

# Anaerobic digestibility of marine microalgae *Phaeodactylum tricornutum* in a lab-scale anaerobic membrane bioreactor

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**Abstract** The biomass of industrially grown *Phaeodactylum tricornutum* was subjected in a novel way to biomethanation at 33°C, i.e., in an anaerobic membrane bioreactor (AnMBR) at a hydraulic retention time of 2.5 days, at solid retention times of 20 to 10 days and at loading rates in the range of 2.6–5.9 g biomass-COD L<sup>-1</sup> day<sup>-1</sup> with membrane fluxes ranging from 1 to 0.8 L m<sup>-2</sup> h<sup>-1</sup>. The total COD recovered as biogas was in the order of 52%. The input suspension was converted to a clear effluent rich in total ammonium nitrogen (546 mg TAN L<sup>-1</sup>) and phosphate (141 mg PO<sub>4</sub>-P L<sup>-1</sup>) usable as liquid fertilizer. The microbial community richness, dynamics, and organization in the reactor were interpreted using the microbial resource management approach. The AnMBR communities were found to be moderate in species richness and low in dynamics and community organization relative to UASB and conventional CSTR sludges. Quantitative polymerase chain reaction analysis revealed that *Methanosaeta* sp. was the dominant acetoclastic methanogen species followed by *Methanosarcina* sp. This work demonstrated that the use of AnMBR for the digestion of algal biomass is possible. The fact that some 50% of the organic matter is not liquefied means that the algal particulates in the digestate constitute a considerable fraction which should be valorized properly, for

instance as slow release organic fertilizer. Overall, 1 kg of algae dry matter (DM) could be valorized in the form of biogas (€2.07), N and P in the effluent (€0.02) and N and P in the digestate (€0.04), thus totaling about €2.13 per kilogram algae DM.

**Keywords** Algae biomass · Mesophilic · Green energy · Biogas · Methane · Microbial resource management

## Introduction

It has been estimated that by the year 2020, biomass will make up for approximately two thirds of the primary renewable energy consumption in Europe (Kautto et al. 2011). The biological conversion of biomass to methane (CH<sub>4</sub>) by anaerobic digestion is considered as one of the most promising technologies in this respect (Verstraete et al. 2005).

Biomass can be classified according to its sources (Gunaseelan 1997). Terrestrial sources include mechanically sorted and hand-sorted municipal solid waste, various types of fruit and vegetable solid wastes, leaves, grass, wood and weed. Aquatic sources cover both marine and freshwater biomass. Aquatic organisms such as microalgae have recently received considerable attention as an alternative feedstock for bio-methanation because these are capable to fixate CO<sub>2</sub> and have certain advantages over terrestrial biomass (Rodolfi et al. 2009; Waltz 2009; Wang et al. 2008). In addition, it has been pointed out that with high productivities (>90 ton dry matter (DM) ha<sup>-1</sup> year<sup>-1</sup>), high conversion efficiencies (>70%), and by using high-rate anaerobic digesters, the production of energy from microalgae biomass can have potential (Zamalloa et al. 2011a). The potential of using microalgae as feedstock can be

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boosted by using wastewater as a source of nitrogen and phosphorus for their cultivation, allowing the recovery of nutrients that can be used as natural fertilizers (Lundquist et al. 2010; Zamalloa et al. 2011a).

Continuous bio-methanation of microalgae has been reported to achieve methane yields ranging between 0.1 and 0.4 LCH<sub>4</sub> g<sup>-1</sup> VS (0.07–0.27 LCH<sub>4</sub> g<sup>-1</sup> COD) which are equivalent to conversion efficiencies of 17–76% at loading rates of about 1.5–5 g COD L<sup>-1</sup> day<sup>-1</sup> (Sialve et al. 2009). Yet, the digestibility is strongly species dependent (Mussgnug et al. 2010; Zamalloa et al. 2011b). The hydraulic retention time (HRT) required for an adequate digestion is between 10 and 30 days in conventional digesters (Sialve et al. 2009). In conventional continuously stirred anaerobic digesters, the solids retention time (SRT) is identical to the HRT. This requires a large reactor volume since a long SRT is needed for the effective destruction of volatile solids. An anaerobic membrane bioreactor (AnMBR) uses a membrane to separate the solids from the sludge suspensions. Therefore, an AnMBR allows operation at a long SRT (up to 50 or more days) due to low biomass wasting rates. This favors the growth of slow-growing microorganisms such as methanogens and also increases the fraction of fermented organic matter (Grundestam and Hellström 2007; Sharrer et al. 2011). Thus, membrane technology allows to control the process better and to produce less residual sludge. It particularly provides a better effluent quality (Gao et al. 2010). Membrane technology is now regarded as a promising process for microalgae separation (Rossi et al. 2008; Rossignol et al. 1999), but it has not been described before in combination with the anaerobic digestion of microalgae biomass. In addition, there is a lack of knowledge regarding the microbial populations in charge of the anaerobic fermentation of microalgae biomass.

In this study, we investigate the performance of the digestion of the marine microalgae *Phaeodactylum tricorutum* in an AnMBR at mesophilic conditions, at moderate organic loading rates and a relatively low hydraulic retention time. The denaturing gradient gel electrophoresis (DGGE) and quantitative polymerase chain reaction (qPCR) were used to (1) determine the relative abundance of the microbial and archaeal populations in the reactors in order to have a better understanding of the bacterial and archaeal communities digesting algae biomass and (2) to apply the microbial resource management (MRM) approach.

