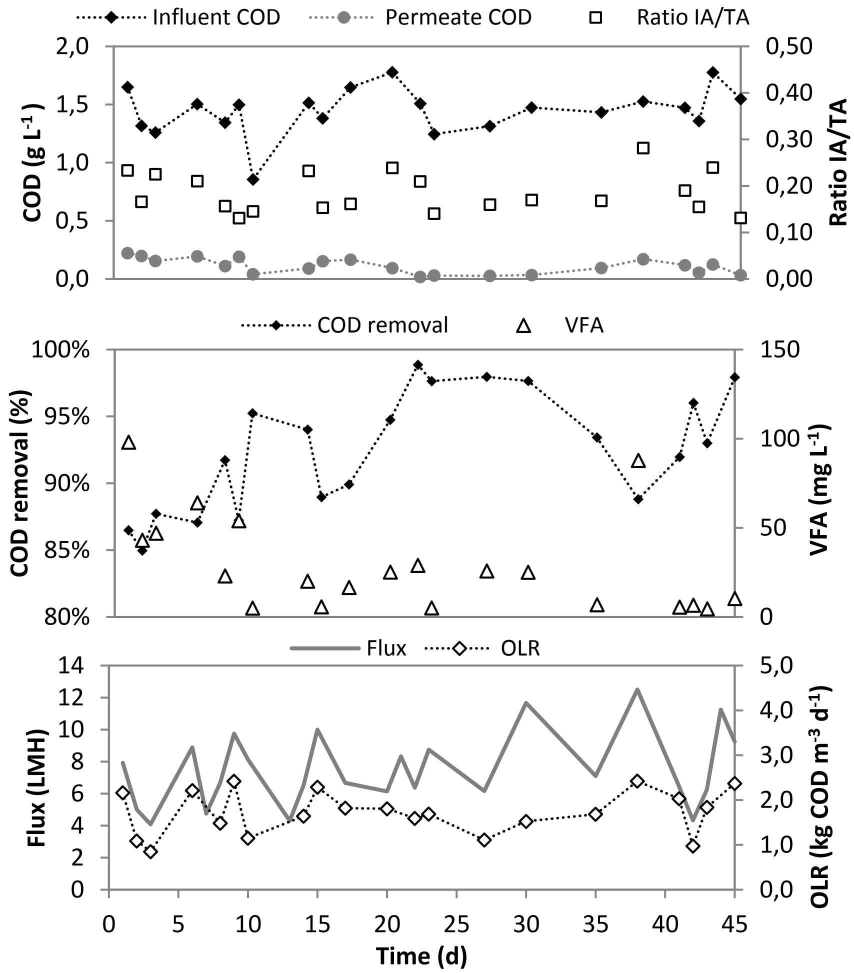


Figure 2. Evolution of the COD, ratio IA/TA, flux and OLR of the UASB-MBR

Membrane performance in the UASB-MBR. Comparing the membrane performance of the UASB-MBR and the CSTR-MBR described previously, the membrane filtration was not significantly improved. The flux decline was slightly better in the UASB-MBR, 1.3±0.8 LMH d^{-1} compared with 2.14±1.62 LMH d^{-1}, although the solids in contact with the membrane unit were lower. A probable reason for this lack of improvement could be that the crossflow velocity in the membrane module was lower when using the UASB than in the CSTR (0.05 vs 0.64 m s^{-1}). Since the retentate was directly connected to the UASB reactor, the flow rate was limited to the upflow velocity required in the UASB, around 1 m h^{-1}. Therefore, the membrane module was not operated at its optimal conditions to reduce the cake layer formed. Hence, membrane cleanings were carried out every 3-4 days when flux decreased below 5 LMH. In Figure 2, it is clearly observed that the OLR tended to oscillate as the flux did, leading to a moderate accumulation of VFA when OLR was high.

Evaluation of biomass acclimation

The specific methanogenic activity (SMA) and the SMP obtained in the batch tests are presented in Table 2. The SMA was similar at 25°C and 35°C, although the SMP was much lower due to less biodegradation and the loss of methane dissolved in the liquid phase. The COD removal percentage was determined to be 75% and the amount of methane dissolved in the liquid phase was 17.5 mg COD L^{-1}, which represented a 1.2% of the methane production. As expected, at 15°C, the SMA and the SMP were notably lower. However, the biodegradation determined was even lower than in the continuous digester (26%). This low biodegradation cannot be attributed to the sorption of methane in the liquid phase, because it was only a 3.6% (18.1 mgCOD L^{-1}). The biodegradation percentage expected in a batch test should be higher than in continuous operation. The COD removal efficiency obtained in the AnMBR was 71% at 15°C and 80% at 25°C. Since the COD removal observed in the BMP test were significantly lower at both temperatures, it suggested that the VFA generated during the batch test inhibited the methanogenic biomass. In fact, the food to microorganism ratio (F/M) was higher in the batch test (1.35 gCOD gSS^{-1} at 15°C and 1.76 gCOD gSS^{-1} at 15°C) than in the AnMBR (0.47 gCOD gSS^{-1} at 15°C and 0.61 gCOD gSS^{-1} at 15°C).

