

Assisting cultivation of photosynthetic microorganisms by microbial fuel cells to enhance nutrients recovery from wastewater

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Abstract

Spirulina was cultivated in cathodic compartments of photo-microbial fuel cells (P-MFC). Anodic compartments were fed with swine-farming wastewater, enriched with sodium acetate (2.34 g_{COD} L⁻¹). Photosynthetic oxygen generation rates were sufficient to sustain cathodic oxygen reduction, significantly improving P-MFC electrochemical performances, as compared to water-cathode control experiments. Power densities (0.8–1 W m⁻²) approached those of air-cathode MFCs, run as control. COD was efficiently removed and only negligible fractions leaked to the cathodic chamber. *Spirulina* growth rates were comparable to those of control (MFC-free) cultures, while pH was significantly (0.5–1 unit) higher in P-MFCs, due to cathodic reactions. Alkaliphilic photosynthetic [microorganisms](#) like *Spirulina* might take advantage of these selective conditions. Electro-migration along with diffusion to the cathodic compartment concurred for the recovery of most nutrients. Only P and Mg were retained in the anodic chamber. A deeper look into electro-osmotic mechanisms should be addressed in future studies.

Keywords: Photo-microbial fuel cells; Wastewater; Nutrients recovery; Microalgae; *Spirulina*

1 Introduction

Photosynthetic microorganisms (PM) cultures can be applied as wastewater treatment technology in lagoons or raceway-like photo-bioreactors. These systems offer several advantages over conventional water treatment processes, including the recovery of nutrients and CO₂ through autotrophic metabolism. When consortia of PM (usually green microalgae or cyanobacteria) and bacteria are employed, syntrophic processes concur to wastewater treatment. On one side, bacteria oxidize organic carbon enriching the medium of CO₂/HCO₃⁻ and nutrients, whereas PM supply O₂ produced by photosynthesis to aerobic bacteria, while maximizing inorganic nutrients uptake and recovery ([Acién et al., 2016](#)). Depending on the type of wastewater, large amounts of K, N, and P are usually present, together with trace elements (B, Cu, Zn, Mo, Fe, Co and Mn), required for the growth of PM. In these systems, mechanical aeration to stimulate organic carbon oxidation could be avoided ([Molinuevo-Salces et al., 2010](#)). Besides, the obtained PM-rich sludge can be a source of added-value hydrolysates (e.g. bio-fertilizers, bio-stimulants and plant growth promoters) ([Acién et al., 2016](#)) or molecules (e.g. natural dyes, anti-oxidants, bio-polymers, carotenoids etc.) ([Ledda et al., 2016](#)).

Several constraints have limited field applications of this bioprocess, especially for the treatment of organic-rich streams, such as animal slurries, agro-food industry wastewater and digestate from anaerobic digestion. The presence of easily bio-available organic carbon at high concentrations (soluble-COD > 1 g/L) favor heterotrophic bacteria growth over PM, easily driving the system to anaerobic conditions, where PM are generally inhibited ([Olguín, 2012](#)). Suspended recalcitrant organic compounds, also, tend to increase water turbidity and limit light penetration into the culture ([Wang et al., 2010](#)). Contamination by pathogenic bacteria present in the wastewater can also limit PM growth and contribute in lowering process efficiency

(Cai et al., 2013). Finally, excess of ammonium and other inorganic compounds can have direct inhibiting effects on PM growth rate (Ledda et al., 2016).

Solutions to these problems have been achieved by diluting the inlet wastewater or significantly lowering wastewater loading rate in the lagoon, i.e. decreasing treatment capacity and increasing treatment costs per volume unit. Otherwise, pretreatments of the wastewater were proposed, including ultra-membranes filtration, UV-sterilization, flocculation of organics, etc. All these solutions contribute to significantly increase in power consumption (Acién et al., 2016).

