A STUDY OF PHOTOSYNTHETIC BIOGAS UPGRADING BASED ON A HIGH RATE ALGAL POND UNDER ALKALINE CONDITIONS: INFLUENCE OF THE ILLUMINATION REGIME

Mariana Franco-Morgado¹, Cynthia Alcántara², Adalberto Noyola¹, Raúl Muñoz², Armando González Sánchez^{1*}

1. Instituto de Ingeniería, Universidad Nacional Autónoma de México, Circuito Escolar, Ciudad Universitaria, 04510 Mexico City, Mexico

2. Department of Chemical Engineering and Environmental Technology, University of Valladolid, C/Dr. Mergelina s/n, 47011 Valladolid, Spain

*Corresponding author: agonzalezs@iingen.unam.mx

ABSTRACT

Microalgal-bacterial processes have emerged as environmental friendly systems for the cost-effective treatment of anaerobic effluents such as biogas and nutrients-laden digestates. Environmental parameters such as temperature, irradiation, nutrient concentration and pH effect the performance of the systems. In this paper, the potential of a microalgal-bacterial photobioreactor operated under high pH (≈ 9.5) and high alkalinity to convert biogas into biomethane was evaluated. The influence of the illumination regime (continuous light supply vs 12 h/12 h light/dark cycles) on the synthetic biogas upgrading efficiency, biomass productivity and nutrient removal efficiency was assessed in a High-Rate Algal Pond interconnected to a biogas absorption bubble column. No significant differences in the removal efficiency of CO_2 and H_2S ($91.5 \pm 2\%$ and $99.5\% \pm 0.5$, respectively) were recorded regardless of the illumination regime. The high fluctuations of the dissolved oxygen concentration during operation under light/dark cycles allowed to evaluate the specific growth rate and the specific partial degradation rate of the microalgae biomass by photosynthesis and respiration, respectively. The respiration reduced the net microalgae biomass productivity under light/dark cycles compared with process operation under the continuous light supply.

Keywords: alkaliphilic algal-bacterial consortium, biogas desulfurization, light/dark cycles

1. INTRODUCTION

Biogas and digestate are the main byproducts from anaerobic digestion of organic waste. The composition of raw biogas varies depending on the type of residue and conditions of anaerobic digestion. A typical raw biogas composition accounts for CH₄ 40-70% vol, CO₂ 15-60% vol, H₂S 0.005-2% vol, and trace contaminants like water, siloxanes and volatile organic compounds (Muñoz et al., 2015). Raw biogas can be used directly to generate heat and/or power or injected into natural gas grids when its quality is comparable with that of natural gas. The high content of CO₂ in raw biogas reduces its specific heating value and increases its transportation costs. Another undesirable biogas component is H₂S, which is a highly corrosive, toxic and malodorous gas (Noyola et al., 2006; Redondo et al., 2008). On the other hand, anaerobic digestates are characterized by a high concentration of nutrients, such as nitrogen (950 mg N-NH₄⁺ L⁻¹) and phosphorus (415 mg P-PO₄³⁻ L⁻¹) which are representative values from anaerobic sludge digestion made in municipal wastewater treatment plants (Uggetti et al., 2014). These aqueous effluents must be treated to avoid eutrophication of inland waterbodies and coastal areas (Batten et al., 2013). In this context, there is crucial need to develop cost-effective and environmentally friendly technologies for biogas upgrading and nutrient removal, based on both the versatile potential of biogas as a renewable energy source and the high eutrophication impact of digestates.

Algal-bacterial processes have emerged as the only technology capable of simultaneously upgrading biogas while recovering nutrients from digestates (Muñoz and Guieysse, 2006). On one hand, microalgae can photosynthetically use the CO₂ present in the biogas as a carbon source while supporting the bacterial oxidation of H₂S via an *in-situ* O₂ supply (González-Sánchez et al., 2008; de Godos et al., 2009; Bahr et al., 2014; Posadas et al., 2013; Hernández et al., 2013). On the other, the use of digestate as a nutrient source for

microalgae and bacteria growth represents an opportunity to mitigate its eutrophication potential (Batten et al., 2013; Franchino et al., 2013; Uggetti et al., 2014). Despite the promising results obtained so far using algal-bacterial photobioreactors, biogas bioconversion to biomethane is still limited by the low CO₂ mass transfer rates of this technology (Yan et al., 2016). For instance, CO₂ removals ranging from 55 to 62 % were recorded by Alcántara et al., (2015) and Lebrero et al., (2016) using High Rate Algal Pond (HRAP) or bubble column algal-bacterial photobioreactors, which hindered the direct injection of the upgraded biogas into natural gas grids.

