Microalgae–bacteria and wastewater treatment (Case Study: Lorestan Province, Iran)

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Abstract

Two combined processes were studied in order to produce second generation biofuels: microalgae biomass production and its further use to produce biogas. Two 5 L photobioreactors for treating wastewater from a potato processing industry (from now on RPP) and from a treated liquid fraction of pig manure (from now on RTE) were inoculated with Chlorella sorokiniana and aerobic bacteria at 24 ± 2.7 °C and 6000 lux for 12 h per day of light supply. The maximum biomass growth was obtained for RTE wastewater, with 26.30 mg dry weight $L^{-1}d^{-1}$. Regarding macromolecular composition of collected biomass, lipid concentration reached 30.20% in RPP and 4.30% in RTE. Anaerobic digestion results showed that methane yield was highly influenced by substrate/inoculum ratio and by lipids concentration of the biomass, with a maximum m`ethane yield of 518 mL CH4 g COD^{-1} added using biomass with a lipid content of 30% and a substrate/inoculum ratio of 0.5.

Keywords: Biofuels, Agro-industrial wastewater, Algal-biomass, Methane production, Lorestan

1. Introduction

The potential of microalgae as an alternative to biofuels is currently subjected to strong research (Sialve et al., 2009). As a matter of fact, algae have a huge number of potential advantages compared to higher plants: (1) it is estimated that the production of algae is ten-fold higher than those of higher plants; (2) algae growth is independent of arable lands, attenuating food and feed competition (Rittman, 2008; Stephens et al., 2010); (3) algae biomass is rich in lipids, proteins and starch, which could be converted into energy utilising thermochemical and biochemical processes and esterification of fatty acids to produce biodiesel (Markou and Georgakakis, 2011). Products obtained through these processes can be considered ‘‘first generation’’ biofuels. However, the use of the resulting biomass to obtain ‘‘second generation’’ biofuels such as methane, is the optimal strategy of the energetic and economic point of view (IEA, 2010). The first study on energetic recovery from algal biomass was published by Golueke et al. (1957), in which the energetic recovery was carried out throughout anaerobic digestion of the biomass. This research effort was reintroduced in the 1970s and 1980s due to the first global energy crisis, and nowadays there is a renewed interest in anaerobic digestion motivated again by the actual fuel crisis and the ability to treat and to convert a wide range of organic wastes into renewable energy, including microalgae biomass. However, microalgae production requires a high amount of nutrients, for which environmental and economic impact may not be suitable (Halleux et al., 2008; Sialve et al., 2009). One alternative to synthetic culture media is to use wastewaters, especially those derived from agro-industrial facilities which usually present high nutrient concentration (Markou and Georgakakis, 2011). In this sense, microalgal–bacterial systems for agro-industrial wastewater treatment have been gaining special attention in last years. In these systems, microalgae produce oxygen during photosynthesis that is used by bacteria metabolism whereas bacteria release CO_2 needed for microalgae growth. Microalgal–bacterial systems, for wastewater treatment avoid the external oxygen supplementation compared to conventional processes, allow nutrients recovery into biomass and reduce CO_2 emissions to the atmosphere by its microalgae use contributing to CO_2 mitigation (Molinuevo-Salces et al., 2010). Therefore, the re-use of this kind of substrates can improve the feasibility to produce microalgae biomass for its further valorization, like anaerobic digestion (Gonzlez-Fernandez et al., 2011). The aim of the present study is to evaluate the integrated system of combining a microalgal–bacterial system for wastewater treatment with anaerobic digestion of the produced biomass. For this purpose, two agro-industrial wastewaters (treated liquid fraction of pig manure and potato processing wastewater) were selected for feeding separately two photobioreactors. The selection of these wastewaters was based on the different phosphorous concentration. The performance of the photobioreactors was evaluated in terms of organic matter and nutrient removal efficiency together with biomass production and biochemical composition. Additionally, anaerobic batch experiments were carried out using the produced biomass in both photobioreactors. Finally, the influence of the substrate/inoculum ratio was determined in terms of methane yield.

