Bioresource Technology 146 (2013) 494–503

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Two-stage anaerobic membrane bioreactor for the treatment of sugarcane vinasse: Assessment on biological activity and filtration performance

Vera Tainá Mota *, Fábio S. Santos, Míriam C.S. Amaral

Department of Sanitary and Environmental Engineering, Universidade Federal de Minas Gerais, Avenida Antônio Carlos, 6627, Bloco 1, 4th floor, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil

HIGHLIGHTS

- A two-stage AnMBR was designed for the treatment of sugarcane vinasse.
- Intermittent feeding was found to be effective to acclimate the microorganisms.
- COD and DOC removals efficiencies were 96.9 ± 0.7% and 95.0 ± 1.1%, respectively.
- Membrane filtration resistance was found to be predominantly removable.
- SMP protein and EPS protein were correlated to membrane filtration resistance.

ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form 20 July 2013
Accepted 24 July 2013
Available online 29 July 2013

Keywords:
Anaerobic digestion
Acidogenesis
Methanogenesis
Submerged anaerobic membrane bioreactor
Vinasse

ABSTRACT

A two-stage submerged anaerobic membrane bioreactor (2-SAnMBR) was designed for the treatment of sugarcane vinasse. For start-up, the flow rate was reduced whenever VFA levels reached critical levels in the methanogenic reactor. After acclimation, the system was operated under a continuous flow. Separation of the stages was observed during the entire period of operation. VFA, COD and DOC levels of raw effluent, acidified effluent and permeate averaged 2141, 3525 and 61 mg VFA L^{-1} (as acetic acid), 15727, 11512 and 488 mg COD L^{-1}, and, 3544, 3533 and 178 mg DOC L^{-1}, respectively. Overall COD and DOC removal efficiencies of 96.9 ± 0.7% and 95.0 ± 1.1%, respectively, were reached. Methane content of the biogas from the acidogenic and methanogenic reactors ranged 0.1–4.6% and 60.1–70.1%, respectively. Removable fouling strongly affected filtration performance and cake layer formation accounted for most of filtration resistance. Membrane resistance was related to presence of protein-like substances and carbohydrates.

1. Introduction

Distillery wastewater is one of the major concerns in alcohol production. For each 1 L of ethanol, approximately 15 L of vinasse are produced (van Haandel, 2005). Important characteristics of sugarcane vinasse include low pH, high levels of BOD, COD, potassium, sulfate and color (Wilkie et al., 2000). The large volumes produced, associated with its high content of biodegradable organic matter, mean that vinasse is a potential source of energy through anaerobic digestion and biogas recovery. In addition to the intrinsic advantages of anaerobic digestion such as low nutrient requirement, small production of excess sludge, lower energy input and generation of methane-rich biogas (Lettinga et al., 1980), anaerobic digestion has been recognized as the most attractive method for vinasse treatment (de Bazúa et al., 1991; Wilkie et al., 2000; van Haandel, 2005). However, anaerobic digestion applied to vinasse treatment is not a well-established technology, and it is put at risk given the fluctuations in the quantity and quality of the vinasse to be processed and the presence of inhibitory compounds.

The instability of anaerobic reactors treating high strength wastewaters is usually associated with an uneven production and consumption of volatile fatty acids. Acidogenic bacteria have the highest growth rates among the microbial consortium and are generally more resistant to environmental stress conditions than syntrophic acetogenic bacteria and methanogenic archea (Ke et al., 2005). Two-stage anaerobic digestion allows the maintenance of optimal conditions for each group of microorganisms involved in each phase of anaerobic digestion, providing improvement in the treatment efficiency, reduction of inhibitory effects of toxic compounds on methanogens (Beccari et al., 1996), higher

* Corresponding author. Address: Rua Carangola, 683/102, CEP 30330–240, Belo Horizonte, Minas Gerais, Brazil. Tel.: +55 31 88329638, +55 31 32237321.
E-mail addresses: vtaina@hotmail.com, vtaina5@gmail.com (V.T. Mota).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.07.110

methane content in biogas (Lun et al., 1995; Yeoh, 1997), tolerance to greater organic load (Ghosh et al., 1985), reduction in the accumulation of propionic acid and more stability when undergoing shock loading (Cohen et al., 1982).

Additionally, the application of membrane retention to anaerobic digestion in the anaerobic membrane bioreactors (AnMBRs) ensures complete retention of biomass and total suspended solids (TSS). The system greatly increases effluent quality in terms of COD and TSS, and favors the maintenance of slow growth microorganisms such as methanogens. Despite the advantages of AnMBRs over conventional anaerobic reactors, this technology is limited mainly by membrane fouling, which affects membrane area requirements and operational costs. While probably very similar to aerobic MBRs, a lot less is known about fouling in anaerobic ones, requiring research to understand how reactor operation influences fouling and release of soluble microbial products (SMP), which accounted for much of the soluble COD (Stuckey, 2012).

Many studies have been performed in order to improve the operational conditions and to identify the factors causing flux reduction in AnMBRs. Jeison and van Lier (2007b) and Jeison et al. (2009) verified that the degree of wastewater acidification strongly affects filtration performance. Submerged and side-stream thermophilic AnMBRs treating acidified wastewater exhibited much better performance than those treating partially or non-acidified wastewater. The authors revealed that acidogenic bacteria, induced by the feed with partially or non-acidified wastewater, grow mostly as individual cells rather than as flocs. This increases the relative amount of smaller particles and sludge viscosity and affects the degree of cake layer deposition, reducing filtration performance.

Based on the results discussed above, it is inferred that in a two-stage AnMBR (2-AnMBR), in which the methanogenic reactor is coupled to a membrane module, the occurrence of acidogenesis in an earlier step could prevent acidogenic biomass growth in the methanogenic reactor, enhancing sludge properties and filtration performance. Studies found in the literature comprise both side-stream 2-AnMBR at 37 °C (Saddoud et al., 2007; Saddoud and Sayadi, 2007) and at 55 °C (Wijekoon et al., 2011), as well as submerged 2-AnMBR at 35 °C (Jeong et al., 2010). In general, they presented good performance for the treatment of high strength wastewaters.

Given the few studies on the application of an acidogenic reactor followed by a methanogenic membrane bioreactor for wastewater treatment, this research aims to contribute to the development of AnMBR technology, raising new concerns about 2-AnMBRs. The present research describes the biological and filtration performance of a two-stage submerged anaerobic membrane bioreactor (2-SAnMBR) treating sugarcane vinasse at room temperature (22 °C). Analyses of the effluent and biogas from both reactors were performed in order to estimate the degree of acidification and organic matter degradation in each phase of anaerobic digestion. The main fouling mechanisms are discussed as well as the influence of soluble microbial products (SMP) and extracellular polymeric substances (EPS) on them.

2. Methods

2.1. Vinasse samples

Vinasse samples were obtained from a distillery located in the state of São Paulo (Brazil), which produces ethanol from sugarcane juice. The plant has the capacity to grind up to 800 thousand tons of cane per harvest. The samples were kept refrigerated at 4 °C. The physicochemical composition of the samples is shown in Table 1.