In treating organic-rich wastewater, an alternative to traditional PM/bacteria cultures is assisting PM cultivations by the introduction of microbial fuel cell (MFC) systems. Such systems have been named photosynthetic algal MFC (Gouveia et al., 2014; Strik et al., 2008) or photo-microbial fuel cells (P-MFC) (Zhang et al., 2011). In P-MFCs, biological anaerobic oxidation of organic carbon is maximized at the anode, by the presence of the counter oxygen reduction reaction at the cathode. Single chamber P-MFCs were proposed by some authors, where the anode is simply buried into an anaerobic sediment, underneath a PM culture (Zhang et al., 2011). Though this single-chamber configuration is the simplest, it doesn't ensure efficient separation between bacteria and PM. This might be a limiting factor, especially for treating wastewater rich in organics and pathogenic-bacteria (e.g. animal manure).

In double chamber P-MFCs, the anodic and cathodic compartments are separated by a membrane or a porous separator (Gajda et al., 2014b; Roy et al., 2016). Such systems can guarantee efficient separation between the PM culture (in the cathodic compartment) and anaerobic bacteria (both in the bulk wastewater and in anodic biofilms), impeding bacterial contamination of the PM environment (Elmekawy et al., 2014). The electrochemical system allows maximizing organic carbon oxidation to $\text{CO}_2/\text{HCO}_3^-$ at the anode, while reducing the oxygen produced by photosynthesis in the separated cathodic chamber. Simultaneously, inorganic carbon and dissolved nutrients are allowed to diffuse through the separator to the cathodic chamber, where PM utilize them as growth medium (see Graphical Abstract).

Oxygen consumption at the cathode is also another useful function in assisting PM cultures by MFCs. Dense PM populations ($>1 \text{ g}_{\text{cells}} \text{ L}^{-1}$) can easily reach inhibiting DO concentrations ($>20 \text{ mg}_{\text{O}_2} \text{ L}^{-1}$) and air-bubbling or degassing systems are used to control DO (Carvalho et al., 2006). The cathode works as self-powered oxygen-consuming interface and has the potential to substitute these energy-consuming processes in photo-bioreactors.

At the same time, the MFC system itself is assisted by the photosynthetic activity at the cathode (Gajda et al., 2014b; Rosenbaum et al., 2010). Double-chamber water-cathode MFCs are known to be limited by low dissolved oxygen (DO) concentrations. DO consumption kinetics at the cathode are generally higher than those of oxygen dissolution from air, leading to DO depletion in the cathodic chamber (Kim et al., 2007). For this reason, open-air cathodes have been largely preferred to enhance current densities as well as COD removal performances in MFC (He et al., 2015; Srikanth et al., 2016). PM have been proposed as oxygen supplier in the cathodic compartment of MFC, to sustain DO consumption by cathodic reduction reaction and light intensity has been correlated to DO in a microalgae-assisted cathode (Gajda et al., 2013; González Del Campo et al., 2013; Wu et al., 2014).

Despite some basic works having been published on P-MFCs and laboratory-scale trials showing evidence of promising results, to our knowledge no prototype or pilot scale experience have been tried yet. Their potential applications, especially in treating organic-rich wastewater while recovering nutrients, are still to be explored. Several fundamental questions remain open on how P-MFC systems work. The aim of this work was to investigate some pertinent questions:

- is photosynthetic oxygen, provided by PM cultivation, sufficient to sustain the performance of the MFC, as compared to air-cathode configurations?
- is PM's growth negatively or positively affected by the presence of the cathode, as compared to a non-assisted PM culture?
- can high concentrations of COD ($>1 \text{ g/L}$) be efficiently removed in the anodic compartment, without diffusing to the cathodic chamber?
- how do important parameters (e.g. DO concentrations, pH, conductivity) vary in the PM culture, thanks to the presence of the cathode? how does DO and pH profiles look like at cathode interface?
- can nutrients be removed from the wastewater? What mechanisms can drive nutrients recovery?

A P-MFC and a control PM culture were compared to understand the effects of the electrochemical system on PM growth. At the same time, an air-cathode and a water-cathode MFC were used as additional control to assess the effects of the PM culture on the electrochemical system. The water-cathode MFC was also considered as control to measure nutrients diffusion from the anolyte to the cathodic chamber, without the influence of the PM culture.

