Elsevier Editorial System(tm) for Bioresource Technology

Manuscript Draft

Manuscript Number: BITE-D-16-03471R2

Title: Photosynthetic membrane-less microbial fuel cells enhance microalgal biomass concentration

Article Type: Original research paper

Keywords: photosynthetic microbial fuel cells, anaerobic digestion, microalgae, wastewater.

Corresponding Author: Dr. Enrica Uggetti, PhD

Corresponding Author's Institution: Universitat Politècnica de Catalunya

First Author: Enrica Uggetti, PhD

Order of Authors: Enrica Uggetti, PhD; Jaume Puigagut, PhD

Abstract: The aim of this study was to quantitatively assess the net increase in microalgal biomass concentration induced by PMFC. The experiment was conducted on six lab-scale PMFC constituted by an anodic chamber simulating an anaerobic digester connected to a cathodic chamber consisting of a mixed algae consortia culture. Three PMFC were operated at closed circuit (PMFC+) whereas three PMFC were left unconnected as control (PMFC-). PMFC+ produced a higher amount of carbon dioxide as a product of the organic matter oxidation that resulted in 1.5 to 3 times higher biomass concentration at the cathode compartment when compared to PMFC-.

Dear Editor,

We have received the comments from the Reviewers, and we have modified the manuscript accordingly. Comments suggested were considered, and the manuscript was carefully modified in order to meet the suggestions. We hope that the revised manuscript fully complies with comments and suggestions addressed by Reviewers. In the following you will find the Reviewers comments and our corresponding Responses.

Yours sincerely,

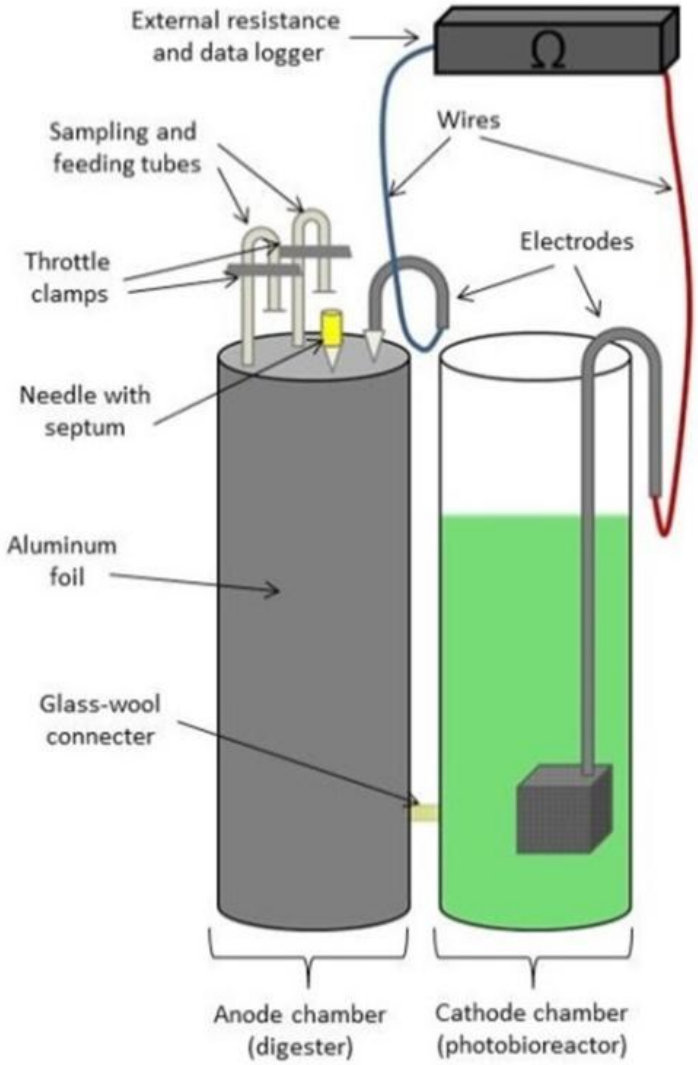
Enrica Uggetti

***Detailed Response to Reviewers**

Remove Fig 4 from revised version by giving details in text only.

Figure 4 has been removed as suggested, values have been added in the text (L 175-178): "The concentration of carbon dioxide at the anode chamber of active PMFC (average value of 6.6 µmol/m3) was significantly higher when compared to unconnected PMFC (average value of 1.6 µmol/m3) ($p<0.05$)."

Graphical Abstract (for review)



Higlights

- Photosynthetic microbial fuel cells PMFC were assessed for microalgae biomass boost.
- PMFC anode was set within an anaerobic digester fed with primary sludge.
- PMFC cathode consisted of a mixed consortia of algae fed with domestic wastewater.
- Active PFMC promoted higher CO_2 transfer, doubling cathodic algae concentrations.
- PMFC may enhance biomass valorization in algal-based treatment system.