2.2. Experimental setup

A schematic of the experimental setup designed for this study is shown as Fig. 1. Two anaerobic reactors made of acrylic were placed in series with the purpose of separating acidogenic and methanogenic stages. Acidogenesis was carried out in an upflow anaerobic reactor with 14 cm diameter and 87 cm height. Valves situated in the side outlets enabled the operation in four levels, corresponding to 2.3 L, 3.8 L, 6.7 L and 11.0 L. The acidogenic reactor (AR) effluent fed the methanogenic reactor by gravity. Methanogenesis was carried out in a continuous stirred anaerobic reactor (IKA RW 16 Basic® stirrer, 250 rpm speed) with 24 cm diameter and 67 cm height. The working volume was controlled by four electric level sensors, corresponding to 7.8 L, 9.3 L, 20.1 L and 24.0 L, which were connected to the peristaltic pump and switched it off if the level was above the selected one. The methanogenic reactor (MR) was fitted with a microfiltration (MF) unit. The membrane module was composed of 205 polyetherimide hollow-fibers with nominal pore size of 0.45 μm and 7 cm length, whose total surface area was 0.045 m^2 (Pam Membranas Seletivas, Brazil). Transmembrane pressure (TMP) was provided by a vacuum tank connected to a pump and was controlled by a valve to regulate air inlet. The vacuum pump maintained the vacuum tank (VT) negatively pressurized so as to allow the suction of permeate. The vacuum in VT was controlled to maintain the permeate flux constant. The filtration took place until the VT upper level was reached. Permeate was then discharged to the permeate tank (PT), which also functioned as a feed tank for back-flush. When back-flush or relaxation was activated, the vacuum line was interrupted. If relaxation was used instead of back-flush the back-flush pump was kept powered off. The time between filtration and back-flush/relaxation was automatically programmed via a timer. The system was maintained at room temperature (general average 22 °C, minimum average 19 °C, maximum average 27 °C).

2.3. Operational conditions

The reactors were inoculated at an initial concentration of 20 g MLVSS L^{-1} with flocculent sludge from a single-stage UASB reactor treating domestic sewage in the Centre for Research and Training on Sanitation UFMG/COPASA – CePTS, located in Belo Horizonte, Brazil. Before starting the 2-SAnMBR operation, an acclimation period was needed in order to select and enrich the microorganism populations associated with acidogenic and methanogenic biochemical reactions in the first and second reactors, respectively. Undiluted vinasse (Sample 1) was used as the feed wastewater. In this period, the treated effluent was collected using a peristaltic pump and transferred to the permeate tank. The settled sludge was recycled daily in the methanogenic reactor. VFA safe levels for anaerobic digestion were maintained in the methanogenic reactor by reducing the flow rate when the VFA reached critical levels (above 1000 mg L^{-1}). During the whole period of operation, the pH in the acidogenic reactor was not controlled, as the pH in the methanogenic reactor was kept above 6.5 by supplying sodium bicarbonate when necessary.

Once start-up was finished, the membrane module was fitted to the methanogenic reactor. The working volumes of the acidogenic and methanogenic reactors were kept at 6.7 L and 24.0 L, respectively. Sample 1 fed the system from day 0 to day 3 and Sample 2 fed the system from day 4 to day 57. The 2-SAnMBR operated under an average organic loading rate (OLR) of 2.5 gCOD L^{-1} d^{-1}, at an infinite sludge age. Most of the time, 40 s of relaxation for each 8 min of filtration was the only strategy used to avoid membrane fouling; the instantaneous flux was 4.8 L m^{-2} h^{-1}, in order to achieve a permeate productivity equivalent to an average flux of 4.4 L m^{-2} h^{-1}.

Table 1
Composition of vinasse samples.

Parameter	Unity	Sample 1	Sample 2
pH	-	4.1	3.9
Conductivity	mS cm^{-1}	5.1	4.8
Color (at pH 6.5)	uH	893.2 (3234.8)	852.2 (2500.2)
Turbidity	NTU	2950.0	3438.0
TCOD (SCOD)	mg L^{-1}	17677.7 (13827.7)	15496.5 (11776.0)
DOC	mg L^{-1}	4205.0	3452.5
VFA	mgHAc L^{-1}	1995.0	2160.0
Soluble N	mg L^{-1}	105.8	50.7
TS (VTS)	g L^{-1}	15.42 (12.07)	11.11 (8.87)
TSS (VSS)	g L^{-1}	2.80 (2.78)	2.99 (2.97)
PO_4^{3-}	mg L^{-1}	64.3	47.0
SO_4^{2-}	mg L^{-1}	1208.0	319.5
K^+	mg L^{-1}	2170.6	1587.4

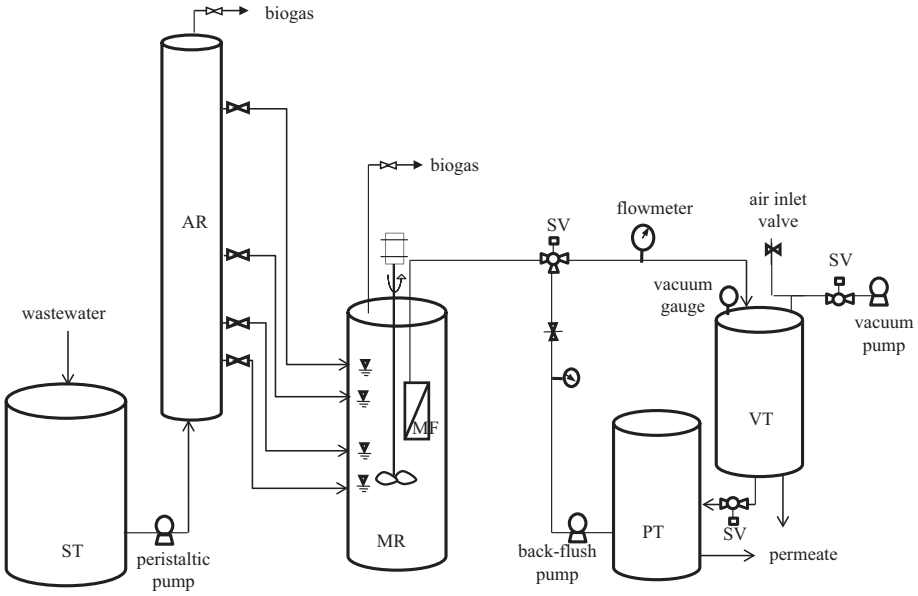

Fig. 1. Schematic diagram of the 2-SAnMBR. ST: storage tank; AR: acidogenic reactor, MR: methanogenic reactor, MF: microfiltration unit, SV: solenoid valve, VT: vacuum tank, PT: permeate tank.

2.4. Analytical procedures

The biological activity of the 2-SAnMBR was evaluated from the biogas composition of both reactors and from analyses of the influent, acidified effluent, treated effluent, soluble microbial products (SMP) and extracellular polymeric substances (EPS) taken from the storage tank, acidogenic reactor, membrane permeate and methanogenic reactor mixed liquor, respectively. SMP and EPS extraction were achieved by centrifugation (Cientec CT-5000) and heating. Mixed liquor from methanogenic reactor samples (50 mL) was centrifuged for 30 min at 6037*g* and the supernatant containing the SMP was collected. EPS gathered in the pellet was extracted using 50 mL 0.05% NaCl solution, heated to 80 °C for 10 min and centrifuged for 30 min at 6037*g*. Analyses were carried out using the supernatant containing the EPS. Fractions of the supernatant samples were passed through 0.45 μm membrane filters (Millipore) and referred to as "$SMP_{0.45}$" and "$EPS_{0.45}$".