In this context, pH has been identified as a critical parameter determining the mass transfer rate of acidic gases such as H₂S and CO₂ in conventional gas-liquid contactors (Bahr et al., 2014; González-Sánchez et al., 2008). Process operation at pH>9 mediate an enhancement in the absorption rates of these biogas contaminants induced by chemical reactions (González-Sánchez and Revah, 2007; Markou et al., 2014). The use of carbonated alkaline cultivation media in algal-bacterial photobioreactors could support a long term efficient biogas upgrading. In these systems, medium acidification induced by CO₂ and H₂S absorption is counterbalanced by microalgae CO₂ uptake in the presence of light and CO₂ stripping under dark conditions. In addition, the use of extreme pH values promotes the stability of microalgae populations by preventing contamination with predators or other photosynthetic microorganisms (Lee, 2001). Despite the above mentioned advantages, the understanding of biogas upgrading using alkaliphilic algal-bacterial consortia is very limited.

This work aimed at evaluating the performance of a novel alkaliphilic microalgae-based process deployed for synthetic biogas upgrading and nutrient removal under different

illumination regimes, assessing both the fate of carbon, nitrogen, phosphorous and sulphur and biomass productivity through mass balances under steady state.

2. MATERIAL AND METHODS

2.1. Microorganisms and culture conditions

An alkali-tolerant microalgae culture (AMC) composed of *Picochlorum sp.* and *Halospirulina sp* was used as a model photosynthetic consortium throughout the entire experiment (de los Cobos-Vasconcelos et al. 2015). The AMC was enriched from Texcoco soda lake in Mexico City. A culture of alkaliphilic sulphur oxidizing bacteria (ASB) was used as a model H₂S degrading consortium (González-Sánchez and Revah, 2007). The mixed AMC/ASB culture was grown in a mineral salt medium (MSM) composed of (g/L): Na₂CO₃ (4.03); NaHCO₃ (13.61); NaCl (1); K₂HPO₄ (1); K₂SO₄ (1); CaCl₂·H₂O (0.04); KNO₃ (2.52); MgCl₂·6H₂O (0.2) and 2 mL of a micronutrient solution (Sorokin et al., 2001). The final pH of the MSM medium was 9.3.

2.2. Experimental set-up

The experimental system consisted of a 25 L HRAP interconnected with a 0.35 L Absorption Bubble Column (ABC) (Figure 1), the ratio of the volumes of the HRAP and ABC was based on the optimized design reported by Serejo et al., (2015). The system was located in a cottage at the roof of the Environmental Engineering building at II-UNAM (México City) where only the illumination was controlled. The HRAP dimensions were 1.25 m long × 0.25 m width × 0.14 cm deep, which entailed an effective illuminated area of 0.28 m². The HRAP was illuminated at a photosynthetically active radiation (PAR) of 500 μmolm⁻²s⁻¹ (model LI250A, LI-COR, USA) provided by LED white-cool light lamps (model 511919, Ecosmart, USA). The cultivation broth in the HRAP was continuously

102 mixed using a six bladed paddle wheel inducing a laminar flow regime at 15 cm s^{-1} . The
103 ABC ($0.80 \text{ m height} \times 0.019 \text{ m}$ of internal diameter) was interconnected to the HRAP via
104 culture broth recirculation using a peristaltic pump (model 7553-80, Cole-Pamer, USA).