2 Materials and methods

Four different system configurations were compared, using lab-scale reactors and electrodes of same dimensions: i) P-MFC, ii) water-cathode-MFC (W-MFC), iii) air-cathode MFC (A-MFC) and iv) control PM culture. All experiments were carried out in duplicates (8 reactors overall). Details on reactors and electrode preparation are reported in the following.

2.1 MFCs configuration

Simple Pyrex® bottles (125 ml volume) with two lateral openings were used as MFC, as described elsewhere (Guerrini et al., 2013). The small opening in the bottles served to insert the anode, while the large opening held the cathode. One single bottle was used for A-MFC and for control PM-culture. Two bottles were coupled for A-MFC and W-MFC configurations. Electrodes were made of carbon cloth (SAATI C1, Appiano Gentile, Italy). Plain carbon cloth ($4 \times 10 \text{ cm}$) was rolled and placed on the bottom of the cell to serve

as anode. Carbon cloth modified by a microporous layer (MPL) made of activated carbon/PTFE mixture was used for cathodes (Santoro et al., 2014). This MPL served to increase the surface area available for O₂ reduction reaction and also as a porous separator between anodic and cathodic compartment. Geometric surface area of the cathode exposed to the anolyte was 3.14 cm². Anode and cathode were electrically connected through an external copper circuit under a load of 100 Ω. Connections were insulated with non-conductive epoxy resin.

2.2 Start-up phase

A start-up phase served to enrich electroactive biofilms in P-MFC, W-MFC and A-MFC systems. All anodic chambers were inoculated in parallel with swine manure (approximately 30 g_{COD} L⁻¹), diluted 1:10 w/w with tap water to obtain a concentration of 3 g_{COD} L⁻¹. The chemical composition of the swine-farming wastewater at the beginning of the experiment is reported in Table 1.

Table 1 Chemical composition of the swine-farming wastewater at the beginning of the experiment.

Element	Total content (µg/L)	Dissolved fraction ^a (µg/L)
Na	86701	70421
Mg	31633	17031
K	274673	221501
Ca	17989	12721
Cr	7	4
Mn	561	190
Fe	10067	971
Co	9	7
Ni	51	37
Cu	1457	118
Zn	10099	974
P	58906	36692
N-NH ₄ ⁺	—	217000

^a Measured after microfiltration (0.2 µm).

In parallel, the cathodic chamber of P-MFC and the control PM culture were inoculated with cyanobacteria belonging to the genus *Arthrospira* (also known as *Spirulina*). *Spirulina* is one of the most used PM in full-scale production with an alkaliphilic nature (grows at pH 9–11) and easy biomass recovery, due to the filamentous cell structure (Rodrigues et al., 2016). Open raceway ponds are the most common and inexpensive cultivation systems (see Graphical Abstract). The PM culture was obtained from a full scale raceway-like plant in Northern Italy (Spirufarm srl, Milan, Italy) and photo-autotrophically cultivated in 1.0 L Erlenmeyer flasks using Zarrouk's culture medium (Zarrouk, 1966). The Zarrouk's medium is free of organic compounds, except for EDTA used as chelating agent. All chemicals were analytical grade and Milli-Q water was used to prepare all solutions. *Spirulina* in culture medium was maintained under stirring by means of a magnetic stirrer, under day-night illumination (photoperiod light:dark = 18:6, light intensity of around 60–80 µE m⁻² s⁻¹) by a white fluorescent lamp (6400 K) and at constant temperature (25 ± 1 °C) (Chen and Zhang, 1997).

2.3 Experiment

All MFCs were operated in parallel in fed-batch mode at 25 ± 1 °C. Several consecutive batch cycles were run (9 cycles), each time adding 3 g L⁻¹ (2.34 g_{COD} L⁻¹) sodium acetate as organic substrate in the anodic chamber. This soluble and bioavailable COD concentration simulate organic-rich wastewater, typical of agro-industrial contexts, e.g. animal manure slurries (Ledda et al., 2013; Markou et al., 2012). When all systems reached stable current production at successive cycles, electrochemical and physico-chemical parameters were measured.