The following parameters were analyzed: chemical oxygen demand (COD) (APHA et al., 2005), proteins (Lowry et al., 1951) and carbohydrates (Dubois et al., 1956) of the influent, acidified effluent, treated effluent, SMP and EPS; pH (pHmeter Qualxtron QX 1500), volatile fatty acids (VFA) (DiLallo method (DiLallo and Albertson, 1961) and Kapp method reported by Buchauer, 1998), total dissolved organic carbon (DOC) and total dissolved nitrogen (TN) (TOC-analyzer Shimadzu, TOC-V CPN and TNM-1) of the influent, acidified effluent and treated effluent; color (spectrophotometer Hach DR2800), turbidity (turbidimeter Hach 2100AN), total solids (TS), volatile total solids (VTS) and fixed total solids (FTS) (APHA et al., 2005), phosphate and sulphate ions (Dionex ion chromatograph, ICS-1000, AS-22 column) and potassium ions (flame atomic absorption spectrometry, SensAA GBC Scientific Equipment) of the influent and treated effluent; bicarbonate alkalinity (Kapp method reported by Buchauer, 1998), intermediary alkalinity to partial alkalinity ratio (IA:PA) (Ripley et al., 1986) and volatile suspended solids (VSS) (APHA et al., 2005) of the methanogenic reactor mixed liquor; and methane percentage in the biogas from the acidogenic and methanogenic reactors (Perkin Elmer chromatograph, TCD detector, carbopax column). Statistical analyses were done using the software Statistica 7. The normal distribution of the results was checked using the Shapiro-Wilk test before applying the other tests. The 95% confidence level was adopted for all tests.

2.5. Membrane-cleaning procedures

Membrane-cleaning procedures were performed when TMP reached 0.5 bar; then, membrane fouling was found to be severe.

Three membrane-cleaning procedures were used to recover membrane permeability: (i) physical cleaning by flushing the membrane surface with tap water until the cake layer was dislodged, (ii) oxidative chemical cleaning with 500 ppm NaOCl for 20 min, (iii) acid chemical cleaning with citric acid at pH 3 for 20 min. Physical cleaning was performed every time that the membrane underwent a cleaning procedure; chemical cleaning was performed optionally.

2.6. Membrane resistance evaluation

Membrane resistance was measured by recording flux rate at a certain TMP. The fraction of each membrane resistance was evaluated based on the resistance-in-series model by Choo and Lee (1996). Intrinsic membrane resistance was determined for the new membrane. Once starting the 2-SAnMBR operation, total membrane resistance (R_t) was experimentally defined as the resistance measured in the biological tank, where $R_t = \Delta P/\mu J$. The R_t presented is the one recorded under the most severe conditions (when TMP reached 0.5 bar), measured right before water flux measurements at each step of the membrane-cleaning procedures. Thus, R_t is the sum of intrinsic membrane resistance (R_m), the internal fouling resistance due to adsorption/adhesion and pore blocking (R_{if}), and the external fouling resistance caused by the solid accumulation/deposition on top of the membrane surface, representing the extent of cake formation during filtration (R_{ef}). Then, the membrane module was extracted from the biological tank and water flux was measured at each step of the three membrane-cleaning procedures described above. Each measurement lasted enough time for the water flux to remain stable for at least 15 min. After physical cleaning, R_m and R_{if} accounted for the whole membrane resistance. Therefore, the difference between the resistance measured in the biological tank ($R_{ef} + R_{if} + R_m$) and the resistance measured after physical cleaning ($R_{if} + R_m$) was the total external fouling resistance (R_{ef}). On days 35, 40 and 44, water flux was also measured after the membrane module was gently rinsed thoroughly with tap water in order to determine external membrane fouling resistance due to highly attached cake layer (R_c); i.e., the one that could not be dislodged by either relaxation or rinsing. Then, the measured resistance was the sum of R_m, R_{if} and R_c. Distinctions among removable, irremovable and irreversible fouling resistances were based on the definition proposed by Meng et al. (2009). Removable fouling can be eliminated by physical cleaning, while irremovable fouling needs to be eliminated by chemical cleaning. Irremovable fouling refers to total internal fouling, caused by pore blocking and strongly attached foulants during filtration. Irreversible fouling is characterized as fouling that cannot be removed by either physical or chemical cleaning. Thus, increased membrane resistance measured after chemical cleaning was considered to be the result of irreversible internal fouling. The difference between total internal fouling resistance (R_{if}) and irreversible internal fouling resistance (R_{iif}) was then named as reversible internal fouling resistance (R_{rif}).

3. Results and discussion

3.1. Start-up

The flow rates throughout the acclimation period are shown in Fig. 2. Initially, the working volumes of the acidogenic and methanogenic reactors were set to 11.0 L and 24.0 L, respectively. A flow rate of 4.8 L d^{-1} was applied up to the 15th day. However, VFA levels in the methanogenic reactor tended to increase progressively and the flow had to be interrupted several times to avoid VFA accumulation and biomass decline since, in previous experiments, the continuous feeding led to a sharp decrease in biomass concentration (data not shown). Then, the flow rate was reduced to 3 L d^{-1} and the ratio of hydraulic retention time (HRT) in the methanogenic reactor to HRT in the acidogenic reactor (HRT_M:HRT_A) was increased from 2.2 to 3.6 by reducing the working volume in the acidogenic reactor to 6.7 L. Before reducing its working volume, the biomass concentration was equal to 21.6 gMLVSS L^{-1} (considering the total working volume: sludge bed and liquid phase). In order to maintain the original sludge concentration of 20 gMLVSS L^{-1}, 103.6 gVSS from the acidogenic reactor was discharged. The upflow configuration seemed to be appropriate to keep the biomass at the bottom of the acidogenic reactor, as no sludge wash-out was observed during the entire period of operation.

A continuous accumulation of VFA in the methanogenic reactor was experienced again, probably because the methanogenic archaea population was not sufficiently enriched and acclimated. On the 28th day, the VFA levels reached the highest level, as high as 1862 mg L^{-1}. A slight decline in the biomass was also observed. The feeding had to be interrupted for 8 days, until the VFA levels were lower than the inhibitory limits (<1000 mg L^{-1}). Flow interruption did not lead to VFA consumption in the acidogenic reactor, indicating that methanogenic activity was almost negligible in this reactor. This was probably due to the high VFA levels and the low pH (4.3 ± 0.2), that made the environmental conditions unfavorable for methanogenic growth. In contrast, in the methanogenic reactor, both the VFA and organic matter levels decreased, reaching over 85% COD and DOC removal efficiencies. Similar strategy was adopted by Spagni et al. (2012) in a SAnMBR treating textile wastewater when it was experimented VFA accumulation in the reactor due azo dye increased concentrations, indicating inhibition of the methanogenic biomass. After switched off the system for 15 days, VFA decreased and COD removal recovered. Although azo dye concentration was increased again, COD removal was never so severely negatively inhibited as during the acclimation and it was reported changes in the microbial community. The process stability after a sufficient period of acclimation was also observed in the present study (see Section 3.2).

On the 38th day, the flow rate was set to 2.0 L d^{-1}. After 10 days, VFA fell to the lowest levels under continuous feeding, suggesting that the VFA consumption rate had become higher than the VFA loading rate in the methanogenic reactor, so the OLR could be increased further. Between the days 55 and 57, the experimental setup underwent structural changes to allow operation with a submerged membrane module. As a result of the feeding interruption for two days, VFA decreased from 880.0 to 126.3 mg L^{-1}.

During the entire period of acclimation, VFA levels in the acidogenic reactor were higher than in the raw effluent, while VFA levels in the methanogenic reactor were the lowest in the overall system, revealing VFA production occurring in the acidogenic reactor and VFA consumption occurring in the methanogenic reactor. Average

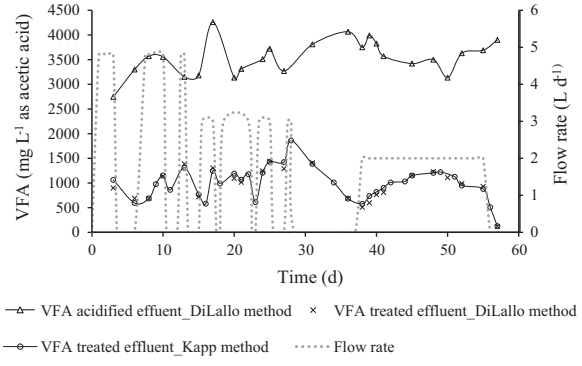

Fig. 2. Constant flow control in response to VFA levels in the methanogenic reactor.