Photosynthetic membrane-less microbial fuel cells enhance microalgal biomass concentration

Enrica Uggetti[1*] and Jaume Puigagut[1]

[1] GEMMA - Environmental Engineering and Microbiology Research Group, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya-BarcelonaTech, c/ Jordi Girona 1-3, Building D1, E-08034, Barcelona, Spain

[*] Corresponding author:

Tel.: +34 934016465

Fax: +34 934017357

E-mail address: enrica.uggetti@upc.edu

Abstract

The aim of this study was to quantitatively assess the net increase in microalgal biomass concentration induced by photosynthetic microbial fuel cells (PMFC). The experiment was conducted on six lab-scale PMFC constituted by an anodic chamber simulating an anaerobic digester connected to a cathodic chamber consisting of a mixed algae consortia culture. Three PMFC were operated at closed circuit ($PMFC^{+}$) whereas three PMFC were left unconnected as control ($PMFC^{-}$). $PMFC^{+}$ produced a higher amount of carbon dioxide as a product of the organic matter oxidation that resulted in 1.5 to 3 times higher biomass concentration at the cathode compartment when compared to $PMFC^{-}$.

Keywords: photosynthetic microbial fuel cells, anaerobic digestion, microalgae, wastewater.

Introduction

Microbial fuel cells (MFC) are bio-electrochemical devices generating electricity from the biodegradation of organic matter under anaerobic conditions. MFC have been widely investigated as a promising technology for wastewater treatment, bioenergy production and for biosensing purposes (Du et al., 2007; Kaur et al., 2013; Kim et al., 2003; Kumlanghan et al., 2007; Liu et al., 2011).

MFC are typically composed of two chambers, the anode and cathode chambers, separated by a proton exchange membrane. However, there are also membrane-less MFC that exploits the naturally generated redox gradient between the anode and the cathode zones to generate electricity. Regardless MFC cell architecture, the organic matter degradation in MFC is performed under anaerobic conditions at the anodic chamber by specific bacteria (exoelectrogens) releasing CO_2, electrons and protons. Electrons flow from the anode (anaerobic compartment) to the cathode (aerobic compartment) through an external circuit generating a current (Logan, 2008). Dissolved oxygen concentration (DO) at the cathode has been highlighted to be a key parameter on cell performance, being 6.6 mg/L the optimal concentration (Gil et al., 2003). Dissolved oxygen is generally provided by atmospheric air in contact with the cathode (air cathode configuration) or by active aeration. Oxygen supply to the anode and CO_2 accumulation at the cathode have been highlighted among the major bottlenecks of MFC (ElMekawy et al., 2014; Venkata Mohan et al., 2014).

Recently, photosynthetic microbial fuel cells (PMFC) integrating microalgae at the cathode chamber have gained interest due to their ability to not only provide an oxygen rich environment but also to remove CO_2 from the cathode compartment through the photosynthetic activity of algae (Gajda et al., 2013). Recent studies reported that even

small amounts of oxygen at the cathode provided by algae resulted in PMFC generating a significant current intensity (Rosenbaum et al., 2010; Venkata Mohan et al., 2009).

Microalgal biomass is nowadays regarded as a promising alternative to fossil fuel resources for energy and bio-products generation (Maity et al., 2014). Furthermore, anaerobic digestion has been recently acknowledged as a suitable strategy to energetically valorize the biomass produced during the treatment of domestic wastewater with algal-based systems (Gonzalez-Fernandez, 2015; Uggetti et al., 2014; Passos et al., 2014). In spite of promising results, the economic feasibility of energy valorization of algal biomass through anaerobic digestion is currently limited by the microalgal production rates and biomass concentration, which, in turn, is highly dependent on the availability of nutrients and carbon concentration. To this regard, wastewater is currently envisaged as an inexpensive source of nutrients to enhance microalgal biomass growth and concentration (Olguin, 2012). Nevertheless, domestic sewage typically contains insufficient inorganic carbon to fully support optimal algal production (3–7 C:N ratio in sewage vs. 6–15 C:N in algal biomass) (Park et al., 2011). For this reason, the external addition of carbon dioxide (CO_2) to microalgal cultures is nowadays recognized as appropriate practice to increase microalgal production.

In this context, PMFC integrating anaerobic digestion at the anodic chamber and a microalgal culture at the cathode is a suitable approach to overcome carbon limitation conditions in algal-based treatment systems without the need for external CO_2 supply. Accordingly, the CO_2 produced at the anode of a PMFC could be diverted to the cathode chamber to enhance microalgae growth. Ultimately, higher algae biomass at the cathode would enhance, in turn, the performance of anaerobic digestion.

The aim of this study was to quantitatively assess the effect of PMFC on the cathodic

74 microalgal biomass concentration. To this end, laboratory experiments were conducted
75 on membrane-less PMFC consisting of an anodic chamber operated as an anaerobic
76 digester that was connected to a cathodic chamber containing a mixed consortium of
77 microalgal biomass.