In the cathodic chamber of P-MFC systems and in the control PM cultures, the *Spirulina* culture was continuously stirred and maintained under day-night regime illumination (16 h light and 8 h dark, controlled by a timer). Artificial solar irradiation was supplied by

two lamps (8 W/50 Hz, 6400 K) placed at 0.1 m distance. Dark conditions were maintained in the anodic compartments by covering glassy reactors with aluminum foil. The PM growth medium was partially replaced with fresh Zarrouk's medium (half volume), when microbial dry mass (DM) exceeded 1.2 g L^{-1} .

Generally, PM biomass productivity and composition mainly depends on some chemical-physical conditions of the growth environment. The most important factors are nutrients availability, pH and alkalinity, light penetration, cell density and temperature (Markou and Georgakakis, 2011). In the present experiment, the purpose was to isolate some of these factors to investigate the eventual effect of the electrochemical system. For this reason, an optimized growth medium (Zarrouk, 1966) was used in P-MFC, to exclude the variable of eventual lack of nutrients.

The W-MFC system served as control for the P-MFC: a) to investigate the effect of photosynthesis on DO concentrations and O_2 reduction reaction at the cathode; b) to monitor the migration of organic and inorganic substances from the anodic to the cathodic chamber. The cathodic chamber was initially filled with water, to allow nutrients detection by ICP-MS. Three times during the experimental period, water was completely replaced with fresh water and analyzed.

The electrochemical systems were characterized for maximum current and power density production, coulombic efficiency (ϵ_c), open-circuit and short-circuit potentials (OCP and SCP, respectively), anode/cathode polarization. All anodic chambers were characterized in terms of soluble COD (sCOD) removal (at the end of each anodic cycle); pH and conductivity trends (measurements every 1–2 days); nutrients removal (at the end of the experiment). The cathodic chambers of P-MFC and W-MFC were monitored for what concerns DO, pH, conductivity (measurements every 1–2 days); COD concentrations (at the end each anodic cycle); and nutrients concentrations (at the end of the experiment). In P-MFC and in the control PM culture, DM was measured every 1–2 days. Microelectrodes were used to measure DO and pH at the interface of the porous separation layer, on both anodic and cathodic side. All details for analytical procedures are reported in Section 2.4.

2.4 Chemical analyses

pH, conductivity and DO in the bulk anodic and cathodic compartments were measured with a pH-meter AMEL Instruments mod. 2335, a conductivity-meter AMEL Instruments mod. 2131 and an oxy-meter AQUALYTIC mod. AI200xi, respectively. pH and DO profiles at the interface of the cathode surface were measured with microelectrodes. A potentiometric microelectrode was used for pH (Guerrini et al., 2014) and an enzymatic O_2 probe was used for the measurement of oxygen (Grattieri et al., 2015). Measurements were performed under open circuit conditions and the positions of microelectrode were determined by a computer controlled micromechanical movement system (NSC-A1 Stepper Motor Controller, Newmark Systems Inc.).

Liquid samples from anodic and cathodic compartment were collected at the beginning and the end of each cycle. sCOD was measured after filtration (0.2 μm nylon filters) using HACH COD vials and HACH DR220 Vis-spectrophotometer, following the standard procedure. For multi-elemental analysis, samples were diluted 1:100 with 0.3 M HNO_3 in Milli-Q water and the concentration of elements was measured by ICP-MS (BRUKER Aurora-M90 ICP-MS). An aliquot of a 2 mg L^{-1} of an internal standard solution (72Ge, 89Y, 159Tb) was added both to samples and calibration curve to give a final concentration of $20 \mu\text{g L}^{-1}$. Typical polyatomic analysis interferences were removed by using CRI (Collision-Reaction-Interface) with an H_2 flow of 75 mL/min^{-1} flown through skimmer cone.

Dry cell mass concentration (DM) of *Spirulina* was measured upon filtration and drying in air. Indirect measurements were performed by a calibration curve with DM and optical density. Optical density of culture was monitored almost daily by recording the absorbance spectra with a HACH DR2000 Vis-spectrophotometer.