105
Figure 1 here

107 **2.3. Operational conditions**

108 The HRAP was initially filled with 21 L of MSM and inoculated with four liters of AMC
109 containing a biomass concentration of $0.12 \text{ g TSS L}^{-1}$. The system was operated as fed-
110 batch under continuous illumination for 39 days. Four liters of algal cultivation broth were
111 drawn by day 20 and replaced with fresh ASB (0.2 g TSS L^{-1}) grown in MSM containing
112 10 g L^{-1} of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) as energy source. The mixed culture was then
113 cultivated in batch mode for 19 days (stage I). From day 40 to 166 (stage II), the HRAP
114 was continuously fed at a hydraulic retention time of 9.5 days with a 1.8 times diluted
115 MSM, supplemented with $0.35 \text{ g Na}_2\text{S}_2\text{O}_3 \text{ L}^{-1}$. At stages I and II only air was fed in the
116 ABC. From day 167 to 269 (stage III) and from day 270 to 399 (stage IV), a gas mixture
117 prepared by a commercial gas supplier (INFRA®), composed of CO_2 (30% vol), H_2S
118 (0.5% vol) and balanced with N_2 was considered as synthetic biogas, it was continuously
119 bubbled at 22 L d^{-1} through a porous stone located at the bottom of the ABC. The column
120 was operated co-currently at a liquid to synthetic biogas volumetric ratio (L/G) of 5 using
121 the algal-bacterial cultivation broth from the HRAP as scrubbing solution. At stage IV, the
122 illumination regime was set at a 12h:12 h light/dark photoperiod while biogas was supplied
123 continuously for 24 h d^{-1} . The last period (stage V) was operated for 117 days under similar
124 operational conditions than those imposed in stage II. Water evaporation rates were

quantified and compensated by addition of distilled water before sampling of the HRAP-ABC system.

Table 1 here

Aliquots of 70 mL of HRAP cultivation broth and influent mineral salt medium were drawn three times per week in order to monitor the concentration of TSS, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-), sulphate (SO_4^{2-}), tiosulphate ($\text{S}_2\text{O}_3^{2-}$) and phosphate (PO_4^{3-}). The pH and dissolved oxygen (DO) concentration of the cultivation broth were on-line recorded every 3 min. The composition of synthetic biogas at the inlet and outlet of the ABC was daily monitored by non-dispersive infrared sensor analysis. Finally, the elemental composition of the algal-bacterial biomass was determined in stage III and IV.

2.4. Analytical Procedures

The concentration of TSS was measured according to Standard Methods (APHA, 2012). Liquid samples were filtered through 0.45 μm Whatman filters prior determination of IC, TOC, TN, PO_4^{3-} , SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ concentrations. The concentration of IC, TOC and TN was determined by a TOC-L CSH analyzer coupled to a TNM-L chemiluminescence module (Shimadzu, Japan). The concentration of N-NO_3^- and N-NO_2^- were measured spectrophotometrically with commercial Nitraver and Nitriver kits, respectively (HACH, USA). $\text{S-S}_2\text{O}_3^{2-}$ concentration was measured by titration according to (Rodier, 1988). The concentration of S-SO_4^{2-} was analyzed according to the Standard Methods (APHA, 2012). P-PO_4^{3-} concentration was analyzed using a Dionex ICS 2000 Ion Chromatograph (USA) equipped with a IonPacAS23 column ($250 \times 4 \text{ mm}$) eluted at 1 mL min^{-1} with a mobile

phase containing CO_3^{2-} and HCO_3^- at 4.5 y 0.8 mM, respectively. The elemental biomass composition (C, N and S content) was determined from the biomass contained in aliquots of 50 mL of cultivation broth centrifuged at 10,000 rpm (Avanti 123, Beckman Coulter, USA) and washed 3 times with Milli-Q water. The salt-free pellets were dried at 105°C for 24h and grinded prior analysis in a Flash 2000 Elemental Analyzer CHNS-O Analyzer (Thermo Scientific, USA). The gas concentration of CO_2 , O_2 and H_2S at the inlet and outlet of the ABC were analyzed with continuous nondispersive infrared sensor gas analyzer (Ultramat 23, Siemens, Germany). The dissolved oxygen concentration and pH were online determined using Applisens Z10023525 (Applikon, The Netherlands), Orion 9107BNMD (Thermo Scientific, USA) and temperature probes.