VFA concentrations in the influent, acidified effluent and treated effluent were 1995, 3541 and 987 mg L^{-1} (as acetic acid), respectively. Sodium bicarbonate was added to the methanogenic reactor on the 9th day at a dosage of 3360 mg$NaHCO_3$ L^{-1}, and on the 17th and 42nd days at a dosage of 1680 mg$NaHCO_3$ L^{-1}; pH remained at 7.0 ± 0.3. Bicarbonate alkalinity input combined with methanogenesis biochemical reactions resulted in the IA:PA ratio in the methanogenic reactor falling from 2.73 on the 3rd day to 0.29 on the 57th day. The methane content in the biogas from the acidogenic and methanogenic reactors also changed from 13.2% and 53.8% on the 10th day to 0.61% and 65.2% on the 57th day, respectively. The average COD, DOC, color and turbidity removals during acclimation were 86.2 ± 5.1%, 84.1 ± 7.1%, 56.1 ± 21.0% and 97.1 ± 2.4%, respectively. The treated effluent samples were filtered through a 45 μm filter (Millipore®) before analysis, in order to allow comparisons between the results of acclimation and of the 2-SAnMBR operation. The end of the start-up period on day 57 is thus considered as day 0 for the 2-SAnMBR operation.

3.2. Treatment performance in the 2-SAnMBR

The flow rate was increased to 4.8 L d^{-1} and the working volume was maintained at 6.7 L in the acidogenic reactor and 24.0 L in the methanogenic reactor. In the acidogenic reactor, pH was not controlled and remained acidic (4.2–4.6); in the methanogenic reactor it was maintained neutral (7.0–7.5) without the need of additional sodium bicarbonate. Acidified effluent and permeate VFA levels are shown in Fig. 3 (a). There was no statistical difference between the DiLallo and Kapp methods, according to Wilcoxon's matched pairs test (p-level = 0.69), regarding the VFA analysis of the treated effluent performed during the acclimation and the 2-SAnMBR operation.

Comparison of the VFA levels in the acidified effluent between the acclimation and the 2-SAnMBR operation periods revealed no statistical differences according to the T-test for independent samples (p-level = 0.87, p-Levene = 0.50) and to the Mann–Whitney U Test (p-level = 0.87). VFA levels of the acidified effluent were always higher than of raw vinasse and VFA levels of permeate remained far beneath the inhibitory limits to methanogens. The separation of acidogenic and methanogenic stages was also confirmed through biogas analyses (Fig. 3 (b)). The IA:PA ratio in the methanogenic reactor was 0.25 ± 0.04, indicating the achievement of a steady-state operating condition. This was likely due to the relatively low OLR, well acclimated biomass and the maintenance of favorable environmental conditions, such as low levels of VFA (65.7 ± 38.4 mg L^{-1} as acetic acid) and neutral pH. As the VFA analyses of the treated effluent did not have normal distribution, only the nonparametric Mann–Whitney U test was applied, which confirmed that permeate VFA levels were statistically lower during the 2-SAnMBR operation than during the acclimation (p-level < 0.001). These results reinforce that methanogenesis is the limiting step of the treatment, as appropriate VFA consumption depends on the maintenance of favorable conditions and biomass acclimation.

The levels and percentage removals of COD and DOC are shown in Fig. 4(a) and (b). COD and DOC levels during 2-SAnMBR operation period in raw effluent, in acidified effluent and in permeate were 15727, 11512 and 488 mgCOD L^{-1}, and 3544, 3533 and 178 mgDOC L^{-1}, respectively. COD removal in the acidogenic reactor was 26.8 ± 5.5%, which is in the same range as reported by Ince (1998), who achieved between 10% and 40% COD removal in the acidogenic reactor of a two-stage anaerobic system treating dairy effluent. However, Ince (1998) reported methane content around 5–15% in the biogas from the acidogenic reactor; this is much higher than what was found in this study that ranged from 0.08% to 4.6% (Fig. 3(b)). Given the low methane content in the biogas from the acidogenic reactor, COD removal through acidogenesis

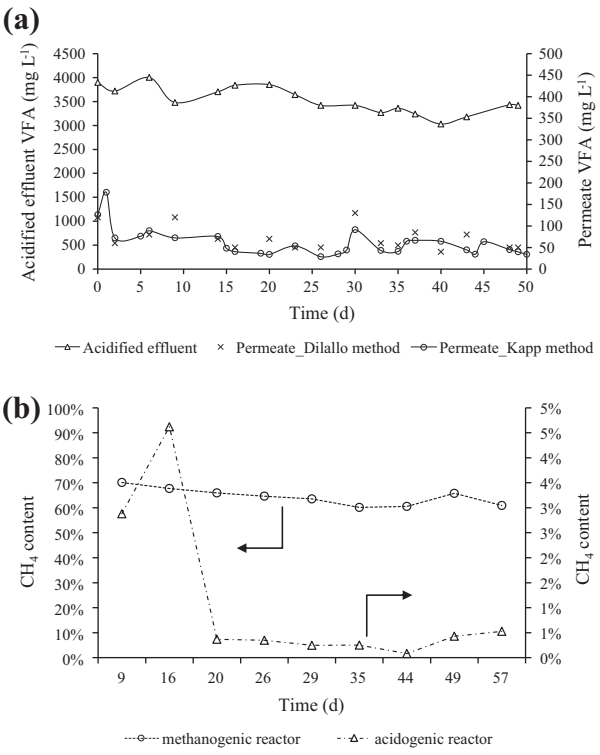

Fig. 3. (a) VFA levels of the acidified effluent and of the permeate. (b) Methane percentage in biogas from acidogenic and methanogenic reactors.

probably occurred because of some biochemical reactions of fermentation and sulfidogenesis (release of COD as hydrogen sulfide in the biogas), biomass incorporation and retention by the filter used to prepare the samples.

The overall removals of COD and DOC achieved in the 2-SAnMBR were 96.9 ± 0.7% and 95.0 ± 1.1%, respectively. COD and DOC fluctuations in the acidified effluent had no impact on the permeate quality, revealing that the overall removal was strictly correlated to the removal occurring in the methanogenic reactor coupled to the membrane module, that presented average COD and DOC removals of 95.7 ± 1.0% and 94.9 ± 1.2%, respectively.