2.5 Electrochemical analysis

For each MFC, the potential difference across a load of 100Ω (R_{ext}) was recorded every 20 min using a multichannel Data Logger (Graphtech midi Logger GL820). The generated current (I) was then calculated by the equation $I = V/R$, where I is the current flowing through the external resistance. Accordingly, the current density was reported with respect to the geometric surface area of the cathode. The Coulombic efficiency ϵ_c was calculated from the generated current and the amount of sCOD removal.

Polarization curves of the MFCs (power curves) were recorded with anode and cathode as working and counter electrode, using a two-electrode configuration. MFCs were allowed to equilibrate at least 2 h at the open circuit potential (OCP). Then the potential was linearly varied from the OCP to 10 mV at a scan rate of 10 mV/min. Internal resistance (R_{int}) of MFCs was then calculated by the equation $P_{max} = R_{int} I_{Pmax}^2$.

Polarization curves of single electrodes were recorded *in situ* on anodes and cathodes using a three-electrode configuration. The anode or the cathode was used as working electrode. A platinum wire and an Ag/AgCl (sat. KCl) electrode were used as counter and reference electrode, respectively. A Luggin capillary was adopted to minimize the ohmic drop into the solution, when polarizations on cathode were recorded. Before recording polarization curves, MFCs were allowed to equilibrate at the OCP for at least 30 min. Potential was then varied at 10 mV/min from the OCP in the anodic or cathodic direction.

An EG&G mod. 273A potentiostat/galvanostat was used to record polarization curves. All the potentials throughout the text are referred to the Ag/AgCl (sat. KCl) electrode.

3 Results and discussion

3.1 Current generation, COD removal and electrochemical properties

Fig. 1 reports the current density trend over the whole experimentation time for each reactor type. MFCs were operated for 77 days, with 9 fed-batch cycles. The acclimation period lasted nearly 40 days (4 fed-batch cycles). During acclimation, the current density produced by all MFCs continuously increased for each consecutive cycle. All MFCs exhibited a prompt increase of current when sodium acetate was added into the anodic chamber, then the current decayed below a negligible value as a consequence of acetate

depletion. Current production and ϵ_c were similar for P-MFCs and A-MFCs (up to 45 A/m²). W-MFCs exhibited lower performances in terms of power generation, ϵ_c and COD removal. Small variations in current density were observed between duplicates.

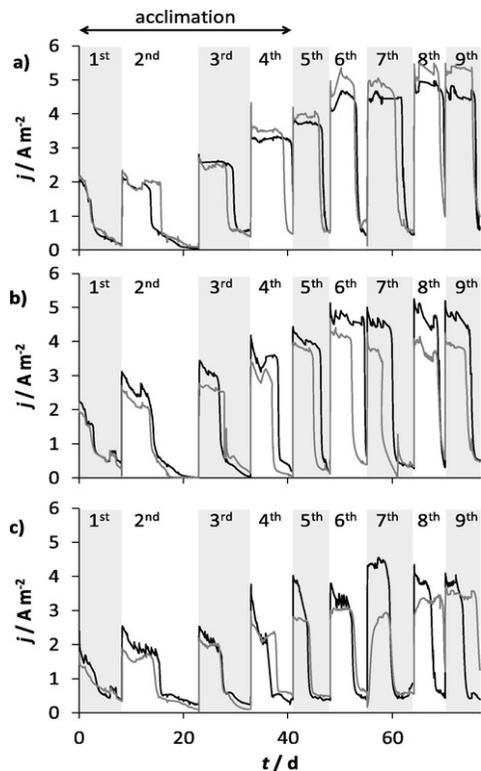


Fig. 1 Comparison of the current density over the whole experimentation period for two MFCs of each type: A-MFC (a); P-MFC (b); W-MFC (c). The duration of the acclimation period and of successive feeding cycles with sodium acetate (3 g/L) are indicated.