78 **Materials and Methods**

79 *Experimental setup*

80 The experimental design consisted of six H-type membrane-less PMFC. Each PMFC
81 consisted of two chambers (anode and cathode) connected by means of a silicon pipe (5
82 cm long and 5 mm inner diameter). The silicon pipe connecting both chambers was
83 filled with glass-wool in order to allow proton and gas exchange between the anode and
84 the cathode chambers (Mohan et al., 2008). Each chamber consisted of a plexiglass
85 cylinder of 20 mm height and an inner diameter of 64 mm (400 mL volume). The anode
86 chamber was batch loaded with 290 mL of primary sludge (TSS: 46 g/L; VSS: 23 g/L)
87 and inoculated with 110 mL of digestate (TSS: 35 g/L; VSS: 13 g/L) from a full-scale
88 anaerobic digester (wastewater treatment plant at Sant Feliu de Llobregat, Barcelona,
89 Spain), sealed and covered with aluminum foil in order to avoid light exposure.
90 Sampling ports for solid and gas extraction were implemented at the upper part of the
91 anaerobic reactor. The cathode was filled up with 400 mL of a mixed consortium of
92 microalgal biomass collected from a pilot high rate algal pond (HRAP) treating real
93 domestic wastewater. A detailed description of the HRAP is out of the scope of the
94 present paper and can be found elsewhere (Passos et al., 2013; Gutierrez et al., 2015). A
95 LED light lamp (90W) was set to illuminate the algae culture. Light cycle was set to
96 provide light/dark periods of 12 hours each. All the chambers were subjected to
97 continuous stirring (Multistirrer 6, Velp Scientifica, Italy).

Electrodes consisted of 14 graphite rods (5 mm diameter and 10 mm long) confined in a stainless steel grid-box (2.5x3x1cm) that worked as electron collector. Stainless steel was marine grade 316 L. The electrodes were connected by means of stainless steel wires (also marine grade 316 L) and the circuit was closed by implementing a 1000 ohms external resistance.

In order to test the effect of PMFC on microalgal biomass concentration at the cathode, three PMFC were operated at closed circuit conditions ($PMFC^{+}$) whereas three of PMFC were operated at open circuit ($PMFC^{-}$) and were used as control conditions.

Experimental procedure, sampling and analysis performed

The anode was fed only at the beginning of the experiment. Concerning the cathode, 50 mL of the mixed liquor were accurately extracted on a daily basis, and the cathodic volume was refilled with the same volume of filtered primary settled wastewater. Therefore, the cathode compartment was operated as a completely mixed algal-based treatment system operated at a hydraulic retention time (HRT) of eight days which is a typical HRT for high rate algal pond systems devoted to secondary wastewater treatment and simultaneous biomass production (García et al., 2000).

At the cathode, the pH was measured on a daily basis (at 10 a.m.) using a Crison Portable 506 pH-meter. Total suspended solids (TSS) were analyzed three times per week according to Standard Methods (APHA;AWWA;WEF, 2005). Nitrites (NO_2^--N), nitrates (NO_3^--N) and orthophosphates (PO_4^{3-}-P) were also analyzed using a DIONEX ICS-1000 ion chromatograph. Ammonium nitrogen (NH_4^+-N) was determined according to the Solorzano method (Solorzano, 1969). Biogas produced within the anodic chamber was weekly analyzed by gas chromatography in order to determine carbon dioxide (CO_2) and methane (CH_4) production (Agilent Technologies 7820A).

Cell voltage across the external resistance of the $PMFC^+$ was continuously monitored by means of a datalogger (CR1000, Campbell Scientific Inc., USA).

In order to assess the statistical significance of experimental results, repeated measures ANOVA test of variance was performed using the Minitab 17.0 Statistical Software. Results were considered statistically significant at $p<0.05$.

Results and Discussion

The three prototypes followed the same voltage pattern. Accordingly, the signal increased during the first eighteen days and then it remained almost constant at around 15 mV for the rest of the study period. Such low values could be attributed to the fact that neither electrodes design nor the electric circuit were optimized. Nevertheless, our results are consistent with that previously reported by Gonzalez del Campo et al. (2013). In that case, a maximum voltage of 16 mV was reached after twenty days of experiment. Figure 1 shows the typical voltage behavior during the light and dark cycles. This pattern was already expected since the microalgal photosynthetic activity during light conditions increases the oxygen concentration at the cathode which, in turn, makes the cell voltage increase. On the contrary, under dark conditions the oxygen derived from algae photosynthesis is no longer produced and the voltage drops.