## Material and methods

### Algae biomass

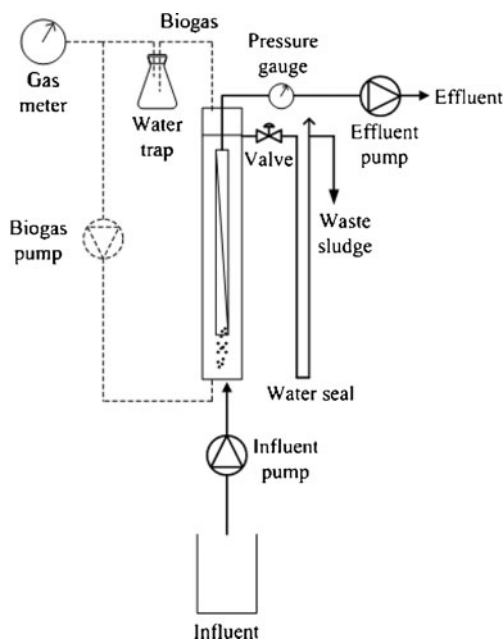
Dry lyophilized biomass of the marine microalga *P. tricorutum* (SBAE, Belgium) was fed to the anaerobic digestion reactor at the respective volumetric organic loading rates (Bv values). *P. tricorutum* (CCAP1055/1) was obtained from the culture collection of the Laboratory of Protistology and Aquatic Ecology, Ghent University. Tap water was used to re-suspend the biomass. Characteristics of the algae biomass are presented in Table 1.

### Anaerobic membrane bioreactor set-up

The anaerobic membrane bioreactor (AnMBR) consisted of a reactor (8 L working volume) made of acrylic panels and a Kubota (Kubota Co. Japan) microfiltration membrane module (0.12 m<sup>2</sup>, 0.4 μm pore size) arranged in parallel (Fig. 1). Two peristaltic pumps (Watson Marlow 313S;

**Table 1** Characteristics of the influent during the experimentation period (*n.d.* not determined)

Parameter	Phase 1 ( <i>n</i> =10)	Phase 2 ( <i>n</i> =15)	Phase 3 ( <i>n</i> =15)
Substrate	Glucose	Algae biomass	Algae biomass
pH	7.09±0.23	7.25±0.17	7.16±0.08
Total chemical oxygen demand, COD (g O <sub>2</sub> L <sup>-1</sup> )	22.5±0.5	16.0±0.3	15.2±0.4
Total Kjendahl nitrogen, TKN (mg L <sup>-1</sup> )	<i>n.d.</i>	1,071±66	999±160
Total phosphorous, TP (mg L <sup>-1</sup> )	<i>n.d.</i>	223±6	236±38
Total solids, TS (g L <sup>-1</sup> )	<i>n.d.</i>	11.0±0.7	10.6±2.5
Volatile solids, VS (g L <sup>-1</sup> )	<i>n.d.</i>	8.1±0.4	8.4±2.1
Ash (g L <sup>-1</sup> )	<i>n.d.</i>	2.9±0.2	2.2±0.5
Conductivity (ms cm <sup>-1</sup> )	<i>n.d.</i>	5.6±0.9	4.7±0.6
TS/VS ratio	<i>n.d.</i>	1.4±0.2	1.4±0.3
COD/VS ratio	<i>n.d.</i>	1.7±0.3	1.9±0.3
COD/N ratio	100/2.5	14.7±2.8	14.7±0.3
COD/P ratio	100/0.25	69.0±10.9	70.2±9.4



**Fig. 1** Scheme of the 8 L lab-scale experimental set-up of the AnMBR

Watson-Marlow Inc., Massachusetts, US) were individually used to feed influent into the anaerobic reactor and withdraw permeate from the reactor. Mixing and membrane scouring to control cake formation were obtained by biogas recirculation using a diaphragm gas pump (KNF, NMP850) through a diffuser located just below the membrane module. The biogas flow rate was approximately  $5 \text{ L min}^{-1}$  ( $0.6 \text{ L min}^{-1} \text{ L}^{-1}$  reactor), which resulted in a superficial velocity of  $16 \text{ m h}^{-1}$  (calculated considering the transversal area of the reactor). Biogas production was measured by means of a gas meter. Transmembrane pressure (TMP) was measured with an analogical gauge (Cole-Parmer International) installed between the membrane module and the permeate pump.

The reactor was inoculated with 500 mL ( $13 \text{ g VSS L}^{-1}$ ) of granular seed sludge harvested from a full-scale anaerobic digester treating potato-processing wastewater (Mydibel, Belgium). The pH, temperature, chemical oxygen demand (COD), volatile fatty acids (VFA), and biogas

production were monitored continuously and reported at STP conditions.

### Experimental operation

The operation of the reactor system was divided into three phases (Table 2): phase 1 (0–20th day) can be characterized as the start-up period; phase 2 (21st–48th day), and phase 3 (49th–70th day) are considered as the experimental period.

#### Start-up

Prior to microalgae biomass experiments, the AnMBR reactor was run with glucose as a substrate for the start-up period of 20 days. During the last days of the start-up period, the reactor was operated at a maximum organic loading rate (Bv) of  $10.4 \pm 0.3 \text{ g COD L}^{-1} \text{ day}^{-1}$  with a COD removal efficiency of 90% and a biogas production rate of  $4.5 \pm 0.1 \text{ L}_{\text{biogas}} \text{ L}_{\text{reactor}}^{-1} \text{ day}^{-1}$ , which indicates a good performance. Thereafter, the reactor was switched to using microalgae biomass as the only substrate.

#### Experimental period

Two experimental runs were carried out in the AnMBR. Only algae biomass without pre-treatment was used for the entire experimental period. The Bv values were varied by changing the COD concentration. During phase 2, the Bv (on COD basis) was increased to a maximum of  $5.8 \pm 0.9 \text{ g COD L}^{-1} \text{ day}^{-1}$ . The HRT was kept constant at about 2.6 days, and the SRT was set to be higher than 20 days. This experiment was performed to estimate the maximum concentration of mixed liquor suspended solid (MLSS) capable to be handled by the reactor.