2.5. Calculations

A series of elemental mass balances under steady state, showed in equations 1, 2, 3 and 4 and expressed as loading rates (Q_i = mass flow rate of component i /total liquid volume), allowed to assess the fate of carbon, nitrogen, phosphorus and sulphur under steady state in the HRAP-ABC.

$$Q_{C_{G_{in}}} + Q_{C_{L_{in}}} = Q_{C_{G_{out}}} + Q_{C_{L_{out}}} + Q_{C_{-X_{out}}} + Q_{C_{stripping}} \quad (1)$$

$$Q_{N_{L_{in}}} = Q_{N_{L_{out}}} + Q_{N_{-X_{out}}} \quad (2)$$

$$Q_{P_{L_{in}}} = Q_{P_{L_{out}}} + Q_{P_{-X_{out}}} \quad (3)$$

$$Q_{S_{G_{in}}} + Q_{S_{L_{in}}} = Q_{S_{G_{out}}} + Q_{S_{L_{out}}} + Q_{S_{-X_{out}}} \quad (4)$$

Where $Q_{i, in}$ and $Q_{i, out}$ represent the inlet and outlet mass loading rates, respectively, of all species (i = carbon-C, nitrogen-N, phosphorous-P and sulphur-S) in liquid (L) and gas (G)

phases. Q_{i_X} represents the loading rate of each i contained in the biomass present in the effluent. $Q_{c, stripping}$ represents the C as CO_2 lost by stripping from the HRAP. The CO_2 stripping from the HRAP was evaluated from equation 5 including the discrete IC measurements together with the corresponding pH. The dissolved CO_2 (DCO_2) was calculated as a function of pH, according to equation 6.

$$CO_2 \text{ stripping rate} = \frac{V_H}{V_T} K_L a_H \left(\frac{CO_{2air}}{H_{CO_2}} - DCO_2 \right) \quad (5)$$

$$[DCO_2] = \frac{IC}{1 + \frac{K_1}{[H^+]} + \frac{K_1 * K_2}{[H^+]^2}} \quad (6)$$

Where V_H and V_T represent the volume of the aqueous phase in the HRAP and total aqueous volume in the HRAP+ABC. H_{CO_2} represents the CO_2 Henry constant (1.1 dimensionless at 20 °C). $K_L a_H$ represents the global volumetric gas-liquid mass transfer coefficient (20 d⁻¹) in the HRAP. $K_L a_H$ was estimated from the abiotic operation of the HRAP-ABC system under steady state, where the continuous supply of the synthetic biogas in the ABC stripped out the oxygen previously absorbed from air in the HRAP. CO_{2air} is the carbon dioxide concentration in air (350 ppm_v = 1.1×10^{-5} mol L⁻¹). K_1 and K_2 are the dissociation constants for the system CO_2 -H₂O (4.66×10^{-7} mol L⁻¹ and 4.67×10^{-11} mol L⁻¹, respectively) and $[H^+]$ is the proton concentration in the cultivation broth (mol L⁻¹).

2.6. Assessment of the specific growth and partial degradation rates of microalgae under light/dark cycles

The fluctuations of the DO measurements (Figure S2) recorded during stage IV under steady state were described using a dynamic oxygen mass-balance applied to the liquid phase (Eq. 7). This mass balance was used to estimate either the volumetric oxygen

production or consumption (r_{O_2}) associated to microalgae biomass metabolism, accounting for the variation on the temperature as well as was recorded from natural environmental fluctuations (supplementary material).

$$\frac{dDO}{dt} = \frac{F}{V_T} (DO_{in} - DO) + \frac{V_C}{V_T} k_L a_C \left(\frac{O_{2g}}{H_{O_2}} - DO \right) + \frac{V_H}{V_T} k_L a_H \left(\frac{O_{2air}}{H_{O_2}} - DO \right) + \frac{V_H}{V_T} r_{O_2} - R_{O_2-S} \quad (7)$$