By contrast, the methane content in the biogas from the methanogenic reactor ranged from 60.1% to 70.1% (Fig. 3(b)). This suggests that two-phase anaerobic digestion contributes to the enrichment of the methane percentage in the methanogenic reactor biogas. Despite the fact that COD and DOC removal rates increased greatly since starting the 2-SAnMBR operation, color removal was only 57.4 ± 15.4% and turbidity removal was 97.2 ± 15.4% (Table 2). In fact, according to the Mann–Whitney U Test, the percentage removals of COD and DOC were statistically higher in the 2-SAnMBR operation than in the acclimation (p-level < 0.0001), while there was no statistical difference between the acclimation and the 2-SAnMBR operation regarding color removal (p-level = 0.89) and turbidity removal (p-level = 0.98). As only color removal had normal distribution throughout the entire period, the T-test for independent samples was also applied. It confirmed no statistical difference between percentage removal during the acclimation and 2-SAnMBR operation periods (p-level = 0.81). These results suggest that vinasse color removal in the anaerobic treatment may not be related to the removal of organic matter; and they are in agreement with the statement that many of the colored compounds present in vinasse are recalcitrant (Gonzalez et al., 2000). Also, the turbidity values of the filtered treated effluent from the acclimation period and of the permeate showed no statistical difference (p-level = 0.61); they were quite

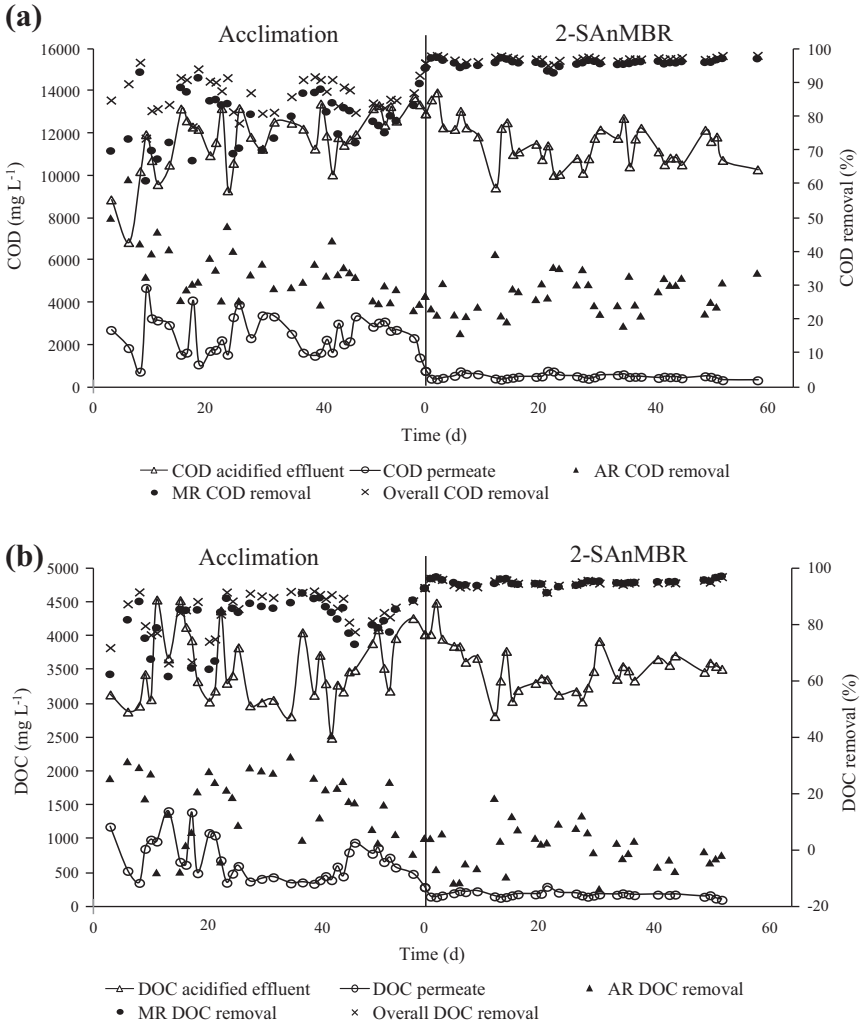

Fig. 4. (a) COD levels and removal during the entire operation period. (b) DOC concentration and removal during the entire operation period.

Table 2
Treatment performance of the 2-SAnMBR.

Parameter	Vinasse	Acidified effluent[a]			Permeate			% Removal
	Aver.	Min.	Aver.	Max.	Min.	Aver.	Max.	Aver. ()[b]
Color (uH)	2562.5[c]	ne[d]	ne	ne	422.5	1115.6	2138.3	57.4 (±15.4)
Turbidity (UNT)	3396.6	ne	ne	ne	12.6	94.4	226.0	97.2 (±2.0)
VTS (g L^{-1})	10.2	ne	ne	ne	0.5	0.7	0.9	93.0 (±2.0)
FTS (g L^{-1})	2.7	ne	ne	ne	1.6	2.5	3.0	5.1 (±26.5)
TN (mg L^{-1})	58.5	25.6	47.9	78.4	37.4	68.7	104.8	−25.8 (±40)
PO_4^{3-} (mg L^{-1})	53.9	ne	ne	ne	1.5	7.4	12.5	85.9 (±8.4)
SO_4^{2-} (mg L^{-1})	674.9	ne	ne	ne	25.1	55.8	72.1	86.6 (±19,5)
K^+ (mg L^{-1})	1733.2	ne	ne	ne	521.9	2041.8	3226.9	−19.6 (±72,2)

[a] The acidified effluent samples were passed through 0.45 μm membrane before the analyses.
[b] () = standard deviation.
[c] The raw vinasse pH was adjusted to 6.5 before color analysis.
[d] ne: not evaluated.

high in both the acclimation (88.0 ± 70.3 NTU) and 2-SAnMBR operation (99.4 ± 64.5 NTU) periods. This suggests the presence of micro-colloidal particles, in the size range from 0.01 to 0.45 microns that pass through the pores of the filters and through the membrane fibers. In addition the correlation between turbidity and color values of permeate was statistically significant according to Spearman test (Spearman's coefficient = 0.90, p-level < 0.0000). Since the increase of organic matter removal reached in the 2-SAnMBR operation period did not lead to an increase in the percentage removals of color and turbidity, it is likely that these micro-colloidal particles are, to some extent, recalcitrant and may interfere with color analysis.

Volatile total solids were largely removed, while fixed total solids remained almost unchangeable (Table 2). Among the inorganic nutrients that were analyzed in the raw vinasse and in the permeate, TN and K^+ appeared at slightly higher levels in the permeate

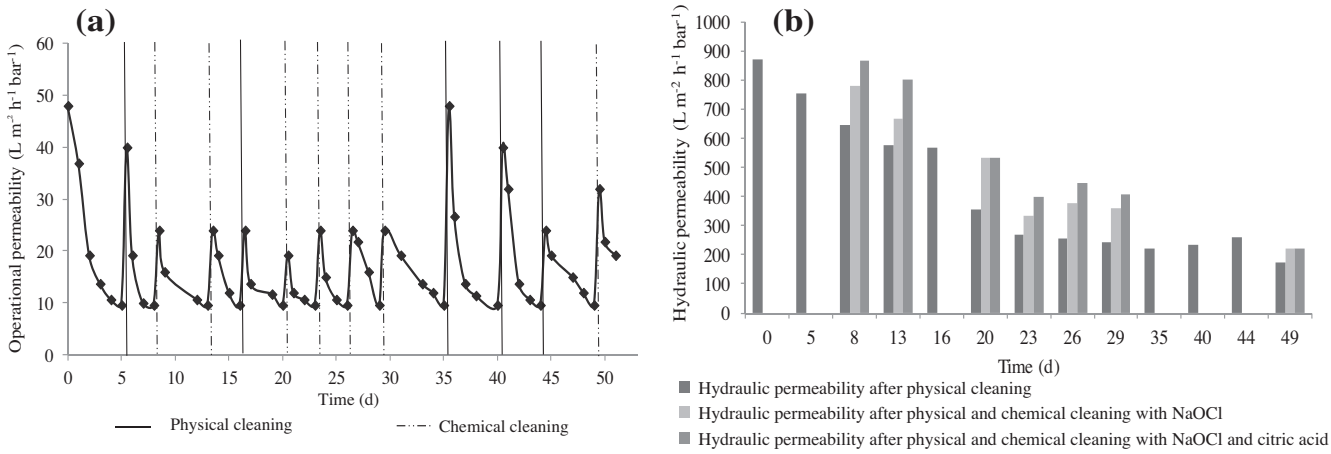

Fig. 5. (a) Operational permeability vs. membrane-cleaning procedures. (b) Effects of membrane cleaning-procedures on hydraulic permeability. Note: chemical cleaning was not performed on days 0, 5, 16, 35, 40 and 44.

than in vinasse. Since the samples had to be filtered (0.45 μm) before analysis, these results suggest hydrolysis and solubilization of nitrogen and potassium-rich compounds present in raw vinasse and in the biomass through microbial metabolism and cell lyses, and bioconversion of particulate organic nitrogen compounds to ammonia. In contrast, PO_4^{3-} and SO_4^{2-} were present in lower concentrations in the permeate. Phosphorus removal mainly occurred due to the biomass uptake and also precipitation as phosphate salts; and sulfate was likely to undergo reduction to sulfide through anaerobic respiration by sulfate-reducing bacteria.