2. Hhg 66 Methods

2.1. Photobioreactors and culture conditions

2.1.1. Microalgae–bacteria inoculum

Chlorella sorokiniana was obtained from the culture collection of the University of Lorestan (Lorestan, Iran). Microalgae inoculum was prepared according to Guieysse et al. (2002). The average temperature was 24 ± 2.7 °C. Before inoculation, microalgae were centrifuged (4000 rpm; Centrifuge 5810R, Eppendorf) for 20 min and resuspended in distilled water. The aerobic sludge was obtained from an activated sludge reactor of the municipal wastewater treatment plant of Khoramabad.

2.1.2. Substrate composition

Treated liquid fraction of pig manure (TE) was collected from a pig manure treatment plant located in (Lorestan, Iran). Treatment consisted of a solid-liquid separation (with addition of coagulants and flocculants) and a treatment of the liquid fraction by nitrification denitrification. Potato processing wastewater (PP) was obtained from a potato industry located in Lorestan, Iran. Wastewaters were homogenized mechanically and stored at 4 °C for further use. Chemical characterization of TE and PP is shown in Table 1.

Table 1

Characterization of treated piggery effluents (TE) and potato processing wastewater (PP). Standard deviation is shown in brackets.

	TE	PP
pH	7.5 (0.3)	5.8 (2.0)
TS (mg L -1)	3319 (147.9)	1603 (388.2)
VS (mg L -1)	1031 (96.5)	903 (320.2)
TCOD (mg L -1)	616 (44.8)	1536 (529.1)
SCOD (mg L -1)	465 (38.5)	745 (227.2)
BOD5 (mg L -1)	63.0 (18.3)	917 (166.9)
TKN (mg L -1)	32.9 (8.0)	33.7 (10.1)
NH4+ -N (mg L -1)	12.3 (1.7)	12.1 (1.7)
NO3- -N (mg L -1)	53.8 (6.1)	N.D.
NO2 - -N (mg L -1)	131.7 (5.7	N.D.
TP (mg L -1)	50.1 (9.0)	4.2 (0.01)
SP (mg L -1)	47.5 (4.4)	3.4 (0.6)

2.1.3. Experimental set-up

The experimental set-up consisted in two photobioreactors, which are open to the atmosphere for a treated liquid fraction of pig manure (RTE) and for a wastewater from a potato processing industry (RPP), with a total working volume of 5 L (17 cm wide, 30 cm long, 10 cm high). Each photobioreactor was illuminated using four fluorescent lamps at 6000 lux (Philips 50 W) for 12 h per day. The lighting of the photobioreactors also provided heating for the cultivation medium. The average temperature was 24.2 ± 2.8 °C. The cultures were gently agitated with magnetic stirrers at 70 rpm. The volume was daily checked and the water lost due to evaporation was corrected by adding distilled water (lower than 4% of culture broth volume). Dissolved oxygen (DO), pH and temperature were monitored in situ. Both photobiorreactors were initially filled with distilled water and inoculated with 26 and 13

mg volatile suspended solids (VSS) L^{-1} of microalgae C. sorokiniana and aerobic sludge, respectively. Right after inoculation, the photobiorreactors were fed with TE (photobioreactor RTE) and PP (photobioreactor RPP). The hydraulic retention time (HRT) was 10 days, corresponding to an ammonium loading rate (ALR) of 1.3 mg $NH4^+$–N L^{-1} day^{-1} for both photobioreactors. Culture broths were collected separately in two settlers for biomass sedimentation. Samples of the influent and effluent from the top of the settlers were collected periodically in order to determine total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total solids (TS), volatile solids (VS), total suspended solids (TSS), VSS, total Kjeldahl nitrogen (TKN), soluble phosphorus (SP), ammonia ($NH4^+$–N), nitrites (NO_2–N) and nitrates (NO_3–N). In addition, TS and VS were monitored periodically in culture broths. Biomass was purged from the bottom of the settlers at the end of the experimental time. These purges were analysed for TS, VS and TKN determination. Moreover, a fraction of collected biomass was lyophilized (Lyoquest 85 Plus Eco, Lorestan) for lipid content determination.