Where V_C represents the volume of the aqueous phase in the ABC (0.3 L), r_{O_2} represents the microalgal oxygen consumption or production rate ($\text{mol L}^{-1} \text{d}^{-1}$), H_{O_2} represents the oxygen Henry constant (32 dimensionless at 20 °C). $K_L a_C$ stands the global volumetric gas-liquid mass transfer coefficient (141 d^{-1}) in the ABC, which was experimentally determined using the gassing-out method. R_{O_2-S} was estimated to $0.0007 \text{ mol O}_2 \text{ L}^{-1} \text{d}^{-1}$ based on the sulphur mass balance under steady state conditions, assuming complete oxidation to sulphate. The evaluation of the specific growth rate (μ_{light}) and the specific partial degradation rate (μ_{dark}) of the microalgae biomass by photosynthesis and by respiration, respectively, was based on the assumption of a 15 % w/w biomass loss by respiration during the 12 h dark period (Grobbelaar and Soeder, 1985) where this percentage depends on the temperatures and irradiances prevailing during the illuminated period. This entails a $\mu_{\text{light}}/\mu_{\text{dark}}$ ratio of -4.2. μ_{light} can be expressed in terms of the photosynthetic oxygen/biomass ($y_{O_2/X}^p$) yield (de los Cobos-Vasconcelos et al., 2015) and the oxygen production rate ($r_{O_2}^p$). Similarly, μ_{dark} can be defined in terms of an endogenous respiration oxygen/biomass ($y_{O_2/X}^r$) yield and its corresponding oxygen respiration rate ($r_{O_2}^r$). Therefore, the overall $y_{O_2/X}^r$ was evaluated according to equation (8) for several light/dark cycles exposed to constant temperature fluctuations. $r_{O_2}^p$ and $r_{O_2}^r$ represent averaged

values for a set of continuous days reaching uniform temperature fluctuations (the evaluation procedure is showed in supplementary material, see the slopes in Figure S3).

$$y_{O_2/X}^r = 4.2 \frac{(r_{O_2}^r)(y_{O_2/X}^p)}{r_{O_2}^p} \quad (8)$$

The net volumetric biomass productivity (P_x) was evaluated from the condition of steady state for a continuous cultivation (see equation 9).

$$P_x = D \cdot X = (\mu_{light} - \mu_{dark}) \cdot X = \frac{r_{O_2}^p}{y_{O_2/X}^p} - \frac{r_{O_2}^r}{y_{O_2/X}^r} \quad (9)$$

Where D is the dilution rate (d^{-1}) and X the experimental biomass concentration (g TSS L^{-1}).

3. RESULTS AND DISCUSSION

3.1. Synthetic biogas upgrading performance

The removal efficiencies of CO_2 and H_2S during stage III accounted for $89 \pm 4 \%$ and $99.5 \pm 0.5\%$, respectively, and $94 \pm 1\%$ and $99.5 \pm 0.5\%$, respectively, during stage IV. The similar pH values recorded during both stages explain the comparable synthetic biogas upgrading performance regardless of the illumination regime. Thus, in spite of the continuous feeding of CO_2 and H_2S during stage III the pH remained constant at ≈ 9.5 likely due to high buffer capacity (alkalinity) of cultivation medium and the high photosynthetic activity of the AMC under continuous illumination. However, pH fluctuated from 9.3 to 9.7 during stage IV as a result of the periodic medium acidification during the dark period due to CO_2 and H_2S absorption, and the microalgae-based regeneration of alkalinity during the light period (see Figure S1). These high CO_2 and H_2S removal efficiencies were also supported by the high L/G ratio of 5, which prevented a deterioration in the CO_2/H_2S mass

transfer induced by a decrease in the pH of the cultivation medium in the ABC (Serejo et al. 2015). However, high L/G ratios often promote a high O₂ stripping from the recirculation medium to the biomethane, with the concomitant contamination of the upgraded biogas. In this study, the oxygen concentration in upgraded synthetic biogas averaged 2.6% v/v during stage III and IV. According to the Mexican regulation (NOM-001-SECRE 2010) oxygen concentration in biomethane must be lower than 0.20% v/v. This requires an optimization of the L/G ratio or the implementation of operational strategies devoted to decrease the oxygen liquid concentration prior recirculation to the ABC (Toledo-Cervantes et al., 2016). The complete removal of H₂S recorded in stage III and IV promoted the accumulation of SO₄²⁻ in the cultivation broth concomitantly with synthetic biogas upgrading. The high DO concentration prevailing in the HRAP during the entire experimental period (even during dark periods DO remained > 2 mg O₂ L⁻¹; see Figure S1) supported a complete oxidation of H₂S to sulphate (González-Sánchez and Revah, 2007) (Table 2). The concentration of S-SO₄²⁻ achieved during steady state in stages III and IV were 0.32 ± 0.02 g L⁻¹ and 0.34 ± 0.05 g L⁻¹, respectively.