Table 2. SMA and SMP obtained in the AnMBR at low temperature treating synthetic winery wastewater

Inoculum	SMA ($kgCH_4$-COD kg^{-1}SS d^{-1})	SMP (Nm^3CH_4 kg^{-1}COD)	CH_4 in biogas (%)
CSTR-MBR at 15°C	0.14	0.09	66
CSTR-MBR at 25°C	0.35	0.26	77
UASB-MBR at 25°C	0.22	0.35	78
CSTR-MBR at 35°C (Basset et al., 2016)	0.36	0.35	81

In Figure 3, the methane production obtained during the different BMP tests is presented. It is clearly observed that the temperature favours a higher SMP and also the activity of the biomass. The SMA was calculated from the slope of the first days of SMP vs time, per amount of biomass (kgSS) added as inoculum. Despite the slope of the SMP at 25°C seemed much lower than at 35°C, the calculated SMA resulted in similar values, because in the BMP tests at low temperatures were prepared with less amount of biomass, reaching a ratio $COD_{substrate}/COD_{inoculum}$ of 1, instead of 0.5 as at mesophilic temperature. Comparing the results of the BMP test with the performance of the continuous AnMBR, it can be stated that the lack of biogas production in the AnMBR was more related to a design problem (the excessive headspace where biogas could be accumulated) than to a question of process efficiency. Although SMP was relatively low in the BMP tests, some biogas production was obtained and should have been observed in the continuous AnMBR.

The BMP test was carried out at 25°C also with the inoculum taken from the UASB. It can be observed in Figure 3 that the SMP in 4 days was 0.18 Nm^3CH_4 kg^{-1}COD, which was close to the SMP determined in the continuous UASB-MBR. Despite the SMP was higher than the one determined for the CSTR-MBR at 25°C; the SMA was 0.22 gCH_4-COD g^{-1}VSS d^{-1}, lower than the one observed for suspended biomass of 0.35 gCH_4-COD g^{-1}VSS d^{-1}. Since the BMP tests were prepared with a ratio $COD_{substrate}/COD_{inoculum}$ around 1, the lack of biodegradation can be attributed to an inhibition by substrate (VFA). For this reason, granular biomass produced more biogas (higher SMP), because the granular shape can reduce the impact high VFA concentration in the mixed liquor, leading to higher removal of COD. However, the SMA was lower than the suspended biomass because it is limited by the diffusion of substrate into the granule.

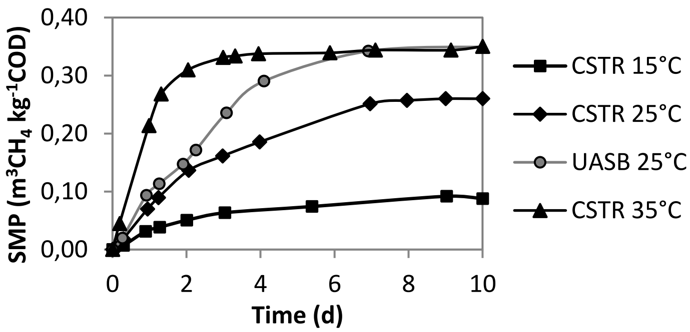
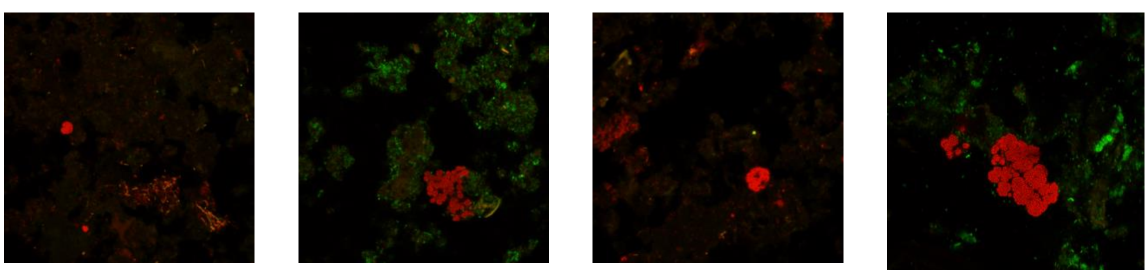