During the first 49 days, no sludge was extracted (except for a small amount for the measurement of solids). Thereafter, due to a high increase in TMP, sludge was extracted in order to control the MLSS according to TMP measurements: the MLSS was set to be approximately  $23.3 \text{ g L}^{-1}$  with an SRT of 10 days (phase 3). During both phase 2 and phase 3, the pH was controlled to be around 7–

**Table 2** Operational parameters during the start-up period and experimental period of the AnMBR

Parameter	Phase 1 ( $n=20$ )	Phase 2 ( $n=20$ )	Phase 3 ( $n=20$ )
Duration (day)	20	28	22
Substrate	Glucose	Algae biomass	Algae biomass
Organic loading rate, Bv ( $\text{g COD L}^{-1} \text{ day}^{-1}$ )	$1.0 \pm 0.2$ – $10.4 \pm 0.3$	$1.0 \pm 0.2$ – $5.8 \pm 0.9$	$6.0 \pm 0.6$
Hydraulic retention time, HRT (days)	$2.5 \pm 0.4$	$2.6 \pm 0.4$	$2.5 \pm 0.1$
Solid retention time, SRT (days)	>20	>20	$8.6 \pm 1.3$
Membrane flux ( $\text{L m}^{-2} \text{ h}^{-1}$ )	$1.1 \pm 0.2$	$1.0 \pm 0.2$	$0.8 \pm 0.2$

7.5 by supplying a buffer (NaHCO<sub>3</sub>) of 0.5 g NaHCO<sub>3</sub> per gram of COD when necessary.

DNA extraction, polymerase chain reaction amplification, and DGGE analysis

DNA extraction and cleaning was performed according to Boon et al. (2000). Gel electrophoresis was performed to confirm DNA presence. Total bacterial primers (P338f with GC-clamp and P518r) (Øvreas et al. 1997) that target all bacteria (to obtain DNA amplicons for further analysis by DGGE) were used. PCRs were performed according to the protocol of Boon et al. (2002). An INGENY phorU2X2 DGGE-system (Goes, The Netherlands) was used for running 8% (w/v) polyacrylamide DGGE gels with a denaturing gradient ranging from 40% to 60% (Bodelier et al. 2005). The obtained DGGE patterns were subsequently processed using Bionumerics software version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium). For the interpretation of the results, three levels of analysis were carried out (Marzorati et al. 2008; Read et al. 2011; Wittebolle et al. 2009): (1) the range-weighted richness (Rr), (2) the dynamics of change (Dy), and (3) community organization (Co).

DNA extraction and quantitative polymerase chain reaction analysis

DNA extraction and quantitative polymerase chain reaction (qPCR) analysis were performed by Bioclear (Groningen, The Netherlands). DNA was extracted using a bead beating method. The DNA was purified using a modified protocol based on a silica-based column and eluted in 50 µL TE.

qPCR analyses were performed for *Methanosaeta* and *Methanosarcina* using modified primers (*Methanosaeta* primers: M0075P0547, *Methanosarcina* primers: P0542P0543). DNA amplification was performed through initial denaturation for 3 min at 94°C, followed by 40 cycles of amplification (30 s denaturation at 94°C; 30 s annealing at 62°C; 1 M elongation at 72°C), and 5 min at 72°C to complete elongation, using the IQ supermix kit from BioRad (Nazareth, Belgium). The limit of detection was in the order of 10<sup>3</sup> 16S rRNA gene copies for all applied primer sets.

Analytical procedures

Liquid samples were taken daily or every second day from the anaerobic reactors. The *n* values reported in the results section refer to the number of samples analyzed through time. Total solids (TS), volatile solids (VS), suspended solids (SS), volatile suspended solids (VSS), total Kjeldhal nitrogen (TKN), total ammonia nitrogen (TAN), chemical

oxygen demand (COD), pH, and conductivity were determined according to Standard Methods (APHA-AWWA-WPCF 1998). Volatile fatty acids (VFA) were, after extraction in diethyl ether, analyzed with a flame ionization detector (FID) gas chromatograph (GC-2014, Shimadzu). Detection limit for VFA analysis was 2 mg L<sup>-1</sup>. Gas chromatography (GC-14B, Shimadzu) with an FID was used for biogas analysis. Gas detection tubes (RAE systems Inc., USA) were used for the detection of hydrogen sulphide and ammonia in the biogas.

## Results

Reactor performance

The results of the operation of the AnMBR reactor are shown in Fig. 2 starting on the first day of algae biomass fermentation (phase 2), i.e., the 21st day of the AnMBR reactor operation. During this phase, the Bv was increased from 1.3±0.4 to a maximum of 5.8±0.9 (Fig. 2a). During this period, the biogas production levels were stabilized at 48.6±7.2% of the maximum theoretical production (on COD basis; 0.35 L CH<sub>4</sub> g<sup>-1</sup> COD converted). Nitrogen (N) and phosphorous (P) concentrations in the influent increased as the Bv increased (Fig. 2c, d). Total ammonium nitrogen (TAN) in the effluent was up to 512±65 mg TAN L<sup>-1</sup>. Total P in the effluent had attained a maximum concentration of 147±43 mg L<sup>-1</sup>.

During phase 3, the biogas production was also stable at 57.0±6.1% of the maximum theoretical production. The TAN concentration in the effluent was on average 546 ± 48 mg TAN L<sup>-1</sup> (Fig. 2c). The concentration of P in the effluent was on average 141±41 mg L<sup>-1</sup>.