2.2. Anaerobic biodegradability experimental set-up

Anaerobic biodegradability assays were carried out at 35 ± 0.3 °C for 50 days in 0.57 L bottles. Quantities were calculated to reach a final volume of 0.25 L, allowing a headspace of 0.32 L for gas accumulation. The bottles were closed with a septum and the headspace flushed with N_2 to remove the O_2. The biogas production was measured by the overpressure in the headspace with time frequency (Colleran et al., 1992). Constant agitation was provided by a shaker at 200 rpm. Anaerobic sludge inoculum was collected at the municipal wastewater treatment plant of khoram abad (Lorestan). Anaerobic sludge presented a TS and VS concentration of 20.0 and 10.2 gL^{-1}, respectively. For these assays, microalgal–bacterial biomass collected from the bottom of the two settlers described in Section 2.1.3 at the end of the experimental period was used. Specifically, biomass was concentrated by centrifugation (9000 rpm; Beckman Coulter, Avanti centrifuge J-30I) for 10 min. For the determination of endogenous methane production, blanks containing only anaerobic sludge were also tested. The influence of the substrate/inoculum ratio (from now on TCOD/VS ratio) was evaluated according to Gonzalez-Fernandez and Garcia-Encina (2009). TCOD/VS ratios ranged between 0.5 and 2.0, which were achieved by keeping a constant inoculum concentration of 2.2 gL^{-1}. Treatments T_1 to T_4 were performed using microalgal biomass produced in photobioreactor RTE, whereas treatments T_5–T_8 were performed using biomass produced in pho- tobioreactor RPP. All experiments were carried out in duplicate and the results were expressed as means.

2.3. Analytical procedures

TS, VS, TSS, VSS, TCOD, SCOD, biological oxygen demand (BOD_5) TKN and SP were analysed in duplicates according to APHA Standard Methods (2005). $NH4^+$-N, NO_2 -N and NO_3 -N concentrations were determined using electrodes, Orion 900/200. DO, pH and temperature in the reactors were determined using a multi-probe system model YSI 556 MPS. Lipids were extracted from the lyophilized biomass with chloroform– methanol following the method proposed by Kochert (1978). Once the extraction was done, the lipids were quantified by gravimetric analysis. Proteins were calculated by multiplying the TKN by 5.95 (Gonzalez-Lṕez et al., 2010). Carbohydrates were estimated by subtracting the percentage of ashes, lipids and proteins out of 100% (Wilkie and Mulbry, 2002). Microalgae identification was carried out by microscopical examination (OLYMPUS IX70, USA) of culture broth samples fixed with formaldehyde at 0.5% and stored at 4 °C prior to analysis, according to

Phytoplankton Manual (Sournia, 1978). Biogas composition was analyzed using a gas chromatograph (Bruker 430-Gc) with a thermal conductivity detector, provided by a CP-Molvsieve5A column (15 m × 0.53 mm 15 lm) followed by a CP-Porabond Q column (25 m × 0.53 mm × 10 lm). Hydrogen (13.6 mLmin^{-1}) was used as carrier gas. The injection port temperature was set at 150 °C and the detector temperature was 175 °C. Total volatile fatty acids (VFA) were analyzed at the end of the assays using a gas chromatograph (Agilent 7820A) equipped with a Teknokroma 10% SP1000 capillary column and a flame ionization detector. Carrier gases were nitrogen, hydrogen and air and the temperature of the injector was 375 °C. The temperature of the oven was set at 160 °C.