Figure 3. BMP tests at different temperatures

Microbial population observed during acclimation. Samples of anaerobic biomass from the CSTR-MBR at each temperature were taken to determine the changes on the microbial population. At 25°C, *Methanosaeta* spp. and *Methanosarcina* spp. were detected, although the amount of *Methanosaeta* spp. was not as predominant as observed in the previous study at 35°C (Basset et al., 2016). In Figure 4a, the *Archaea* probe showed two different shapes of microorganisms, rounded and elongated. The overlapping with *Methanosaeta* spp. revealed that the elongated ones corresponded to this specie, marked in yellow. In Figure 4b, it is observed most of *Bacteria* in green colour, and in red colour *Methanosarcina*, which corresponded to the rounded *Archaea* determined previously. The probes of *Methanomicrobiales* and *Methanobacteriales* resulted negative. The microbial population observed at 15°C, was very similar. The only difference observed was the absence of *Methanosaeta* spp. (Figure 4c) and the only methanogenic specie determined was *Methanosarcina* spp. (Figure 4d). The probes of *Methanomicrobiales* and *Methanobacteriales* resulted negative.



(a) Archea and Methanosaeta spp. (25°C) (b) Bacteria and Methanosarcina spp. (25°C) (c) Archaea and Methanosaeta spp. (15°C) (d) Bacteria and Methanosarcina spp. (15°C)

Figure 4. FISH image of *Archaea* (ARC915)-*Methanosaeta* spp. (MX825) (a) and *Bacteria* (EUB338)-*Methanosarcina* spp. (MS821) at 25°C (b); and *Archaea* (ARC915)-*Methanosaeta* spp. (MX825) (c) and *Bacteria* (EUB338)-*Methanosarcina* spp. (MS821) at 15°C (d)

The mesophilic inoculum that contained mostly *Methanosaeta* spp. turned to *Methanosarcina* spp. relatively fast, after 30 days at 25°C both species were determined, and after 80 days only *Methanosarcina* spp. appeared. The evolution of the biomass was because *Methanosarcina* spp. is more tolerant to high acid concentrations, so the growth rate is higher than *Methanosaeta* spp. (Janssen, 2003). Despite Zhang et al. (2012) determined that *Methanomicrobiales* played an important role in psychrophilic anaerobic digestion, the enrichment in acetotrophic methanogens as *Methanosarcina* spp., can be attributed to the acetate concentration in the AnMBR. Smith et al. (2013) determined that a mesophilic inoculum can be used for psychrophilic anaerobic digestion. The development of mesophilic psychrotolerant populations may have a negative impact on the COD removal efficiency at low temperature, although their capacity to acclimatise to changes in temperatures rapidly is an advantage to ensure stable performance along the year.

CONCLUSIONS

CSTR-MBR and UASB-MBR configurations were successfully applied to treat winery wastewater at low temperatures and resulted in a COD removal of 80% and 71% at 25°C and 15°C, respectively. Due to the VFA accumulation and methane retained in the liquid phase, the effluent COD not always accomplished the legal standards. Therefore, a polishing post-treatment would be necessary to recover methane and meet the legislation.

By using UASB-MBR configuration higher removal rates and biogas production could be reached mainly due to the capacity to retain more biomass in the reactor. A higher degree of fouling was observed compared with the mesophilic AnMBR. Although at lower temperatures less fouling was expected, the oscillations of organic load probably promoted EPS production that increased fouling.

From the BMP tests, it can be concluded that the SMA decreased at low temperatures compared to the mesophilic one, especially at 15°C. However, SMA obtained from the CSTR-MBR at 25°C was higher than the granular biomass activity from the UASB-MBR, probably caused by the substrate diffusion rate into the granule. The microbial population in the CSTR-MBR shifted from *Methanosaeta* spp. to *Methanosarcina* spp., because the higher amount of VFA favoured the development of acetotrophic methanogens with higher growth rate under high acetate concentration.

ACKNOWLEDGEMENTS

This research was carried out within the framework of the Red Novedar (CTQ2014-51693-REDC) project. Núria Basset is grateful to the Ministerio de Educación, Cultura y Deporte for her predoctoral FPU grant.

REFERENCES

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., Stahl, D.A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56, 1919–1925.

An, Y., Yang, F., Bucciali, B., Wong, F., 2009. Municipal Wastewater Treatment Using a UASB Coupled with Cross-Flow Membrane Filtration. J. Environ. Eng. 135, 86–91.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, a J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927–34.

Artiga, P., Ficara, E., Malpei, F., Garrido, J.M., Méndez, R., 2005. Treatment of two industrial wastewaters in a submerged membrane bioreactor. Desalination 179, 161–169.

Bandara, W.M.K.R.T.W., Kindaichi, T., Satoh, H., Sasakawa, M., Nakahara, Y., Takahashi, M., Okabe, S., 2012. Anaerobic treatment of municipal wastewater at ambient temperature: Analysis of archaeal community structure and recovery of dissolved methane. Water Res. 46, 5756–64.