The microalgal biomass concentration, here expressed as the concentration of total suspended solids (TSS) (Figure 2), was always significantly higher for the $PMFC^+$ (roughly between 1.5 and 3 times higher biomass concentration) than for the $PMFC^-$ ($p<0.05$). The biomass concentration of $PMFC^+$ rapidly increased from about 120 mg TSS/L up to about 350 mg TSS/L, while for the $PMFC^-$ biomass concentration slowly increased up to 145 mg TSS/L. This corresponds to productivities of about 29 mg TSS/L·d and 0.25 mg TSS/L·d for $PMFC^+$ and $PMFC^-$, respectively. Such low

146 productivities can be attributed to nutrients limitation.

147 Ammonium nitrogen and nitrites measured during the experiment (Table 1) indicated
148 significant differences between the effluent of closed and open circuit PMFC. Effluent
149 concentrations of ammonium were very low under both conditions, indicating that the
150 ammonium supplied was immediately consumed either by microalgae and/or oxidized
151 via nitrification.

152 Nitrates concentrations were significantly higher for the $PMFC^-$ (around 30 mg NO_3^--
153 N/L) when compared to the $PMFC^+$ (around 3 mg NO_3^--N/L). This was probably due to
154 the fact that, in MFC, nitrate can be used as an electron acceptor at the cathode (Fang et
155 al., 2011). Indeed, it has been demonstrated that exoelectrogenic bacteria can use both
156 nitrite and nitrate as electron acceptor for nitrogen reduction, depending on the electrons
157 flow (Puig et al., 2011).

158 Orthophosphates were entirely consumed in $PMFC^+$, while small residual amounts
159 (up to 2.1 mg PO_4^{3-}P/L) were detected in $PMFC^-$. The higher amount of biomass present
160 in the closed PMFC, combined with phosphates precipitation caused by the high pH (9–
161 10.5) probably contributed to the decrease of orthophosphates (Lau et al., 1997).
162 Moreover, considering the higher pH values recorded in $PMFC^+$ (Figure 3), it is
163 possible that phosphates precipitation occurred as well. Likewise, the pH indicates more
164 microalgal activity for $PMFC^+$, where values oscillated between 9 and 10 (Figure 3).

165 Algal biomass concentration here reported for the active PMFC is consistent to that
166 stated in current literature. Accordingly, Gonzalez del Campo et al. (2013) found
167 microalgal concentrations oscillating between 150 and 350 mg TSS/L. These authors,
168 unlike in our current experiment, worked with a monoculture of the algae *Chlorella*
169 *vulgaris* at the cathode chamber and fed the anode chamber with a synthetic fruit

processing industry wastewater. Furthermore, it is important to note that Gonzales del Campo and co-authors (2013) externally supplied CO_2 in order to promote the algal growth, while in the present study the only source of extra CO_2 provided was the one produced at the anode. Indeed, in our experimental setup, the glass wool placed between the two chambers was permeable to gasses, allowing the transfer to the cathode part of the CO_2 produced by the bacterial activity at the anode. The concentration of carbon dioxide at the anode chamber of active PMFC (average value of 6.6 µmol/m^3) was significantly higher when compared to unconnected PMFC (average value of 1.6 µmol/m^3) ($p<0.05$). Accordingly, CO_2 is generated in PMFC as an end product of the organic matter oxidation, regardless the carbon source provided (glucose, acetate or real wastewater) (Freguia et al., 2007). In this case, authors believe that the higher amount of CO_2 produced in the $PMFC^+$ was responsible for higher CO_2 concentrations available for microalgal consumption at the cathode, and thus enhanced microalgal biomass. This result is consistent with that described by Cui et al. (2014) which found that the supply of CO_2 produced at the anode doubled the microalgal concentration at the cathode (approximately from 500 to 1000 mg/L).

Unexpectedly, the production of methane was also significantly higher ($p<0.05$) for the $PMFC^+$ (10 µmol/m^3) when compared to the $PMFC^-$ (0.9 µmol/m^3). This may be due to the fact that PMFC enhanced not only exoelectrogenic bacteria metabolism, but also methanogenic-related metabolism. Indeed, the coexistence of exoelectrogenic and methanogenic bacteria was recently stated by Chung and Okabe (2009) reporting images of methanogenic archaea colonizing the anode surface in concomitance with several eubacteria. Moreover, at the temperatures at which the experiment was conducted (around 25°C), exoelectrogens have been demonstrated to be less competitive than methanogens for substrate (Karluvali et al., 2015). Moreover, in recent

studies, Rotaru et al. (2014a, 2014b) demonstrated that *Methanosaeta* species and *Methanosarcina barkeri* (being both methane producing bacteria) can directly accept electrons through biological electrical connections for the reduction of carbon dioxide to methane and that direct interspecies electron transfer can predominate over interspecies H_2/formate transfer during anaerobic digestion. Similarly, Corbella et al. (2015) found a higher abundance of *Methanosaeta* species in active membrane-less microbial fuel cells implemented in constructed wetlands.

Microbial interactions at electrode level are still under discussion in current literature. Therefore, authors suggest that further analysis on the microbial community shall be performed to light on the synthrophic relationships that might be taking place under the experimental conditions here considered. Overall, results here reported indicated a positive effect of active PMFC on both CO_2 and CH_4 production probably due to the enhancement of both exoelectrogenic and methanogenic metabolic activities.