3.3. Filtration performance in the 2-SAnMBR

In the first week, back-flush was applied for 15 s, for every 15 min of filtration. However, the formation of precipitates was observed in the permeate storage tank. As these relaxation instead of back-flush was applied to control membrane fouling during operation filtration. Biogas sparging was not done so that the system would consume minimum energy; also, this prevents the loss of dissolved methane in the effluent as well as the reintroduction of gases that could adversely affect acetogenesis (H_2), methanogenesis (H_2S) and the pH of the medium (CO_2). However, a drastic reduction in membrane permeability in the biological tank occurred throughout the operation period, thus requiring a routine cleaning of the membrane (Fig. 5(a)). Chemical cleaning with NaOCl and citric acid was no more effective to recover operational permeability than physical cleaning only. This reveals that removable fouling was the main factor in determining operational permeability and solids larger than the membrane pores were strongly affecting membrane filtration performance, since the physical cleaning procedure is a superficial treatment and can only remove the material accumulated on the membrane surface (Jeison and van Lier, 2007a). On the other hand, the chemical cleaning procedure contributed to the recovery of hydraulic permeability (Fig. 5(b)). Membrane-cleaning with citric acid, as compared to sodium hypochlorite only, promoted additional recovery of hydraulic permeability. This suggests that inorganic substances contribute to internal fouling.

The resistance-in-series model was applied in order to evaluate the role of each component of membrane resistance (Table 3). External fouling was the major mechanism of filtration resistance and, when it was evaluated, the strongly attached cake layer accounted for over 70% of the overall filtration resistance. These results are in agreement with those by Jeison and van Lier (2007a) that showed that cake formation was entirely governing the applicable flux in a submerged AnMBR treating acidified wastewater at mesophilic range. Cake layer formation in AnMBRs is most likely to occur due to operation at high biomass concentrations (Charfi et al., 2012); also due to the presence of high levels of fine colloidal matter (Shen, 2011) and inorganic precipitation that may strengthen the cake layer (Choo and Lee, 1996). Although internal fouling accounted for less than 5% of total membrane filtration resistance, it tended to increase over time. This was especially true for irreversible internal fouling (Table 3), which resulted in a progressive reduction of hydraulic permeability (Fig. 5(b)).

3.4. SMP and EPS

Analyses of SMP and EPS fractions were performed on the same days as the resistance-in-series measurements. Although the term "SMP" refers to products released by the microorganisms, this fraction may contain both partially- and non-converted substrates. The Pearson correlation and multiple regression tests were applied to verify the correlation of the internal fouling and cake layer resistances with the COD, protein and carbohydrate content in SMP and EPS. The nonparametric Spearman's rank correlation test was also applied to the results that did not have normal distribution. Higher correlation coefficients regarding membrane fouling were related to SMP and EPS protein-like substances. Fig. 6(a) presents the internal fouling resistance after each physical cleaning procedure, and the protein concentrations in total SMP and total EPS. According to the Pearson correlation test, there was a statistically significant correlation between internal fouling resistance and protein concentration in total SMP (p-level < 0.001), $SMP_{0.45}$ (p-level = 0.003), total EPS (p-level = 0.002) and $EPS_{0.45}$ (p-level = 0.001). The correlation between R_{if} and SMP protein was positive; the Pearson's coefficient was equal to 0.88 and 0.78 for total SMP and $SMP_{0.45}$, respectively. On the contrary, the Pearson's coefficient was equal to −0.79 and −0.81 for the correlation between R_{if} and protein concentration in total EPS and $EPS_{0.45}$, respectively. The Spearman test confirmed a significant negative correlation between total EPS protein and R_{if} (Spearman coefficient = −0.61, p-level = 0.037). Regarding carbohydrates, a statistically significant correlation was only found between R_{if} and total EPS carbohydrate, corresponding to a Pearson's coefficient of −0.71 (p-level = 0.009) and a Spearman's coefficient of −0.75 (p-level = 0.005). Concerning COD analyses, R_{if} had a moderate correlation only to total SMP COD, which was equivalent to a Pearson's coefficient of −0.65 (p-level = 0.022).

Table 3
A series of membrane resistances.

Time (d)	Type of cleaning	External fouling resistance[a,b,c]		Internal fouling resistance[a,b,c]		
		Total R_{ef} (m^{-1})	R_c (m^{-1})	Total R_{if} (m^{-1})	R_{rif} (m^{-1})	R_{iif} (m^{-1})
5	Physical	$3.52.10^{13}$ (98.7)	ne[d]	$6.25.10^{10}$ (0.2)	ne	ne
8	Chemical	$3.52.10^{13}$ (98.4)	ne	$1.45.10^{11}$ (0.4)	$1.43.10^{11}$ (0.4)	$1.39.10^{9}$ (0.0)
13	Chemical	$3.51.10^{13}$ (98.3)	ne	$2.09.10^{11}$ (0.6)	$1.73.10^{11}$ (0.5)	$3.60.10^{10}$ (0.1)
16	Physical	$3.51.10^{13}$ (98.2)	ne	$2.21.10^{11}$ (0.6)	ne	ne
20	Chemical	$3.47.10^{13}$ (97.2)	ne	$5.99.10^{11}$ (1.7)	$3.38.10^{11}$ (0.9)	$2.61.10^{11}$ (0.7)
23	Chemical	$3.44.10^{13}$ (96.2)	ne	$9.36.10^{11}$ (2.6)	$4.50.10^{11}$ (1.3)	$4.86.10^{11}$ (1.4)
26	Chemical	$3.43.10^{13}$ (96.1)	ne	$9.95.10^{11}$ (2.8)	$5.99.10^{11}$ (1.7)	$3.96.10^{11}$ (1.1)
29	Chemical	$3.42.10^{13}$ (95.9)	ne	$1.08.10^{12}$ (3.0)	$6.12.10^{11}$ (1.7)	$4.70.10^{11}$ (1.3)
35	Physical	$3.41.10^{13}$ (95.4)	$2.75.10^{13}$ (76.9)	$1.21.10^{12}$ (3.4)	ne	ne
40	Physical	$3.42.10^{13}$ (95.7)	$2.71.10^{13}$ (75.8)	$1.11.10^{12}$ (3.1)	ne	ne
44	Physical	$3.43.10^{13}$ (96.1)	$3.24.10^{13}$ (90.6)	$9.75.10^{11}$ (2.7)	ne	ne
49	Chemical	$3.37.10^{13}$ (94.2)	ne	$1.65.10^{13}$ (4.6)	$4.42.10^{11}$ (1.2)	$1.21.10^{12}$ (3.4)

[a] (): the percentage of total membrane filtration resistance.
[b] Total membrane filtration resistance: 3.57.1013 m^{-1} (P = 0.5 bar; J = 4.8 l m^{-2} h^{-1}; $\mu_{permeate}$ = 1.05 mPa s).
[c] New membrane resistance: 4.14–1011 m^{-1} (1.2% of Rt).
[d] ne: not evaluated.