After acclimation (from the fourth cycle on), the performances of all MFCs increased in terms of current generation. The maximum current density achieved in each fed-batch cycle settled at about 5 A/m² for A-MFCs and P-MFCs and 4 A/m² for W-MFCs. The consumption of organic substrate in anodic chamber occurred with high efficiency. Considering an average of cycles 5–9 (days 40–88), the COD removal resulted of 89 ± 1% for A-MFC and P-MFC and 87 ± 1% for W-MFC, respectively, with no significant differences among all systems (Table 2). The increased performances of MFCs with respect to acclimation period led to significant increases of ϵ_c . In particular, ϵ_c was significantly ($P < 0.05$) higher in A-MFC as compared to P-MFC and P-MFC was higher than in W-MFC (Table 2). No remarkable differences were found in the ranges of pH and conductivity, measured in the anodic chambers of all systems (Table 2).

Table 2 Electrochemical and chemical parameters after acclimation period (days 40–88). Average or range in duplicate trials. Numbers in the same line followed by same letters are not significantly different (Tukey test $p < 0.05$).

	P-MFC	A-MFC	W-MFC	PM culture	
$P_{max} / W m^{-2}$	0.85 ± 0.29 ^a	0.98 ± 0.13 ^a	0.51 ± 0.11 ^b	—	
R_{int} / Ω	287	210	429	—	
Cell SCP / mV	−442 ± 60 ^a	−289 ± 26 ^b	−444 ± 59 ^a	—	
OCP / mV		Anode / e	−524 ± 30 ^a	−531 ± 49 ^a	−542 ± 25 ^a
		Cathode / e	26 ± 44 ^a	25 ± 44 ^a	27 ± 44 ^a
Coulombic Efficiency (ϵ_c)			17.0 ± 3.3 ^{ab}	20.4 ± 3.9 ^a	13.4 ± 3.1 ^b

sCOD removal from anodic chamber / %		89 ± 1 ^a	89 ± 1 ^a	87 ± 1 ^a	–
sCOD concentration in cathodic chamber / mg _{COD} L ⁻¹		359 ± 113 ^a	–	48 ± 34 ^b	221 ± 84 ^a
DO concentration range in cathodic chamber / mg _{O₂} L ⁻¹		10–32	–	2–6	12–30
Bulk pH range	Anodic	7.4–8.9	8.1–8.7	7.6–8.8	–
	Cathodic	9.7–11.8	–	8.6–12.5	8.9–10.6
Bulk Conductivity range / mS cm ⁻¹	Anodic	6.8–8.2	8.0–9.6	5.2–9.3	–
	Cathodic	10.4–20.0	–	0.4–3.9	16–18

When reproducible trends at successive cycles were observed, MFCs were fully characterized for electrochemical and chemical parameters. Fig. 2 shows power curves and anode/cathode single-electrode polarization curves recorded for the MFCs at maximum power production, in a representative cycle. From power curves, maximum power density (P_{max}) and internal resistance (R_{int}) were obtained (Table 2). Average P_{max} was only slightly higher ($P < 0.05$) in A-MFC, as compared to P-MFC, and they were both remarkably higher than W-MFC. Vice versa, R_{int} was very different for the three systems, the lowest for A-MFC. Average SCP was ($P < 0.05$) less negative in A-MFC as compared to both P-MFC and W-MFC (Table 2).

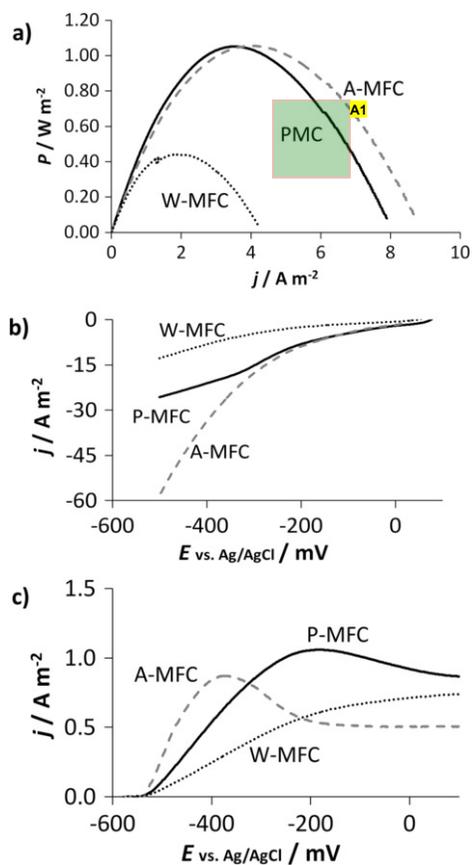


Fig. 2 Electrochemical characterization of MFC systems at day 72: Power curves of MFCs (a); polarization curves recorded on cathodes (b) and anodes (c).