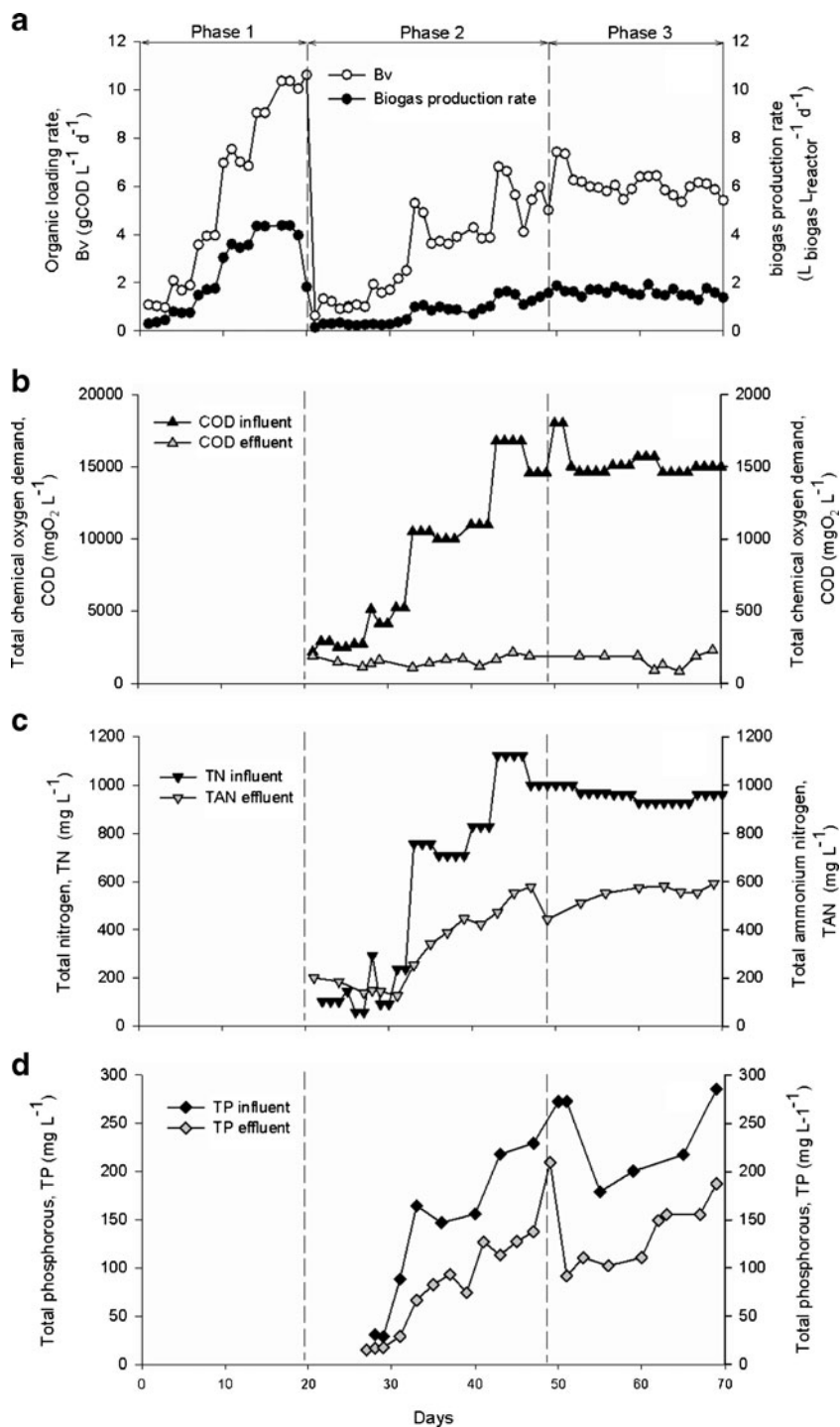
During the experimental period, phase 2 and 3, the residual COD in the effluent COD was below 200 mg L<sup>-1</sup> (Fig. 2b). In addition, the residual short chain fatty acid (VFA) concentration in the effluent was minimal (below detection limit of the method, i.e., <2 mg L<sup>-1</sup>), the sugars content was 4.7±1.1 mg C L<sup>-1</sup> (data not shown), and the conductivity was 7.6 ± 0.2 ms cm<sup>-1</sup>. The pH was kept stable between 7.2 ± 0.2.

The gas composition during the experimental period remained fairly stable; 75.3±2.6% of methane (CH<sub>4</sub>), 24.8±2.6% of carbon dioxide (CO<sub>2</sub>), and traces of hydrogen sulphide (H<sub>2</sub>S) reaching a maximum average of 0.41±0.05%. No ammonia and H<sub>2</sub> were detected in the biogas.

Membrane performance

Membrane performance was characterized by recording transmembrane pressure (TMP) values and fluxes values (Fig. 3) during the experimental period. During the start-up