At the same time, the extent of external fouling resistance due to cake layer consolidation (R_c), defined as the resistance due to cake formation that could not be removed by gentle membrane rinsing with tap water (measured on days 35, 40 and 44), seemed to be positively correlated to total EPS protein and inversely correlated to $SMP_{0.45}$ COD (Fig. 6(b)). Despite the few measurements, these correlations were statistically significant at the 95% confidence level. This corresponds to a Pearson's coefficient of 0.99 and p-level equal to 0.026 regarding R_c and total EPS protein; and, to a Pearson's coefficient of −0.99 and p-level equal to 0.014 regarding R_c and $SMP_{0.45}$ COD. The positive correlation between R_c and EPS protein, and the negative correlation between R_{if} and EPS protein, are probably because EPS contributes to the aggregation of the biomass, biofilm formation and sorption of organic and inorganic compounds (Flemming and Wingender, 2010), which may lead to the reduction of fouling by particles dispersed in the environment and to the strengthening of the cake layer. In addition, cake layer can prevent the occurrence of internal fouling and reduce the influence of membrane properties on filtration performance, since the membrane surface is no longer in direct contact with the suspension (Jeison and van Lier, 2007b).

Fig. 6(c) shows the content of protein-like substances, carbohydrates and COD present in SMP, EPS and also in acidified effluent, permeate, and raw and filtered vinasse. For acidified effluent and permeate, only those values of COD that corresponded to those analyzed on the days that the SMP and EPS fractions were assessed were considered. Due to the large differences among the samples, the values are in logarithmic scale.

The protein-like substances detected by Lowry's method in the acidified effluent were higher than those in the raw vinasse. This suggests that at least a fraction of the protein-like substances was released from the microbial metabolism. These results are in accordance with Wu and Zhou (2010) who showed that protein substances composed the majority of the SMPs and accumulated at the bottom of the UASB reactor treating distillery wastewater. This indicated that the acidogens might have contributed more to the production of SMPs, while the methanogens played a more important role in SMP consumption. Since the acidified effluent corresponds to the soluble fraction of the acidogenic reactor mixed liquor, the separation of the stages may have prevented higher levels of the protein-like substances released due to metabolism by the acidogenic bacteria in the methanogenic reactor, which also may have contributed to diminishing the membrane fouling caused by the protein-like substances. In regard to the other analyses of carbohydrates and proteins of the acidified effluent, permeate, SMP and EPS, it was not possible to distinguish the fractions coming from the microbial metabolism and from the substrate. As mentioned before, the term SMP is inaccurate since this fraction may contain compounds other than those released from microbial metabolism.

Despite the MLVSS concentration in the methanogenic reactor being stabilized at 13.5 gMLVSS L^{-1}, the average carbohydrate levels in $SMP_{0.45}$ equal to 58 ± 8 mg L^{-1} were close to those found by Martin-Garcia et al. (2011). They found 47 ± 14 mg L^{-1} in a flocculent AnMBR treating domestic wastewater at a sludge concentration of 7.7 gMLSS L^{-1} and an OLR of 0.5 gCOD L^{-1} d^{-1}. However, in terms of $SMP_{0.45}$ protein, the average of 451 ± 112 mg L^{-1} was much higher than reported by these authors, which was 108 ± 27 mg L^{-1}. This discrepancy can be attributed in part to the fact that most of the residual carbohydrate and protein-like substances were likely to be microbial products and non-converted substrate, respectively. This is supported by the fact that in the biodegradability tests performed before the operation, in which it was running in parallel reactors fed with glucose and vinasse under anaerobic conditions, the results showed that carbohydrates from vinasse were completely biodegradable, while 15–20% of protein-like substances detected by Lowry method from vinasse were recalcitrant (Mota et al., 2011). Also, the overall COD removal, ranging from 92% to 95% achieved in the biodegradability tests, suggests that the residual COD in permeate is mainly composed of recalcitrant compounds from vinasse and substances released by microbial metabolism.

TN concentration in the acidified effluent was lower than the concentration in the raw effluent (Table 2), in contrast to what was observed for protein-like substances (Fig. 6(c)). Also, the higher concentrations of TN were present in permeate, which showed the lowest concentrations of protein-like substances. Hence, these results suggest the presence of other nitrogen rich-compounds in permeate and/or the interference of non-nitrogen rich compounds in the Lowry's method, such as reducing agents and biosurfactants, that could lead to the overestimation of the protein levels in acidified effluent.

Although the pore sizes of the membrane were equal to the pore sizes of the filters used to separate the $SMP_{0.45}$ samples (0.45 μm), it was notable that the levels of COD, proteins and carbohydrates in permeate, corresponding to 465 ± 71 mg L^{-1}, 316 ± 89 and 39 ± 5 mg L^{-1}, respectively, were always inferior to those present in $SMP_{0.45}$. These differences were statistically significant according to the T-test for dependent samples ($p < 0.001$). As the COD levels in soluble SMP did not have normal distribution, the

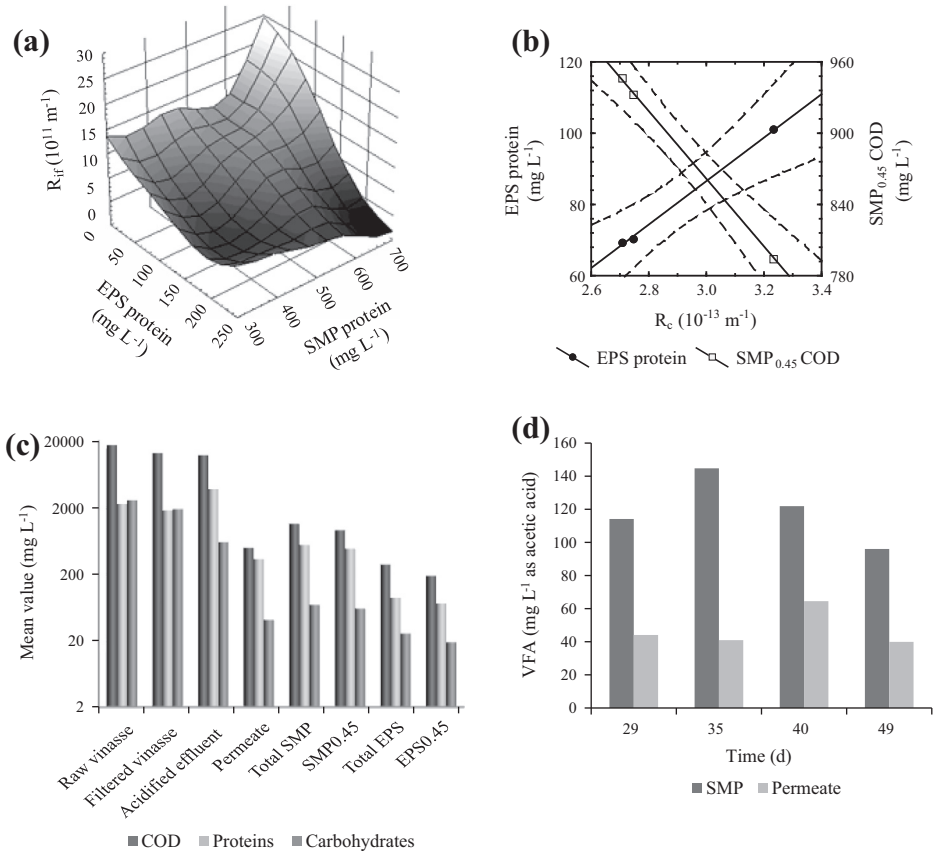

Fig. 6. (a) Total internal fouling resistance after physical cleaning-procedure vs. SMP protein and EPS protein. (b) Cake layer resistance measurements vs. SMP COD and EPS protein. (c) Levels of COD, protein and carbohydrate in vinasse, acidified effluent, permeate, SMP and EPS. (d) SMP and permeate VFA levels.