Conclusions

PFMC operated at closed circuit conditions enhanced metabolic activities at the anode compartment (anaerobic digester) that resulted in higher CO_2 and CH_4 transferred to the algae consortium at the cathode compartment. PMFC operated at closed circuit showed greater biomass concentrations than those operated under open circuit conditions (roughly between 1.5 and 3 times higher biomass concentrations). This result evidence the potential application of PMFC as a strategy to increase biomass production in algal-based treatment systems that may lead to a greater degree of energy biomass valorization through anaerobic digestion.

Acknowledgements

This study was funded by the Spanish Ministry of Science and Innovation (MICINN) (projects CTM2010-17750 and CTQ2014-57293-C3-3-R). Enrica Uggetti is grateful to the Ministry of Economy and Competitiveness for her scholarship (IJCI-2014-21594). Authors kindly acknowledge Anne Delmarre for her contribution during experiment deployment.

References

1. APHA;AWWA;WEF. 2005 Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association, Washington, DC.
2. Chung, K., Okabe, S., 2009. Continuous power generation and microbial community structure of the anode biofilms in a three-stage microbial fuel cell system. Appl. Microbiol. Biotechnol. 83, 965–977.
3. Corbella, C., Guivernau, M., Viñas, M., Puigagut, J., 2015. Operational, design and microbial aspects related to power production with microbial fuel cells implemented in constructed wetlands. Water Res. 84 (1), 232-242.
4. Cui, Y., Rashid, N., Hu, N., Rehman, M.S.U, Han, J., 2014. Electricity generation and microalgae cultivation in microbial fuel cell using microalgae-enriched anode and bio-cathode. Energ. Conv. Manag. 79, 674-680.
5. Du, Z., Li, H., Gu, T., 2007. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. Biotechnol. Adv. 25, 464-482.
6. ElMekawy, A., Hegab, H.M., Vanbroekhoven C., Pant, D., 2014. Techno-productive potential of photosynthetic microbial fuel cells through different configurations. Energ. Reviews 39, 617-627.

241 7. Fang, C., Min, B., Angelidaki, I., 2011. Nitrate as an oxidant in the cathode
242 chamber of a microbial fuel cell for both power generation and nutrient removal
243 purposes. Appl. Biochem. Biotechnol. 164, 464-474.
244 8. Freguia S, Rabaey K, Yuan Z, Keller J., 2007. Electron and carbon balances in
245 microbial fuel cells reveal temporary bacterial storage behavior during electricity
246 generation. Environ. Sci. Technol. 41, 2915–21.
247 9. Gajda, I., Greenman, J., Melhuish, C., Ieropoulos, I., 2013. Photosynthetic
248 cathodes for Microbial Fuel Cells. Int. J. Hydrog. Energ. 38, 1559-1564.
249 10. García, J.; Mujeriego, R.; Hernández-Mariné, M., 2000. High rate algal pond
250 operating strategies for urban wastewater nitrogen removal. J. Appl. Phycol., 12,
251 331–339.
252 11. Gonzalez del Campo, A., Cañizares, P., Rodrigo, M.A., Fernández, F., J., Lobato,
253 J., 2013. Microbial fuel cell with an algae-assisted cathode: A preliminary
254 assessment. J. Power. Sourc. 242, 638-645.
255 12. Gonzalez-Fernandez, C., Sialve, B., Molinuevo-Salces, B., 2015. Anaerobic
256 digestion of microalgal biomass: Challenges, opportunities and research needs.
257 Biores. Technol. 198, 896-906.
258 13. Gil, G.C., Chang, I.S., Kim, B.H., Kim, M., Jang, J.K., Park, H.S., Kim, H.J.,
259 2003. Operational parameters affecting the performance of a mediator-less
260 microbial fuel cell. Biosens. Bioelectron. 18, 327–34.
261 14. Gutiérrez, R., Passos, F., Uggetti, E., Ferrer I., García J., 2015. Harvesting
262 microalgae from wastewater treatment systems with natural flocculants: Effect on
263 biomass settling and biogas production. Algal Res. 9, 204-211.
264 15. Kaur, A., Ibrahim, S., Pickett, C.J., Michie, J.., Dinsdale, R.M., Guwy, A.J.,
265 Premier, G.C., 2014. Anode modification to improve the performance of a

microbial fuelcell volatile fatty acid biosensor. Sensors Actuators B 201, 266–273.

16. Karluvalı, A., Köroğlu, E.O., Manav, N., Çetinkaya, A.J., Özkaya, B., 2015. Electricity generation from organic fraction of municipal solid wastes in tubular microbial fuel cell. Separ. Pur. Technol. 156 (2), 502-5011.

17. Kim, B., H., Chang, I.N., Gil, G.C., Park, H.S., Kim, H.J., 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. Biotechnol. Letters 25, 541–545.