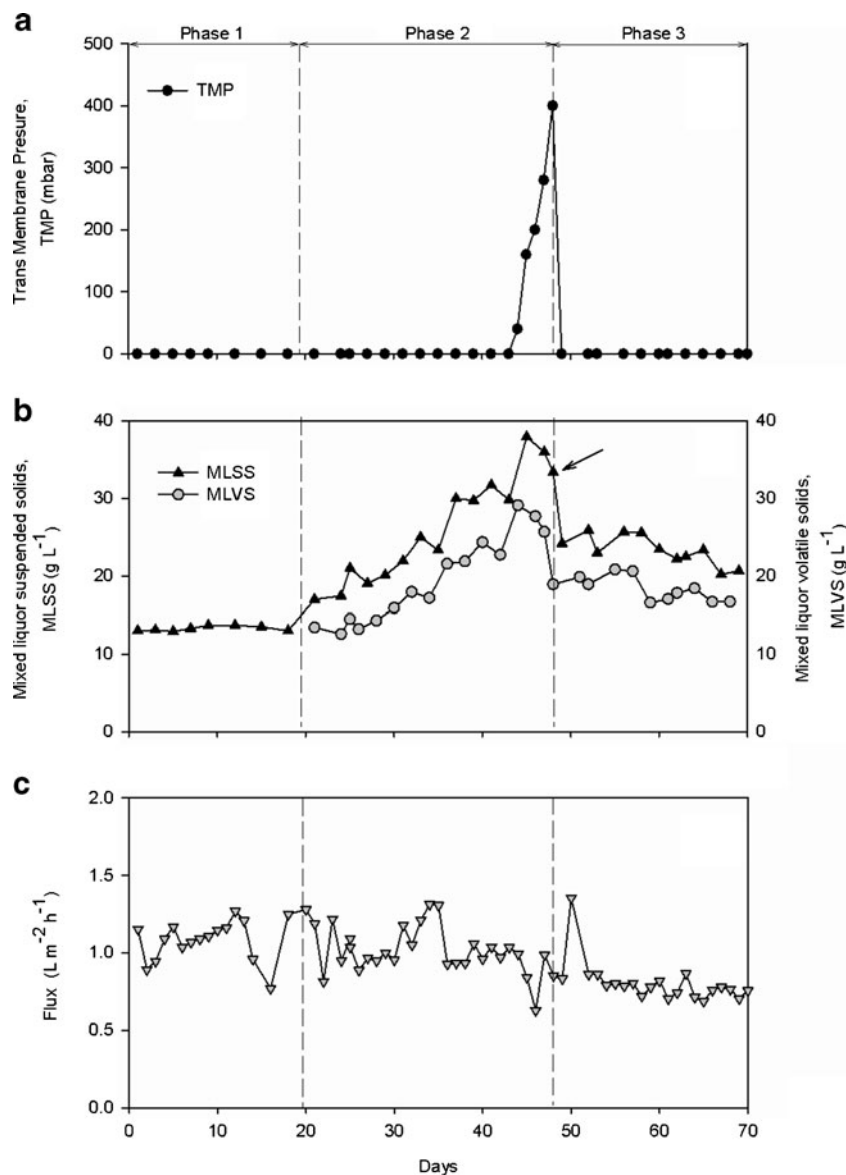
**Fig. 2** Performance of AnMBR reactor during the experimental period; loading rate, Bv, and biogas production rate (a); influent and effluent total COD (b); influent total nitrogen and effluent total ammonium (c) and influent and effluent total phosphorous



phase, the TMP values remained low at 0 mbar with an average flux of  $1.1 \pm 0.2 \text{ L m}^{-2} \text{ h}^{-1}$  (Table 2) and mixed liquor suspended solids (MLSS) of about  $13 \pm 0.2 \text{ g L}^{-1}$ . During phase 2, the TMP showed an abrupt increase to about 400 mbar with a low flux of about  $0.5 \text{ L m}^{-2} \text{ h}^{-1}$ . The MLSS continuously increased up to  $35 \text{ g L}^{-1}$  during the first 49 days due to (1) no withdrawal of sludge and (2) the accumulation of algae biomass in the reactor. Thereafter,

due to the TMP build-up, filtration was switched off, and some MLSS was withdrawn in order to decrease this concentration to approximately  $23.3 \pm 1.9 \text{ g L}^{-1}$ . At this MLSS concentration, the TMP returned to its initial low value of 0 mbar (Fig. 3). During phase 3, the MLSS was controlled by decreasing the flux to  $0.8 \pm 0.2 \text{ L m}^{-2} \text{ h}^{-1}$ , keeping the influent flow rate constant and wasting the difference of MLSS. Concomitantly, the SRT was calculat-

**Fig. 3** Evolution of trans membrane pressure, TMP (a); evolution of the solids in the reactor measured as mixed liquor suspended solids, MLSS (b); the arrow indicates MLSS withdrawal and evolution of the permeate flux (c)



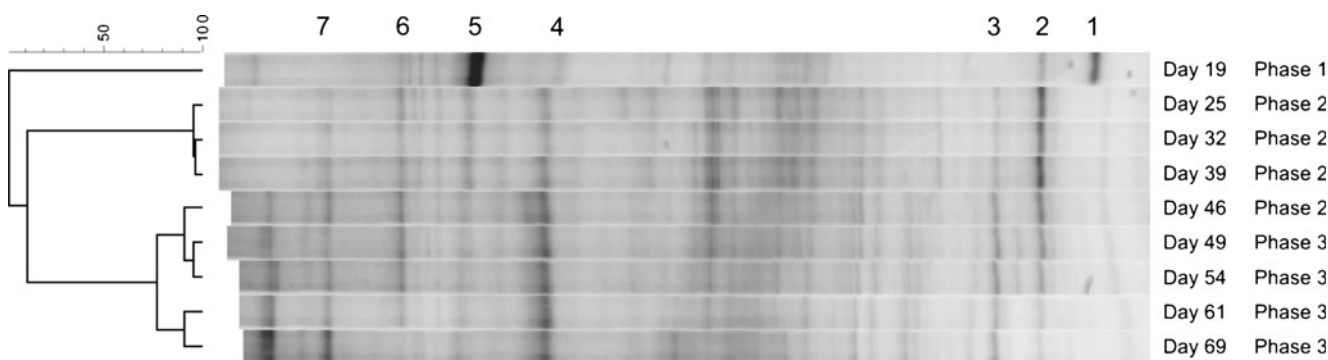
ed to be  $8.6 \pm 1.3$  days. For these conditions, the TMP was kept at 0 mbar for the rest of the test period (Fig. 3).

#### Effect of microalgae biomass on the bacterial community

The DGGE pattern indicated a strong shift in the microbial community structure during the operational time (Fig. 4). A total of 17 detectable DGGE bands were observed at the end of the start-up phase on day 19. In phase 2, 23 bands were detected after day 46. The number of detectable bands did not strongly change (24 bands) at the end of phase 3 on day 69 compared with the previous phase. Of these, seven predominant bands were identified. Some band intensities decreased (i.e., bands 1, 2, and 5) and other band intensities increased (i.e., bands 3, 4, 6, and 7) during the whole operational period. Cluster analysis of the DGGE finger-

prints indicated that after changing the substrate from glucose (day 19) to algae biomass, specific bacterial populations became dominant. This was illustrated by a low similarity (30%) (Fig. 4). Analysis of the fingerprint similarity revealed that the presence of algae as the only substrate for the fermentation process clearly affected the bacterial community composition. However, when the community had been established, there were no further strong changes in the community during the experimental period (day 19 to day 69) as shown by the high similarity (>90%).