3. Results and discussion

3.1. Photobioreactor performance

3.1.1. Organic matter removal

The organic loading rates (OLR) applied to photobioreactors during the whole experimental time were 0.06 and 0.15 g TCOD L^{-1} day^{-1} for RTE and RPP, respectively. The highest COD removalefficiencies were observed when treating potato processing wastewater. More specifically, TCOD removal was 62.3% for RTE and 84.8% for RPP (Table 2). SCOD removal efficiencies accounted for 58.1% and 86.1% for RTE and RPP, respectively. As stated by other authors, higher COD removal efficiency observed in photobioreactor RPP could be attributed to the higher influent strength (Molinuevo-Salces et al., 2010; Wang et al., 2012). Additionally, BOD5/TCOD ratio in PP wastewater (0.59) was higher than in TE (0.10) and therefore, organic matter was more degradable. As shown in Fig. 1a, DO was never limited in reactor RTE. Therefore, the remaining COD was acting as recalcitrant for this system. COD removal efficiencies observed in reactor RTE were slightly lower than those reported by De Godos et al. (2009) when treating piggery wastewaters (76%). These better COD removal behaviors could be probably promoted by higher pig manure biodegradability. In the case of photobioreactor RPP, DO decreased during the experimental time up to values below 1 mg O_2 L^{-1} at day 23 (Fig. 1a), which indicated that microalgae limited the system and higher COD removal efficiencies could be achieved with a proper consortia development.

Table 2

COD removal, ammonium, removal, ammonium removed by stripping, nitrification, denitrification and soluble phosphorous removal in the two photobioreactors. Standard deviation is shown in brackets.

	RTE%	RPP%
Removal TCOD	62.3 (2.0)	84.8 (3.2)
Removal SCOD	58.1 (4.5)	86.1 (2.6)
Removal NH4+ - N	82.7 (3.0)	>95
Removal by stripping	25.4 (8.0)	2.9 (1.3)
Removal by nitrification	75.7 (11.8)	-
Removal by denitrification	53.8 (10.1)	-
Removal SP	58.0 (7.5)	80.7 (12.3)