3.2. Photobioreactor performance

The batch cultivation during stage I supported an increase in biomass concentration in the HRAP-ABC system from 0.12 g TSS L⁻¹ to 0.77 g TSS L⁻¹ by day 39, concomitant with a decrease in IC concentration from 1.25 g C L⁻¹ to 0.21 g C L⁻¹ (Figure 2). Process operation under continuous illumination at a HRT of 9.5 days entailed steady state biomass concentration of 0.72 ± 0.04 g TSS L⁻¹ during stage II. The continuous supply of MSM mediated an increase of the IC concentration in the HRAP, which stabilized at 1.13 ± 0.09 g C L⁻¹ from day 120 till 166. Synthetic biogas supply during stage III under continuous illumination resulted in an increase in biomass concentration up to 1.23 ± 0.05 g TSS L⁻¹,

which suggested that microalgae growth was limited by CO₂ concentration during stage II at the high pH value prevailing in the cultivation broth (pH=10.03 ± 0.16). The 50% reduction in light energy supply mediated by process operation under 12h/12h light /dark cycles induced a decrease in biomass concentration from 1.23 ± 0.05 g TSS L⁻¹ to steady state values of 0.23 ± 0.05 g TSS L⁻¹ by the end of stage IV. This decrease in TSS concentration entailed a reduction in the volumetric biomass productivity from 0.129 to 0.023 g TSS L⁻¹ d⁻¹, but did not impact on the concentration of IC in the HRAP, which remained constant at 1.02 ± 0.06 g C L⁻¹ (Table 2 and Figure 2) probably due to the CO₂ stripping as it is explained below. A detailed explanation of the mechanisms underlying the unexpectedly low biomass productivity is provided below. Process operation under continuous illumination during stage V supported an increase in biomass concentration and productivities up to steady state values of 0.94 ± 0.09 g TSS L⁻¹ and 0.098 g TSS L⁻¹ d⁻¹ (Table 2). The absence of synthetic biogas supply during stage V resulted in an increase in the pH up to 10.23 ± 0.05 (see Figure S1) and in the expected decrease in IC concentration to steady state values of 0.86 ± 0.12 g C L⁻¹ via biomass assimilation (Figure 2).

Figure 2 here

The average removal efficiencies of nitrogen and phosphorous during stages III, IV and V are shown in Table 2. Similar nutrient removal efficiencies were achieved during stage III and V, while the decrease in biomass productivity recorded in stage IV resulted in a significant decrease in the removal efficiency of phosphate. A potential N₂O production in the system was also ruled out due to the relatively high DO present in the HRAP during

stage IV ($> 2\text{mg O}_2 \text{ L}^{-1}$, see Figure S1), which prevented nitrate denitrification (Wang et al., 2008).

Table 2 here

3.3. C, N, P and S mass balances

Mass balance calculations for the main elemental components were conducted in the experimental system under steady state in stages III, IV and V.

Figure 3 here

The carbon present as CO_2 in the synthetic biogas supplied to the ABC accounted for approx. 40 % of the inorganic carbon inlet load in stage III and IV (Figure 3 a). The dissolved inorganic carbon present in the HRAP effluent accounted for 58 % of the output carbon from the system. On the other, the carbon contained in the biomass present in the liquid effluent represented 30 % of the output carbon during continuous illumination, while this share decreased to 7 % under light/dark cycles as a result of the limited microalgal productivity. This limited microalgae growth during stage IV resulted in an enhanced carbon stripping from the HRAP surface, which increased from 5 to 12 % (values referred to the total carbon output). The restoration of continuous illumination in the absence of synthetic biogas in stage V increased the share of inorganic carbon assimilated into biomass, which accounted for 37 % of the total output carbon, thereby minimizing carbon stripping from the HRAP surface. The nitrogen assimilated as biomass accounted for 54, 14 and 50 % of the output nitrogen in stages III, IV and V, respectively (Figure 3b). Similarly,