The range-weighted richness index (Rr) was calculated from the DGGE pattern of each sampling point (i.e., each lane in the gel) to characterize the communities in the AnMBR (Fig. 5a). When using glucose as a carbon source, the Rr was relatively low (Rr=17, day 19). When



**Fig. 4** Cluster analysis of DGGE fingerprints of bacterial community in the AnMBR reactor at different sampling times (day 19 to day 69). Cluster analysis (WARD algorithm) of the DGGE patterns was performed based on the Pearson correlation and expressed as percentage

submitting algae biomass to digestion, the  $R_r$  values slightly increased but kept the same level over time ( $R_r=21$ –24, day 25–69).

The values of the dynamics of change (Dy) were calculated based on the comparison tool moving-window analysis by correlating between similarities at a specific sampling point (Fig. 5b). The Dy value was high just after switching the substrate from glucose to algae biomass (Dy=70% per 7 days, day 25). The Dy kept fairly constant during the experimental period, i.e., about 10% per sampling point.

The community organization (Co) coefficient was estimated based on the Gini value. Relatively high Co was observed at the end of the start-up period (Co=50, day 19). During the experimental period, Co values were lower than at the start-up. By the end of phase 2, the Co coefficient decreased from 34 on day 25 down to 24 on day 49. By the end of phase 3, Co values slightly increased up to 28 on day 69.

#### Effect of microalgae biomass on the archaea community

*Methanosaeta* and *Methanosarcina* were quantified using qPCR (Fig. 6). Results indicated that *Methanosaeta* sp. copy numbers were about  $10^9$  copies per gram of sample at the end of the start-up (phase 1) and  $10^8$  copies per gram of sample at the end of phase 3 (day 69). Thus, *Methanosaeta* sp. 16S rRNA gene copies constituted the highest percentage (99%) of the total 16S rRNA gene copies of the acetoclastic methanogens in the start-up phase. This high percentage of *Methanosaeta* sp. 16S rRNA gene copies remained at the same level during phase 2 (day 25–46) with a slight decrease (93%) by the end of phase 3 (day 69). Compared to *Methanosaeta* sp., only few *Methanosarcina* sp. 16S rRNA gene copies ( $\leq 10^7$ ) were detected during the whole experimentation period.

It can be observed that by the end of phase 3 (day 69), the total amount of acetoclastic methanogens decreased in concentration compared with the initial concentration (start-up, day 19). Their levels decreased from approximately  $10^9$

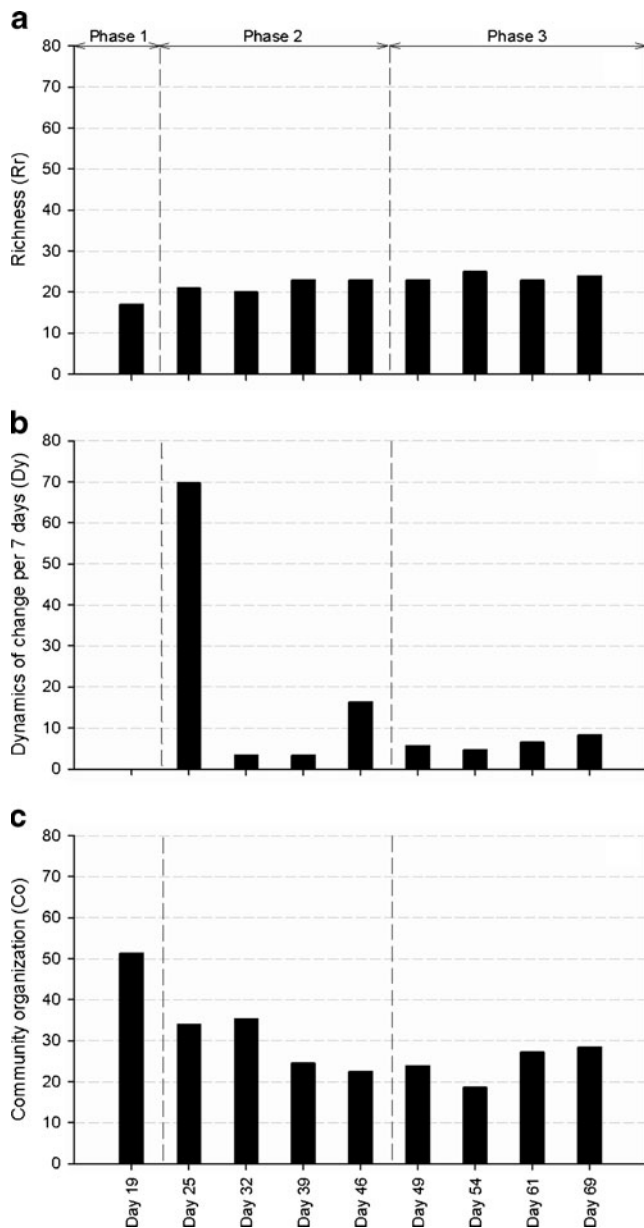
per gram of sample in the start-up phase to  $10^8$  per gram of sample 16S rRNA gene copies at the end of phase 3.