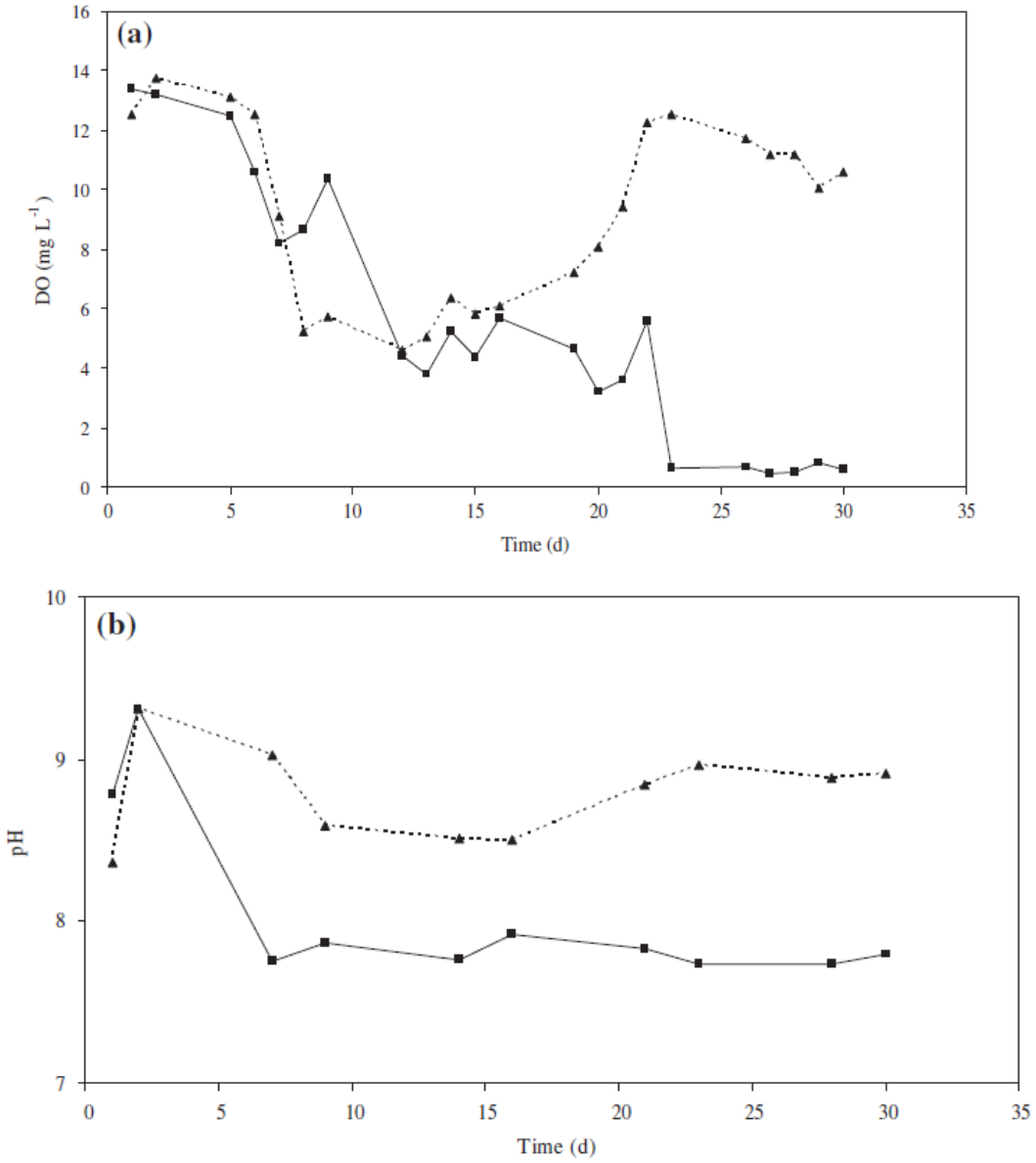

Fig.1. (a) Dissolved oxygen and (b) PH measured in situ in photo bioreactor RTE (discontinuous line) and RPP (continuous line).