18. Kumlanghan, A.; Liu, J.., Thavarungkul, P., Kanatharana, P., Mattiasson, B., 2007. Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter. Biosens. Bioelectron. 22 (12), 2939–2944.

19. Lau, P.S., Tam, N.F.Y., Wong, Y.S., 1997. Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized Chlorella vulgaris. Environ. Technol. 18, 945–951.

20. Liu, Z., Liu, J., Zhang, S., Xing, S.H., Su, Z., 2011. Microbial fuel cell based biosensor for in situ monitoring of anaerobic digestion process. Biores. Technol. 102, 10221-29.

21. Logan, B.E. Microbial Fuel Cells. 1st ed. New Jersey: Wiley Interscience, 2008.

22. Maity, J.P., Bundschuh, J., Chen, C.Y., Bhattachary, P., 2014. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. Energ. 78, 104-113.

23. Mohan, S.V., Raghavulu, S.V., Sarma, P.N., 20808. Biochemical evaluation of bioelectricity production process from anaerobic wastewater treatment in a single chambered microbial fuel cell (MFC) employing glass wool membrane. Biosens. Bioelectron. 23 (9). 1326–1332.

291 24. Olguin, E., 2012. Dual purpose microalgae–bacteria-based systems that treat
292 wastewater and produce biodiesel and chemical products within a Biorefinery.
293 Biotechnol. Adv. 30 (5), 1031-1046.
294 25. Park, J. B. K., Craggs, R. J., Shilton, A.N., 2011. Recycling algae to improve
295 species control and harvest efficiency from a high rate algal pond. Water Res., 45
296 (20), 6637–6649.
297 26. Passos, F., García, J., Ferrer, I., 2013. Biogas production from microalgae grown
298 in wastewater: effect of microwave pretreatment. Appl. Energ., 108, 168–175.
299 27. Passos, F., Uggetti, E., Carrère, H., Ferrer, I., 2014. Pretreatment of microalgae to
300 improve biogas production: A review. Biores. Technol. 172, 403-412.
301 28. Puig, S., Serra, M., Vilar-Sanz, A., Cabré, M., Bañeras, L., Colprim, J., Balaguer,
302 M.D., 2011. Autotrophic nitrite removal in the cathode of microbial fuel cells.
303 Biores. Techonol. 102 (6), 4462-4467.
304 29. Rosenbaum, M., He, Z., Angenent, L., 2010. Light energy to bioelectricity
305 photosynthetic microbial fuel cells. Curr. Opin. Biotechnol. 21, 259–264.
306 30. Rotaru, A., Shrestha, P., Liu,F., Shrestha, M., Shrestha, D., Embree, M., Zengler,
307 K., Wardman, C., Nevin, K.P., Lovley, D.R., 2014a. A new model for electron
308 flow during anaerobic digestion: direct interspecies electron transfer to
309 Methanosaeta for the reduction of carbon dioxide to methane. Energ. Environ. Sci.
310 7, 408–415.
311 31. Rotaru, A. Shrestha, P., Liu, F., Markovaite, B., Chen, S., Nevin, K., Lovley, D.,
312 2014b. Direct interspecies electron transfer between Geobacter metallireducens
313 and Methanosarcina barkeri. Appl. Environ. Microbiol. 80 (15), 4599–4605.
314 32. Solorzano, L., 1969. Determination of ammonia in natural waters by the
315 phenolhypochlorite method, Limnol. Oceanogr. 14, 799.

316 33. Uggetti, E., Sialve, B., Trably, E., Steyer, J.P., 2014. Integrating microalgae
317 production with anaerobic digestion: a biorefinery approach. Biofuels, Bioprod.,
318 Bioref. 8 (4), 516-529.
319 34. Venkata Mohan, S., Srikanth, S., Raghuvulu, S.V., Mohanakrishna, G., Kiran
320 Kumar, A., Sarma, P.N., 2009. Evaluation of the potential of various aquatic eco-
321 systems in harnessing bioelectricity through benthic fuel cell: effect of electrode
322 assembly and water characteristics. Bioresour. Technol. 100, 2240–2246.
323 35. Venkata Mohan, S., Srikanth, S., Chiranjeevi, P., Arora, S., Chandra, R., 2014.
324 Algal biocathode for in situ terminal electron acceptor (TEA) production:
325 Synergetic association of bacteria–microalgae metabolism for the functioning of
326 biofuel cell. Biores. Technol. 166, 566–574.