Basset, N., López-Palau, S., Dosta, J., Mata-Álvarez, J., 2014. Comparison of aerobic granulation and anaerobic membrane bioreactor technologies for winery wastewater treatment. Water Sci. Technol. 69, 320–7.

Basset, N., Santos, E., Dosta, J., Mata-Álvarez, J., 2016. Start-up and operation of an AnMBR for winery wastewater treatment. Ecol. Eng. 86, 279–289.
Cerón-Vivas, A., Morgan-Sagastume, J.M., Noyola, A., 2012. Intermittent filtration and gas bubbling for fouling reduction in anaerobic membrane bioreactors. J. Memb. Sci. 424, 136–142.
Dereli, R.K., Ersahin, M.E., Ozgun, H., Ozturk, I., Jeison, D., van der Zee, F., van Lier, J.B., 2012. Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters. Bioresour. Technol. 122, 160–70.
Gerardi, M.H., 2003. The Microbiology of Anaerobic Digesters. John Wiley & Sons, Inc., Hoboken, New Jersey. ISBN: 0-471-20693-8
Giménez, J.B., Robles, a, Carretero, L., Durán, F., Ruano, M. V, Gatti, M.N., Ribes, J., Ferrer, J., Seco, a, 2011. Experimental study of the anaerobic urban wastewater treatment in a submerged hollow-fibre membrane bioreactor at pilot scale. Bioresour. Technol. 102, 8799–806.
Janssen, P.H., 2003. Selective enrichment and purification of cultures of Methanosaeta spp. J. Microbiol. Methods 52, 239–244.
Keyser, M., Witthuhn, R.C., Ronquest, L.-C., Britz, T.J., 2003. Treatment of winery effluent with upflow anaerobic sludge blanket (UASB) – granular sludges enriched with Enterobacter sakazakii. Biotechnol. Lett. 25, 1893–1898.
Lettinga, G., Rebac, S., Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnol. 19, 363–70.
Lin, H., Zhang, M., Wang, F., Meng, F., Liao, B.-Q., Hong, H., Chen, J., Gao, W., 2014. A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: Characteristics, roles in membrane fouling and control strategies. J. Memb. Sci. 460, 110–125.
McKeown, R.M., Hughes, D., Collins, G., Mahony, T., O'Flaherty, V., 2012. Low-temperature anaerobic digestion for wastewater treatment. Curr. Opin. Biotechnol. 23, 444–51.
Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Res. 43, 1489–512.
Moletta, R., 2005. Winery and distillery wastewater treatment by anaerobic digestion. Water Sci. Technol. 51, 137–144.
Ng, H.Y., Tan, T.W., Ong, S.L., 2006. Membrane Fouling of Submerged Membrane Bioreactors: Impact of Mean Cell Residence Time and the Contributing Factors. Environ. Sci. Technol. 40, 2706–2713.
Ozgun, H., Dereli, R.K., Ersahin, M.E., Kinaci, C., Spanjers, H., van Lier, J.B., 2013. A review of anaerobic membrane bioreactors for municipal wastewater treatment: Integration options, limitations and expectations. Sep. Purif. Technol. 118, 89–104.
Robles, A., Ruano, M.V., Ribes, J., Ferrer, J., 2013. Performance of industrial scale hollow-fibre membranes in a submerged anaerobic MBR (HF-SAnMBR) system at mesophilic and psychrophilic conditions. Sep. Purif. Technol. 104, 290–296.
Salazar-Peláez, M.L., Morgan-Sagastume, J.M., Noyola, A., 2011. Influence of hydraulic retention time on fouling in a UASB coupled with an external ultrafiltration membrane treating synthetic municipal wastewater. Desalination 277, 164–170.
Sander, R., 2015. Compilation of Henry's law constants (version 4.0) for water as solvent. Atmos. Chem. Phys. 15, 4399–4981.
Smith, A.L., Skerlos, S.J., Raskin, L., 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. Water Res. 47, 1655–65.
Smith, A.L., Stadler, L.B., Love, N.G., Skerlos, S.J., Raskin, L., 2012. Perspectives on anaerobic membrane bioreactor treatment of domestic wastewater : A critical review. Bioresour. Technol. 122, 149–159.
Stuckey, D.C., 2012. Recent developments in anaerobic membrane reactors. Bioresour. Technol. 122, 137–48.
Wang, Z., Wu, Z., Tang, S., 2010. Impact of Temperature Seasonal Change on Sludge Characteristics and Membrane Fouling in a Submerged Membrane Bioreactor. Sep. Sci. Technol. 45, 920–927.
Zhang, D., Zhu, W., Tang, C., Suo, Y., Gao, L., Yuan, X., Wang, X., Cui, Z., 2012. Bioreactor performance and methanogenic population dynamics in a low-temperature (5-18 °C) anaerobic fixed-bed reactor. Bioresour. Technol. 104, 136–43.