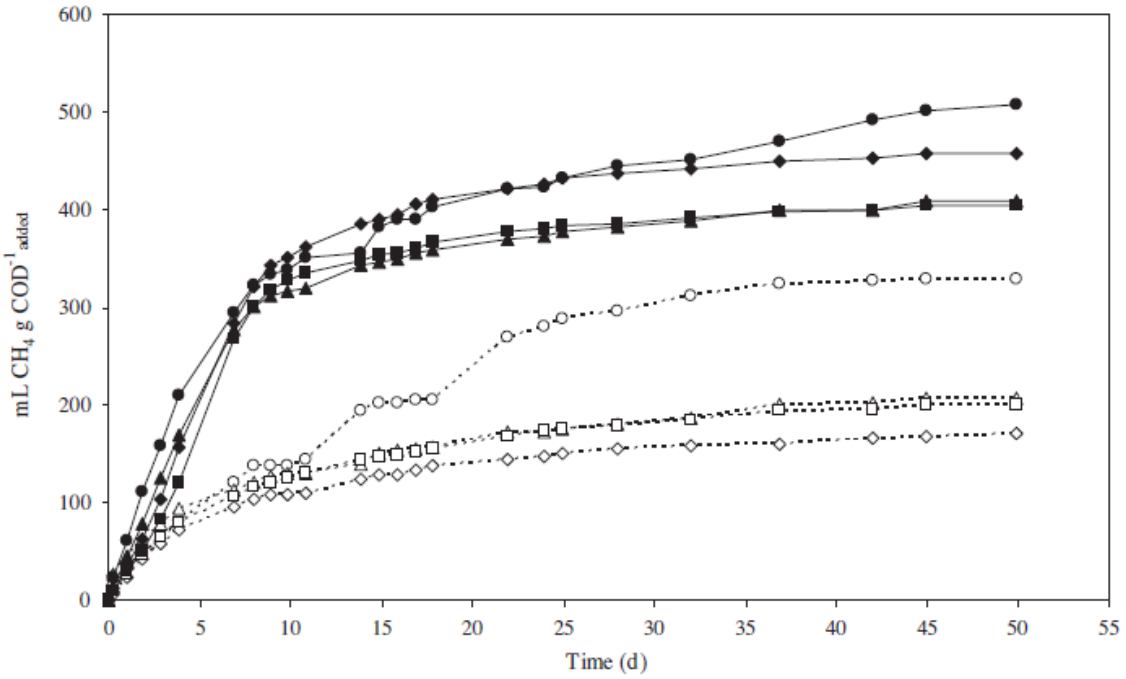

Fig2. Net methane yield in the evaluated treatments for RTE (discontinuous line) ND FOR RPP (continuous line) at substrate / inoculum ration 0f 0.5 (•), 1.0 (▲), 1.5 (◊) and 2.0 (■) g TCOD/ g VS.

3.1.2. Nutrient removal

ALR was similar in both photobioreactors (1.2 mgNH_4 +–N$L^{-1}d^{-1}$). Ammonium was removed up to 82.7% in RTE and it was almost exhausted in RPP (Table 2). These high removal efficiencies were expected since ALR was low in comparison with those applied in other studies (Sialve et al., 2009; Xin et al., 2010; Wang et al., 2012). In order to quantify NH_4 +–N stripping, the free ammonia concentration was calculated according to Hansen et al. (1998). The results indicated that ammonia volatilization was not the main mechanism for ammonium removal since ammonia stripping accounted for 25% in RTE and for 3% in RPP (Table 2). This difference was attributed to the higher pH achieved in reactor RTE (8.8) compared to RPP (8.0), as a consequence of the higher SCOD removals in photobioreactor RPP (Fig. 1b), and therefore to higher bacterial activity in this reactor. Hence, biomass nitrogen assimilation was determined by the daily TKN increase, being higher in RPP (0.25 mg TKN g^{-1} TS d^{-1}) than in RTE (0.08 mg TKN g^{-1} TS d^{-1}). Photobioreactor RTE showed a nitrite removal efficiency higher than 75%. Even when ammonia stripping and assimilation were indicated as the main mechanisms for nitrogen removal in open reactors (Molinuevo-Salces et al., 2010), the present study demonstrates that denitrification also occurs when pH ranges between 8.0 and 8.8. In this manner, denitrification accounted for 53.8% in RTE. It should be noted that denitrification requires low DO in the medium and RTE presented DO concentration up to 4 mg O2 L^{-1} (Fig. 1a). Nevertheless, DO could be much lower in the flocks formed during the treatment (De Kreuk et al., 2005; Molinuevo-Salces et al., 2010). Additionally, even when nitrate is not the preferred nitrogen form for microalgae uptake (Travieso et al., 2006), this process could also have contributed to the nitrogen removal from the culture broth. In photobioreactor RPP, NO_2 –N or NO_3–N was not detected during the whole experimental time, which indicated that nitrification process did not occur. As shown in Table 2, SP removal efficiency was 58.0% and 80.7% in RTE and RPP, respectively. These

results were similar to those from Wang et al. (2012) who obtained a SP removal efficiency of 60.6% when treating diluted piggery wastewater with similar COD concentration using the microalgae Chlorella pyrenoidosa. These differences in phosphorous removal could be attributed to the higher SP loading rate applied in RTE (4.75 mg L^{-1} d^{-1}) than in RPP (0.34 mg L^{-1} d^{-1}). SP removals achieved in the present study were high compared with those reported by De Godos et al. (2009), who obtained efficiencies of 10% working with high rate algal ponds. In this sense, it should be noticed that high pH achieved in both reactors may be involved in PO43_ precipitation (Nurdogan and Oswald, 1995).