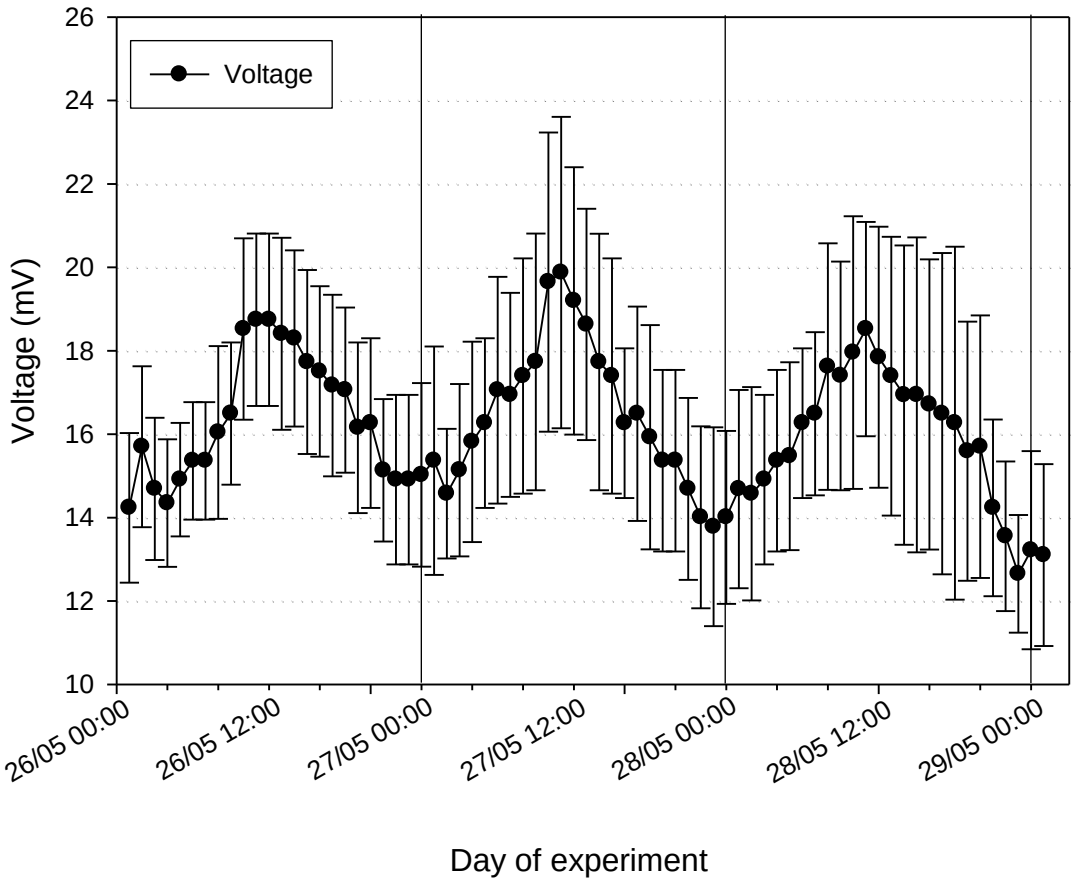
327

Table 1. Average concentration of water quality parameters measured at the influent and effluent of the cathode chamber along the study period. Note that statistical significance is given for the comparison among PMFC+ and PMFC-.

	Influent	**Effluent**		
		$PMFC^+$	$PMFC^-$	P-value
NH_4^+-N (mg/L)	31±2.9	0.5±0.4	0.1±0.1	<0.05
NO_2^--N (mg/L)	n.d.	6.2±3.3	1.0±0.7	<0.05
NO_3^--N (mg/L)	n.d.	3.4±2.7	25.9±6.8	<0.05
PO_4^{3-}-P (mg/L)	4.4±0.6	0.0±0.2	1.1±1.0	<0.05