Annotations:

A1. please, replace PMC with P-MFC

Single-electrode polarizations revealed differences in electrochemical behavior among different systems (Fig. 2b and c). Cathodes of W-MFC exhibited very low performances, in agreement with lower power production and lower Coulombic efficiencies. Higher currents were recorded for A-MFC and P-MFC in the investigated potential range. Polarization curves overlapped, until the potential of -300 mV. At lower potentials, A-MFC showed an exponential current increase that was not recorded in P-MFC. Anodic curves (Fig. 2c) highlighted other differences among the three systems. Lower current with a diffusion limited plateau were evident for anodes of W-MFC. In the case of A-MFC, an oxidation peak typical of redox mediator on carbon based anode (Fricke et al., 2008) is visible at -400 mV. For P-MFC, lower anodic currents were observed in the range of operating potentials of MFCs (Table 2). At potential less negative than -300 mV the anodic current of P-MFC is higher than for A-MFC and a shift of the anodic peak was observed towards higher potentials.

3.2 P-MFC vs. A-MFC performances

Along all the experiment, in P-MFCs photosynthetic oxygen production rate kept DO concentrations high enough to sustain ORR at the cathode, at comparable extents to the air-exposed cathodes of A-MFC. Normally, the replenishment of O₂ as reactant for ORR might be more difficult in the water bulk as compared to air-exposed cathodes, due to slower mass-transport rates. Other phenomena could concur in limiting DO mass transfer to the cathodic surface, such as possible formation of biofilm at the porous interface, as well as salt deposition over long term MFC operation. However, here an ORR limitation in P-MFC, as compared to A-MFC, was evident from cathode polarizations only at potentials lower than -300 mV vs. Ag/AgCl (Fig. 2b). Also, DO was always higher than saturation levels, even at cathode interface (Fig. 4a). Even if cathodic ORR was limited by mass-transport up to some extent, the electrochemical performances of P-MFC were still comparable to A-MFC in terms of power generation, internal resistance, Coulombic efficiencies and sCOD removal (Table 2). This means that the P-MFC configuration can be used without significant lack of performance of the MFC system, as compared to air-breathing cathode configuration.