3.1.3. Biomass productivity and biochemical profile

Biomass growth was measured as the dry weight (total solids) of produced biomass per day and litre of the photobioreactor. RTE produced 26.3 mg dry weight (DW) L^{-1} d^{-1} while RPP produced 18.8 mg DW $L^{-1}d^{-1}$. Hence, the higher phosphorous availability in RTE resulted in higher biomass growth. These result significant protein content reduction (from 8% up to 54% of proteins) in different microalgae species. In this study, carbohydrates were the main cellular component obtained for both photobioreactors. Percentage of carbohydrates was found to be 2-fold higher in biomass produced in RTE than in RPP (data not shown), as a consequence of the lower content in lipids and proteins. Therefore, it was detected that the characteristics of wastewater presented a high influence on macromolecular composition of microalgal–bacterial biomass produced. Specifically, the use of a substrate with low phosphorous concentration was elucidated as an important factor affecting percentage of lipids in biomass, and therefore, it could determine the further valuation of this added-value product.

3.2. Overall anaerobic biodegradability performance

3.2.1. Biogas production and methane yields

Anaerobic experiments lasted for 50 days. Table 3 shows the accumulated biogas production at substrate/inoculum ratio from 0.5 to 2.0 g TCOD/g VS when using biomass produced in photobioreactor RTE (T_1–T_4) and RPP (T_5–T_8). The methane volumes were corrected by subtracting the mean methane volume of the blanks (endogenous production) and were converted to standard temperature and pressure (STP, 0 °C and 760 mm Hg). Methane yields were calculated by dividing the corrected methane volume by TCOD added to each digester. As seen in Table 3 and Fig. 2, the highest methane yields were achieved by the treatments T1 and T5 corresponding to a substrate/inoculum ratio of 0.5 g TCOD/g VS ratio. The rest of treatments showed a similar methane production, varying between 172.0 and 207.2 mL CH4 g COD^{-1} added in the case of the digestion of the biomass produced in photobioreactor RTE and between 404.6 and 460.1 mL CH4 g COD^{-1} added for biomass from RPP. According to Gonzalez-Fernandez and Garcia-Encina (2009), high COD/VS ratios were responsible for methane production delay due to the accumulation of VFA. Therefore, the substrate/inoculum ratio can be an essential parameter to influence the methane yield in the batch anaerobic digestion of microalgal–bacterial biomass. The same conclusion was obtained by previous research using different substrates (Raposo et al., 2006, 2008; Foster-Carneiro et al., 2008). Biochemical composition of microalgal-biomass also determined methane yield obtained. Specifically, methane yield increased between 157% and 268% in the case of the biomass from RPP in comparison to biomass obtained from RTE (Fig. 2). This fact could be explained by the lower lipid content of biomass from RTE than from RPP as stated before. Regarding this, several authors reported that lipids showed a higher biogas production potential compared with proteins and carbohydrates (Cirne et al., 2007; Li et al., 2002). The

results herein demonstrated that an increase in lipid content of digested, also increases the potential methane yield. However, an excess of the percentage of lipids in biomass could lead to VFA accumulation, causing the inhibition of the anaerobic process (Park and Li, 2012). The percentage of methane in biogas varied between 71.9% and 76.1% for biomass from RTE and between 76.5% and 77.0% for biomass from RPP (data not shown). These results revealed a good conversion of the microalgal–bacterial biomass into methane. High methane content in anaerobic digester implies a steady balance of methane and carbon dioxide, which are products of methanogenesis and acetogenesis, respectively (Park and Li, 2012). The values obtained in present study were similar to those reported by Sialve et al. (2009).