the phosphorous fixed in the biomass represented 17, 3 and 14 % of the output phosphorous in stages III, IV and V, respectively. For both nutrients, the share in the output streams was correlated with biomass productivity since no difference in the biomass N and P content was recorded. Finally, the H₂S present in the synthetic biogas accounted for 12 and 10% of the inlet Sulphur in stages III and IV, respectively. This share was significantly lower than the sulphur present as thiosulphate in the feed. Both reduced forms of sulphur were completely oxidized to sulphate, which represented 99.5% of the output sulphur in stage III, IV and V.

3.4. Effect of light/dark cycles on the biomass productivity

During the illuminated period in stage IV, microalgal photosynthesis was responsible of a net oxygen production in the HRAP. However, in the dark period, the microalgae and sulfide oxidizing bacteria consumed by respiration the dissolved oxygen present in the cultivation broth, with a concomitant production of CO₂ (Bahr et al., 2014; Masojídek and Koblízek, 2004). Hence, the dynamic balance between microalgae growth and endogenous biomass consumption entailed a steady state biomass concentration 0.2 g TSS L⁻¹, which were significantly lower than the expected value derived from a 50% reduction in light supply. In the absence of light supply, the microalgae consumed their intracellular organic carbon (i.e. carbohydrates) for cell maintenance, which was likely responsible of the lower biomass concentrations recorded. Figure 4, based on the estimated volumetric oxygen production and consumption (rO₂) rates from equation 7, shows that endogenous respiration activity was similar to the photosynthetic activity responsible of microalgae growth.

Figure 4 here

Two different temperature fluctuation trends can be observed in Figure 4, since the HRAP-ABC was exposed to the daily outdoors temperature fluctuations. These variations in the temperature of the cultivation broth influenced the extent of the oxygen production and endogenous respiration rates of the algal-bacterial consortium. From day 370 to 377, the temperature fluctuated from 13 to 24°C, while for days 387 to 396 the range was 16 to 28°C. This difference of 4 °C in the temperature fluctuation range induced a 60 % increase in both μ_{light} , μ_{dark} and biomass productivity (Table 3). Therefore, the microalgae productivities estimated were highly affected by the lower temperatures, which correlated with the decrease in the photosynthetic growth rates. The endogenous respiration yield, $y_{O_2/X}^r$, was up to three times higher than the photosynthetic oxygen production yield, $y_{O_2/X}^p$, and showed an inverse correlation with temperature. The $y_{O_2/X}^r$ here estimated were in agreement with the respiration yields typically reported in microalgal cultures (0.0034-0.124 mol O₂/gTSS) (Le Borgne and Pruvost, 2013; Ruiz-Martinez et al., 2016). The contribution of sulfide-oxidizing biomass to the total biomass productivity of the system was estimated to 4% (on a weight basis). Therefore, the role of this bacterial group on CO₂ fixation was negligible compared to photosynthetic CO₂ fixation (González-Sánchez and Revah, 2007). The novel methodology for the calculation of microalgae biomass productivity here developed based on continuous DO measurements was validated by the empirical biomass productivity estimated on the empirical measurements of the dilution rate and effluent biomass concentration (Table 3).

Table 3 here

4. Conclusions

Alkaliphic algal-bacterial consortia were shown as an effective platform to bioconvert biogas into biomethane. Microalgal photosynthesis was able to effectively regenerate the alkalinity consumed during CO₂ and H₂S absorption even during light/dark illumination regimes similar to those prevailing under outdoors conditions. Carbon and nutrient recovery was a function of the biomass productivity, which itself depended on the photobioreactor illumination regime and temperature. The endogenous microalgae respiration during the dark period was likely responsible of the higher decrease in biomass compared to that expected from a 50% reduction in light supply.

Acknowledgements

This research was supported by the National Science Foundation of Mexico (CB-Conacyt 168288), Mexican Secretary of Marine (SEMAR-Conacyt 207151), Mexican Secretary of Energy (SENER-Conacyt 247006) and by the internal project IIUNAM 4328. The analytical assistance of Dr. Juan Gabriel Vigueras Ramírez and the financial support of projects CTM2015-70442-R (MINECO-FEDER) and VA024U14 are also gratefully acknowledged. This research work was done in the IIUNAM-Environmental Laboratory with ISO9000 certificate.