Wilcoxon Matched Pair Test was also applied, which confirmed the differences ($p = 0.001$). Although the VFA levels in the supernatant of the methanogenic reactor (SMP) were analyzed for only four samples, these levels were much higher than that in permeate (Fig. 6(d)). This phenomenon is explained by the additional retention and/or degradation of these compounds in the cake layer that acts a secondary dynamic membrane.

4. Conclusions

The strategy adopted for acclimation of acidogens and methanogens showed to be simple and effective. Not controlling the pH in the acidogenic reactor did not stop VFA production, and probably inhibited methanogenic activity in it. The 2-SAnMBR reached a satisfactory stability and treatment efficiency. Parts of the recalcitrant compounds included micro-colloidal particles, colored compounds and protein-like substances. Removable fouling resistance was the main mechanism affecting filtration performance. Regarding SMP and EPS, the most relevant effect on membrane resistance was related to protein like-substances. The acidogenic reactor exhibited the highest abundance of these compounds, suggesting the release of SMP by acidogens.

Acknowledgements

This research was financially supported by the Brazilian government through the provision of a personal scholarship from the National Council of Technological and Scientific Development (CNPq) and through support from the Research Support Foundation of the State of Minas Gerais (Fapemig). The authors acknowledge Pam Membranas Seletivas for supplying the experimental apparatus.

References

APHA, AWWA, WEF, 2005. Standards Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington DC, USA.
Beccari, M., Bonemazzi, F., Majone, M., Riccardi, C., 1996. Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. Water Res. 30, 183–189.
Buchauer, K., 1998. A comparison of two simple titration procedures to determine volatile fatty acids in effluents to wastewater and sludge treatment processes. Water SA 24, 49–56.
Charfi, A., Amar, N.B., Harmand, J., 2012. Analysis of fouling mechanisms in anaerobic membrane bioreactors. Water Res. 46, 2637–2650.
Choo, K.H., Lee, C.H., 1996. Membrane fouling mechanisms in the membrane-coupled anaerobic bioreactor. Water Res. 30, 1771–1780.
Cohen, A., Breure, A.M., van Andel, J.G., van Deursen, A., 1982. Influence of phase separation on the anaerobic digestion of glucose, 2. Stability and kinetic responses to shock loadings. Water Res. 16, 449–455.
De Bazúa, C.D., Cabrero, M.A., Poggi, H.M., 1991. Vinasses biological treatment by anaerobic and aerobic processes: laboratory and pilot-plant tests. Bioresour. Technol. 35, 87–93.
Dilallo, R., Albertson, O.E., 1961. Volatile acids by direct tritation. J. Water Pollut. Con. F. 33, 356–365.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, P., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Flemming, H.C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623–633.
Ghosh, S., Ombregt, J.P., Pipyn, P., 1985. Methane production from industrial wastes by two-phase anaerobic digestion. Water Res. 19, 1083–1088.
Gonzalez, T., Terron, M.C., Yague, S., Zapico, E., Galletti, G.C., Gonzalez, A.E., 2000. Pyrolysis/gas chromatography/mass spectrometry monitoring of fungal-biotreated distillery wastewater using *Trametes* sp. I-62 (CECT 20197). Rapid Commun. Mass Sp. 14, 1417–1424.

Ince, O., 1998. Performance of a two-phase anaerobic digestion system when treating dairy wastewater. Water Res. 32, 2707–2713.
Jeison, D., Plugge, C.M., Pereira, A., van Lier, J.B., 2009. Effects of the acidogenic biomass on the performance of an anaerobic membrane bioreactor for wastewater treatment. Bioresour. Technol. 100, 1951–1956.
Jeison, D., van Lier, J.B., 2007a. Cake formation and consolidation: main factors governing the applicable flux in anaerobic submerged membrane bioreactors treating acidified wastewaters. Sep. Purif. Technol. 56, 71–78.
Jeison, D., van Lier, J.B., 2007b. Thermophilic treatment of acidified and partially acidified wastewater using an anaerobic submerged MBR: factors affecting long term operational flux. Water Res. 41, 3868–3879.
Jeong, E., Kim, H., Nama, J., Shin, H., 2010. Enhancement of bioenergy production and effluent quality by integrating optimized acidification with submerged anaerobic membrane bioreactor. Bioresour. Technol. 101, S7–S12.
Ke, S., Shi, Z., Fang, H.H.P., 2005. Applications of two-phase anaerobic degradation in industrial wastewater treatment. Int. J. Environ. Pollut. 23, 65–80.
Lettinga, G., Vanvelsen, A.F.M., Hobma, S.W., Dezeeuw, W., Klapwijk, A., 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. Biotechnol. Bioeng. 22, 699–734.
Lowry, O.H., Rosenbrough, N.J., Farr, R.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Lun, S.Y., Wu, J., Chen, J., 1995. Anaerobic waste treatment: efficient separation of the acid and methane forming phases using two UASB reactors. Process Biochem. 30, 523–529.
Martin-Garcia, I., Monsalvo, V., Pidou, M., Le-Clech, P., Judd, S.J., Mcadama, E.J., Jefferson, B., 2011. Impact of membrane configuration on fouling in anaerobic membrane bioreactors. J. Membr. Sci. 382, 41–49.
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, 1489–1512.
Mota, V.T., Amorim, T.A., Amaral, M.C.S., 2011. Analysis of anaerobic biodegradation and residual organic compounds in sugarcane vinasse. Proceedings of the X latin American workshop and symposium on anaerobic digestion, Ouro Preto, Brazil. International Water Association, Londres.
Ripley, L.E., Boyle, W.C., Converse, J.C., 1986. Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. Res. J. Water Pollut. C. 58, 406–411.
Saddoud, A., Hassaïri, I., Sayadi, S., 2007. Anaerobic membrane reactor with phase separation for the treatment of cheese whey. Bioresour. Technol. 98, 2102–2108.
Saddoud, A., Sayadi, S., 2007. Application of acidogenic fixed-bed reactor prior to anaerobic membrane bioreactor for sustainable slaughterhouse wastewater treatment. J. Hazard. Mater. 149, 700–706.
Shen, L.H., 2011. Hydrogen (H_2) production and membrane fouling in fermentative H_2-producing membrane bioreactors. PhD thesis (Doctor of Philosophy). University of Toronto, Canada.
Spagni, A., Casu, S., Grilli, S., 2012. Decolourisation of textile wastewater in a submerged anaerobic membrane bioreactor. Bioresour. Technol. 117, 180–185.
Stuckey, D.C., 2012. Recent developments in anaerobic membrane reactors. Bioresour. Technol. 122, 137–148.
van Haandel, A.C., 2005. Integrated energy production and reduction of the environmental impact at alcohol distillery plants. Water Sci. Technol. 52, 49–57.
Wijekoon, K.C., Visvanathan, C., Abeynayaka, A., 2011. Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor. Bioresour. Technol. 102, 5353–5360.
Wilkie, A.C., Riedesel, K.J., Owens, J.M., 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass Bioenergy 19, 63–102.
Wu, B., Zhou, W., 2010. Investigation of soluble microbial products in anaerobic wastewater treatment effluents. J. Chem. Technol. Biotechnol. 85, 1597–1603.
Yeoh, B.G., 1997. Two-phase anaerobic treatment of cane-molasses alcohol stillage anaerobic digestion VIII. Water Sci. Technol. 36, 441–448.