table 3

methane yields and total solid removal efficiency obtained after anaerobic process of biomass produced in RTE (T1-T4) and (T5-T8).			
Substrate / I noculum ration (g TCOD/ g VS)		% TS removal	Methhane yield (m L CH4 g COD -1 added
T1	0.5	12.24 (0.37)	329.8
T2	1.0	18.36 (2.92)	207.2
T3	1.5	17.17 (2.92)	172.0
T4	2.0	21.33 (1.80)	200.5
T5	0.5	15.81 (5.09)	517.5
T6	1.0	23.32 (1.48)	408.2
T7	1.5	24.14 (1.34)	460.1
T8	2.0	24.97 (1.82)	404.6

3.2.2. Solid removal efficiency

TS removal efficiency improved with the increase of substrate/inoculum ratio (Table 3). For biomass produced in RTE, TS removal efficiency increased from 12.2% to 21.3% when substrate/inoculums ratio increased from 0.5 to 2.0. In the case of biomass produced in reactor RPP, TS removal efficiency increased from 15.8% to 25.0% when substrate/inoculum ratio increased from 0.5 to 2.0. These findings were in accordance with previous results obtained by Gonzalez-Fernandez et al. (2011), who accomplished TS removal efficiencies of 14.7–32.9% by co-digesting algal biomass with swine manure.

3.2.3. Process stability

As seen from Table 4, all final pH values ranged from 7.3 to 7.8. These values were compatible with the normal growth of anaerobic microorganisms. Ammonia could mainly influence the anaerobic digestion by affecting acetate-utilizing methanogenic Archaea, hydrogen-utilizing methanogens and syntrophic bacteria (Zeng et al., 2010). The inhibitory concentrations of ammonia are reported to be between 1.7 and 5 g NH_4^+_NL^{-1} (Stams et al., 2003). From Table 4, initial and final ammonia concentrations are too low to inhibit anaerobic digestion. Finally, many authors have observed that VFA are one of the most important parameter for the accurate control of anaerobic digestion, having a direct relation with the digester performance (Zeng et al., 2010). In the present study, no VFA were detected in the samples after digestion, which indicated that the anaerobic digestion process was complete in all treatments.

Table 4

pH and NH4+ -N during anaerobic process of biomass produced in RTE (T1-T4) and RPP (T5-T8).				
	PH		NH4+ -N (mgL -1)	
	Initial	Final	Initial	Final
T0	8.0	7.7	153.0 (1.4)	162.5 (2.9)
T1	7.9	7.8	168.0 (1.4)	190.0 (1.5)
T2	8.0	7.7	161.5 (0.7)	198.0 (2.6)
T3	8.0	7.8	175.0 (0.0)	236.0 (0.5)
T4	8.1	7.6	166.5 (0.7)	241.0 (3.0)
T5	7.6	7.6	155.0 (0.0)	167.0 (1.6)
T6	7.6	7.4	165.0 (0.0)	181.5(1.0)
T7	7.5	7.3	160.5 (0.7)	210.8 (5.1)
T8	7.4	7.4	172.5 (0.7)	229.8 (1.7)

4. Conclusions

Microalgae–bacteria consortia presented high organic matter and nutrient removal efficiencies in agro-industrial wastewater treatment. Low phosphorous concentration in wastewater led to an increase in the lipid content of produced biomass. Moreover, batch anaerobic digestion assays indicated that methane yield was determined by lipid content and substrate/inoculum ratio. The highest methane yield (518 mL CH_4 g COD^{-1} added) was obtained using biomass with a lipid percentage of 30% and a substrate/inoculum ratio of 0.5. In conclusion, the selection of a suitable agro-industrial wastewater for microalgae growth, attending to the nutrient concentration, could determine biomass macromolecular composition and, therefore, its potential valorization for biofuel production.

Acknowledgements

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