## Discussion

The influence of algae biomass in the performance of the AnMBR

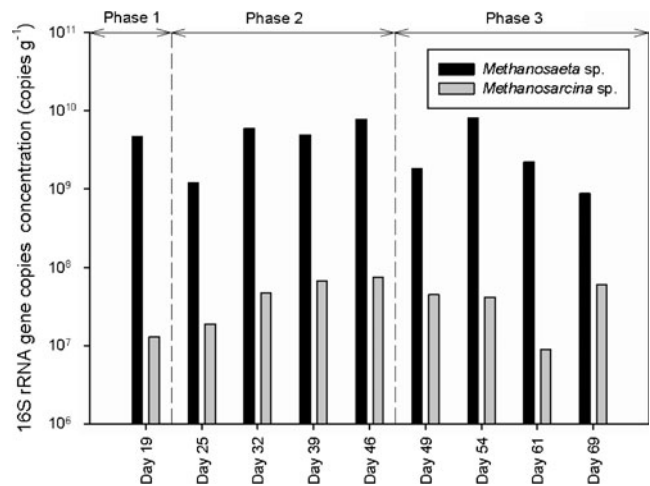
The AnMBR reactor was operated for 50 days in two phases using microalgae *P. tricornutum* as substrate. A steady state was achieved during each phase of this study. The pH in the reactor was kept stable during the whole period of operation at 7–7.5 which is considered as the most appropriate range for digestion. The performance of the reactor fermenting the algae was constant with about  $52.2 \pm 0.1\%$  COD removed (phase 2 and 3). This indicated a good stability of the process. It must be noted that the SRT in phase 2 can be estimated to be on average 25 days (on the basis of biomass removal by mixed liquor withdraw) and in phase 3 on average 10 days. The performance obtained in this study was comparable to the ones obtained in our previous study using the same microalgae strain and using a hybrid reactor (combining a sludge blanket and a carrier bed) operated with a Bv of  $1.9 \text{ g COD L}^{-1} \text{ day}^{-1}$  and an HRT of 2.2 days (Zamalloa et al. 2011b). The biogas production was constant with a composition of about 70% of  $\text{CH}_4$  and 0.4% of  $\text{H}_2\text{S}$  and without detectable concentrations of  $\text{H}_2$  and  $\text{NH}_3$ .  $\text{H}_2\text{S}$  is a corrosive gas and can damage energy co-generation equipment or other installations. If the concentration of  $\text{H}_2\text{S}$  is higher than 250 ppm (0.025%), it is recommendable to treat the gas prior to combustion (Gayh et al. 2010). Thus, the biogas will need a selective removal of  $\text{H}_2\text{S}$  gas.

The obtained effluent (permeate) quality was good with no detectable concentrations of suspended solid and with low residual COD (see supporting material Fig. S1). Low concentrations of VFA (data not shown) and soluble COD were obtained which indicated that the soluble COD was due to particulates. The low concentrations of VFA also



**Fig. 5** Comparisons between microbial parameters from DGGE. **a** Richness (Rr), **b** dynamics of change per sampling point (Dy), and **c** community organization (Co)

indicated that the HRT applied is sufficient to allow the conversion to methane. During digestion, N and P are released from the solid phase into the liquid phase, and ammonium was detected as the main nitrogen form in the liquid phase. The N released (as TAN) in the effluent accounted for  $47.4 \pm 9.7\%$  of the ingoing total N. Total P had a mineralization of  $50 \pm 11.2\%$ . N and P mineralization levels were consistent with the COD conversion matching the mass balance. The applied HRT not only allowed for a good conversion of the readily biodegradable fraction of the influent but also kept the concentration of TAN below an inhibitory concentration.



**Fig. 6** Results of group-specific quantitative PCR of the DNA samples. 16S rRNA gene copies numbers were determined specific for *Methanosaeta* sp. and for *Methanosarcina* sp.

The MLSS concentration was initially  $13 \text{ g L}^{-1}$  and then gradually increased to  $35 \text{ g L}^{-1}$  during phase 2 (Fig. 3b). At this MLSS concentration, the TMP reached a maximum of 400 mbar which was considered the maximum that can be handled by this Kubota membrane (Kubota Co. Japan). Lubbecke et al. (1995) and Ueda et al. (1996) observed that at MLSS concentrations higher than  $30 \text{ g L}^{-1}$ , the negative impact on the TMP was more related to the viscosity of the mixed liquor. During phase 3, the MLSS was decreased (by wasting part of the sludge) to a practical concentration (i.e.,  $23.3 \text{ g L}^{-1}$ ), and this concentration was kept constant in the reactor (by opening valve #1 to waste the appropriate amount of sludge, see Fig. 1). The TMP returned to 0 mbar without any extra cleaning, which demonstrated that the membrane fouling was reversible. Scouring by gas bubbling was thus sufficient to restore membrane filtration. These results indicate that the MLSS concentration has a direct impact on the flux through the membrane. MLSS concentration plays an important role in the cake formation resulting in a TMP increase or permeate flux decrease (Chang et al. 2001; Shimizu et al. 1993). Although a wide range of MLSS in AnMBR treating high solid wastes (sludge, pig manure, slaughterhouse effluent, etc.) are reported in literature, i.e., between 10 and  $40 \text{ g MLSS L}^{-1}$  (Liao et al. 2006), below  $25 \text{ g L}^{-1}$  of MLSS seemed to be adequate in this study to avoid membrane fouling. The operational fluxes were quite low ( $0.8 \text{ L m}^{-2} \text{ h}^{-1}$  in phase 3), but this was related to the imposed HRT and can possibly be increased provided the level of MLSS in the reactor is monitored.

The influence of algae biomass on the bacterial community dynamics

The behavior of microbial communities in function of time was studied in the AnMBR fermenting the microalgae



suspension. DGGE fingerprinting results (Fig. 4) indicated that bacterial populations changed more significantly in the reactor when changing the substrate (day 19). For instance, intensities of band 1 and 5 decreased significantly after the substrate change which suggests that these two species are the most metabolically active bacteria of the community. This finding correlated with the observation of Fernández et al. (1999) and Delbès et al. (2000) that few major bacteria are dominant when anaerobic reactors are fed only with glucose. Furthermore, slight differences in the predominant bacterial populations were also observed in phase 2 and phase 3 when the substrate was only algae biomass. For example, bands 2 and 6 decreased in intensity, whereas bands 3 and 7 increased in intensity by the end of phase 3, presumably reflecting the change in the SRT. Variations in operational conditions such as organic loading rate have been reported to contribute to changes in the bacterial populations in anaerobic reactors (Fernández et al. 1999; McHugh et al. 2004). However, as pointed out by Delbès et al. (2000), several factors could have affected the bacterial activity and interfered with the DGGE results, such as (1) the time necessary for metabolic change; (2) the management of the ribosome pool, and (3) the applied substrate concentrations.