Appendix A. Supplementary material

Supplementary material to this article can be found online

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Legends of figures

Figure 1. Experimental set-up used for the alkaliphilic synthetic biogas upgrading. Dashed lines represent synthetic biogas streams and continuous lines represent liquid streams.

Figure 2. Time course of the concentration of biomass (Δ) and inorganic carbon ($\bullet$) during the operation of the HRAP-ABC.

Figure 3. Carbon (a), nitrogen (b), phosphorous (c) and sulphur (d) mass balances for stages III, IV and V under steady state in the experimental HRAP-ABC. Numbers above bars represent the respective input and output loading rates ($\text{g L}^{-1}\text{d}^{-1}$).

Figure 4. Time course of the estimated oxygen production and consumption rates (a) and temperature (b) in the cultivation broth during the light and dark periods. Shade areas represent dark periods.

Table 1. Operational conditions deployed in the experimental system HRAP-ABC.

Stage	Period (d)	MSM condition	Operation mode	Illumination	Synthetic biogas supply	
I	0-39	(a)	Fed-batch	24 h	No	
II	40-166	(b)	Continuous HRT = 9.5 d	24 h	No	
III	167-269	(b)	Continuous HRT = 9.5 d	24 h	Yes	GRT= 23 min
IV	270-399	(b)	Continuous HRT = 9.5 d	12 h/12 h light/dark	Yes	
V	400-517	(b)	Continuous HRT = 9.5 d	24 h	No	

(a) Cultivation in MSM

(b) Cultivation in 1.8 diluted MSM containing $0.35 \text{ g Na}_2\text{S}_2\text{O}_3 \text{ L}^{-1}$

GRT-Gas residence time; HRT-Hydraulic residence time

Table 2. Summary of the environmental and operational parameters in the HRAP-ABC system under steady state during Stages III, IV and V.

Parameter	Stage III	Stage IV	Stage V
P_x (g L ⁻¹ d ⁻¹)	0.129 ± 0.005	0.023 ± 0.001	0.098 ± 0.012
X (g TSS L ⁻¹)	1.23 ± 0.05	0.23 ± 0.05	0.94 ± 0.09
DO (mg L ⁻¹)	10.8 ± 0.6	Light/Dark 11.4 ± 0.5 / 2.8 ± 0.1	10.8 ± 1.3
pH	9.53 ± 0.05	Light/Dark 9.71 ± 0.00 / 9.39 ± 0.00	10.23 ± 0.05
T (°C)	19.2 ± 2.6	22.8 ± 4.0	23.9 ± 1.7
N-NO ₃ ⁻ removal (%)	52 ± 9	ND	55 ± 21
P-PO ₄ ³⁻ removal (%)	24 ± 8	12 ± 7	29 ± 11
C-biomass (%w)	44.7	44.5	50.0
N-biomass (%w)	8.7	7.7	8.0
S-biomass (%w)	0.8	0.6	0.1
P-biomass (%w)*	1.0	1.0	1.0

* (Grobbelaar, 2004)

ND: not determined

Table 3. Influence of photosynthesis and respiration on the kinetic parameters and biomass productivity during light/dark cycles, based on the assumption of a 15 % w/w biomass lost by respiration during a 12 h dark period.

Temp. Range (°C)	Kinetic parameters					Biomass productivity (P_x)	
	$r_{O_2}^p$	$r_{O_2}^r$	$y_{O_2/X}^r$	μ_{light}	μ_{dark}	$\frac{r_{O_2}^p}{y_{O_2/X}^p} - \frac{r_{O_2}^r}{y_{O_2/X}^r}$	$D \cdot X$
	(mol L ⁻¹ d ⁻¹)		(mol g ⁻¹)	d ⁻¹		g L ⁻¹ d ⁻¹	
13 to 24	0.0010	0.0009	0.2441	0.0786	-0.0184	0.0120	0.0208
16 to 28	0.0016	0.0006	0.1017	0.1258	-0.0295	0.0193	0.0208