328

329

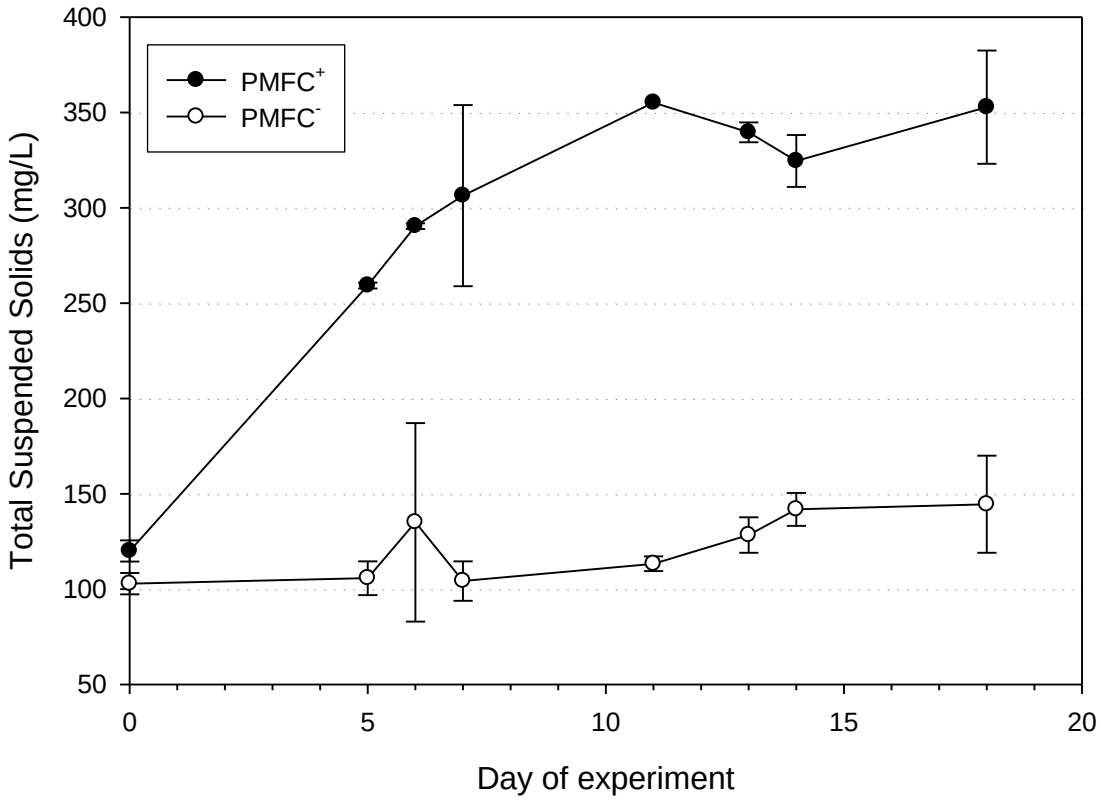


330

331 Figure 1. Behavior of the voltage recorded in the three closed PMFC during three

332 light and dark cycles (average and standard deviation).

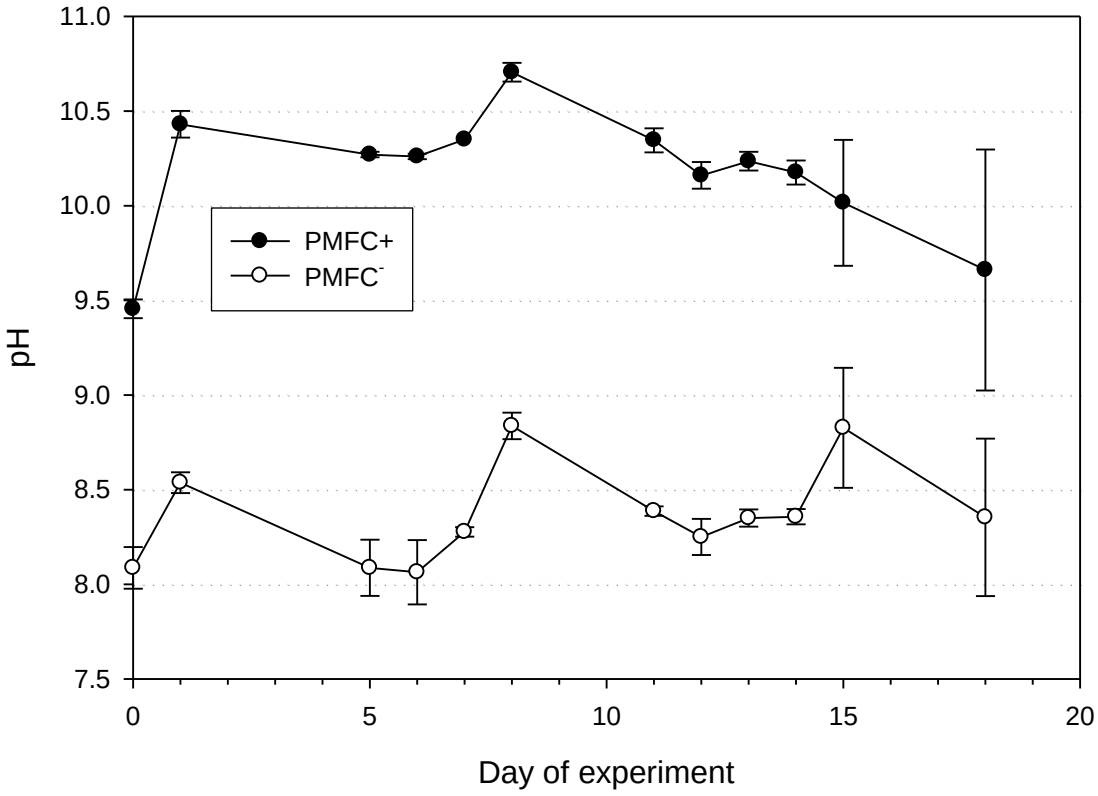
333



334

335 Figure 2. Average and standard deviation of total suspended solids concentrations
336 measured at the cathode compartment along the experiment for the PMFC operated at
337 close circuit (solid circles) and the PMFC operated at open circuit (open circles). Note:
338 each value averages three replicates.

339



340

341 Figure 3. Daily average pH measured for the PMFC+ and the PMFC- along the

342 experiment.

343