The parameters of analysis estimated to interpret the community composition, dynamics, and organization were  $R_r$ ,  $D_y$ , and  $C_o$ . The utilization of algae biomass had an impact on the bacterial community structure in the AnMBR, which was characterized by a medium  $R_r$  during the experimental period (i.e., average 22). It appears that the bacterial community was quite specialized compared to other digestion systems with higher  $R_r$  (>20) (Carballa et al. 2011; Pycke et al. 2011).

The  $D_y$ , based on moving window analysis, demonstrate that bacterial communities evolve with time. For instance, the analysis of samples taken just after the shift to algae as a substrate (Fig. 5b, day 19–25) showed a high  $D_y$  value (about 70% change per week), which indicated the adaptation towards the new conditions. During the experimental period (day 20–70), the  $D_y$  values can be considered low range, i.e., on average, 7% change per week. Hence, it can be inferred that neither the change in the SRT nor the substrate destabilized the community in the AnMBR during the operational period (phase 2–3). Low  $D_y$  values indicate a stable community, but the latter can hamper the overall adaptability to changing conditions (Verstraete et al. 2007).

The  $C_o$  value in the AnMBR during the start-up period was estimated to be around 50, which represents a moderately organized community. During the experimental period, the  $C_o$  values were lower than during the start-up (phase 1), i.e., averaging a value of 27, which suggests a rather even bacterial community (low abundance distribu-

tion) in the reactor. Interestingly,  $C_o$  values tended to slightly increase by the end of phase 3 (day 69) which could be related to the decrease of the SRT. Usually, moderately organized communities ( $C_o=45–60$ ) are reported for well-working digesters (Carballa et al. 2011; Pycke et al. 2011). The low  $C_o$  values obtained in this study might decrease the capability of effectively converting all the organics supplied.

#### The influence of algae biomass in archaeal community dynamics

qPCR results showed that *Methanosaeta* sp. cells were dominant in the AnMBR regardless of the substrate used (Fig. 6). Only *Methanosaeta* and *Methanosarcina* of the methanogenic genera are known to produce methane solely from acetate by an acetoclastic reaction (Conklin et al. 2006). *Methanosaeta* is well known to have a competitive advantage over *Methanosarcina* in environments with low acetate concentrations (Griffin et al. 1998; Yu et al. 2006). This is in accordance with the findings of this study since the concentrations of acetate in the AnMBR were low, allowing *Methanosaeta* sp. to be dominant. Interestingly, both *Methanosaeta* sp. and *Methanosarcina* sp. 16S rRNA gene copies were comparably higher than the ones found in continuously fed, mesophilic digesters fed with triticale silage ( $10^5$  copy numbers of *Methanosaeta* and  $10^2$  copy numbers of *Methanosarcina* per gram of sample) and synthetic dairy waste water with sludge ( $10^8$  copy numbers of *Methanosaeta* and  $10^6$  copy numbers of *Methanosarcina* per gram of sample) (Bialek et al. 2011; Klocke et al. 2008). This higher concentration of acetoclastic methanogens was probably due to the AnMBR configuration and require further research. Previous studies have suggested that high densities of methanogens are necessary for a balanced and successful anaerobic biological treatment in particular when the system is subjected to perturbations (McHugh et al. 2004; Nozhevnikova et al. 2000).

#### Perspectives and opportunities

Overall, the biogas digestion efficiency of the *Phaedactylum tricornutum* did not yield the desirable 75% necessary for adequate operation of a microalgae to biogas energy producing system (Zamalloa et al. 2011a). The configuration with the AnMBR gave no better conversions than other attached growth systems tested and reported before (Zamalloa et al. 2011b). Yet, the AnMBR allowed an effluent free of suspended solids with a putative value as N and P natural liquid fertilizer (average composition per kilogram DM algae digested; 0.031 kg N and 0.008 kg P). Alternatively, the waste sludge extracted from the AnMBR can have value as a mixed liquor containing stable organics

(humus) which might serve as slow release fertilizer (average composition per kilogram DM algae digested, 0.734 kg COD, 0.059 kg N, and 0.019 kg P). The potential of this has to be demonstrated further. It must, however, be recognized that both types of liquors are low in strength and will require up-concentration before they can qualify as an acceptable commercial fertilizer. Yet their respective mineral nutrient value can be estimated at €0.3 m<sup>-3</sup> and €1.3 m<sup>-3</sup> of the effluent and digestate, respectively (Table S1). Estimations of costs to produce these fertilizers need to include capital and operational expenses and to that extent pilot scale tests are required to make reliable estimations. Overall, 1 kg of algae DM has an estimated value of biogas (€2.07), N and P in the effluent (€0.02), and N and P in the digestate (€0.04); thus, a total gross value of €2.13 kg<sup>-1</sup> algae DM.

Marine microorganisms can be utilized for the treatment of nutrient-rich brine wastewater (Rodolfi et al. 2009). Nutrient rich wastewater is generated by different industries such as the fish canning industry, the pesticide industry, the wet lime-gypsum desulphurization industry, etc. (Windey et al. 2005). In this context, microalgae can be used alone or in combination with biological processes to treat these waste streams. It remains to be examined to what extent the production of marine algae on such brines, and their subsequent processing to biogas on the one hand and to rapid respectively slow fertilizer on the other hand, offer possibilities for the food industry to design effective closed cycle water processes.

To conclude, this work demonstrated for the first time that the use of AnMBR for the digestion of algal biomass is feasible. Yet, the fact that some 50% of the organic matter was not readily liquefied means that the particulates in the digestate constitute a considerable fraction which should be valorised properly, for instance, as slow release organic fertilizer. The bacterial community was found to be rather restrictive, stable, and not very organized. The archaeal community was dominated by *Methanosaeta* sp.

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