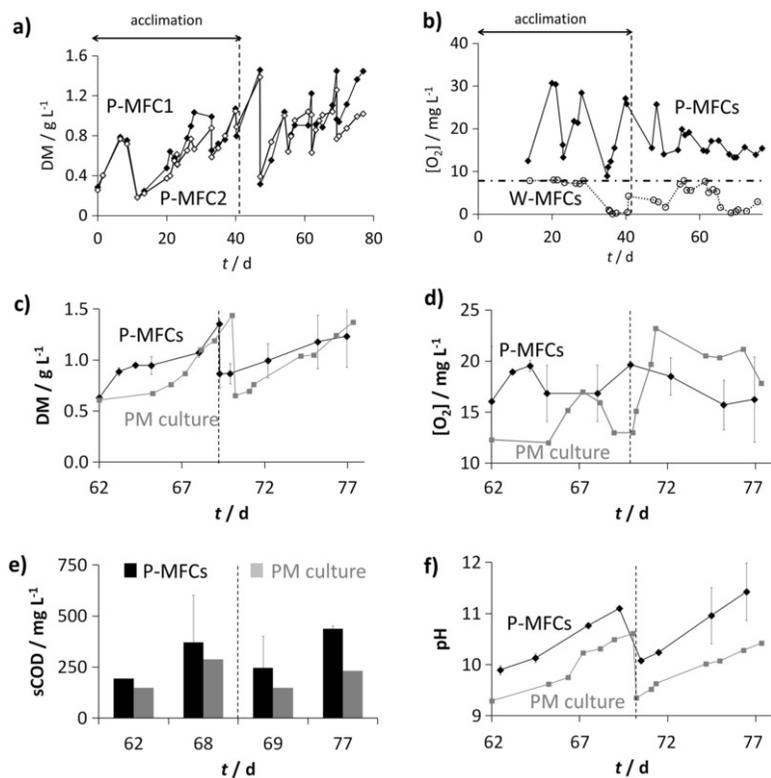


Fig. 3 *Spirulina* growth conditions in P-MFCs, compared to W-MFCs and MFC-free PM cultures: dry mass (DM) trends in duplicate P-MFCs (a); dissolved oxygen (DO) in the bulk catholyte of P-MFCs and W-MFCs (b); MFC-free PM culture compared to PMFCs for *Spirulina* growth as DM (c); dissolved oxygen DO (d); soluble-COD (e); pH (f). Dash-dotted line refers to water saturation DO concentration at experimental conditions.

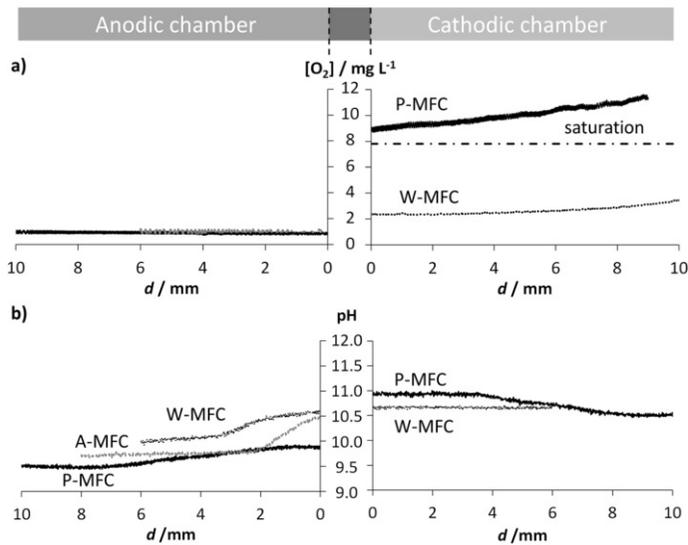


Fig. 4 Dissolved oxygen DO (a) and pH (b) profiles approaching the cathode of MFCs from both the anodic and cathodic compartment (dashed-dotted line refers to DO oxygen at saturation in water). The x-axis reports the distance from the cathode surface.

The direct effect of photosynthetic oxygen on cathodic ORR was already observed in previous experiments (Fu et al., 2009; Gajda et al., 2014c). Here, photosynthesis played a fundamental role in sustaining DO consumption at cathode interface (Fig. 4a), with significantly improved performances of P-MFC, as compared to W-MFC (Fig. 2). In absence of PM (W-MFC), both bulk-phase and interface DO concentrations remained always far below saturation (Figs. 3b and 4a).

Different electrochemical behavior was also observed at anodes (Fig. 2c). In these MFC systems, anodic and cathodic microbial communities can mutually influence each other, affecting the performances of the whole system (Cristiani et al., 2013). The exposition of the cathodes to different DO concentrations led to different ORR efficiencies (P-MFC, A-MFC, W-MFC), which accounted for modifications in anodic bacteria responsible for the degradation of organic substrates. The consequence of this was evident by the significant increase in Coulombic efficiency for increasing cathodic and anodic performances in P-MFC as compared to W-MFC (Table 2).

3.3 Conditions and growth of the PM cultures

During acclimation, the PM mass concentration in the cathodic chamber of P-MFCs was brought to 1.4 g/L (Fig. 3). Further on, PM concentration was maintained in the range 0.6–1.4 g/L for several semi-batch cycles (Fig. 3a). DM concentration increased almost linearly at each cycle. Moreover, no significant differences in PM growth were detected in duplicates P-MFCs.

The PM growth was characterized in details in P-MFCs and compared to the control PM culture (not-assisted by MFC). Fig. 3c-f shows the variation of key-parameters in two representative PM growth cycles (days 62–78). Equal *Spirulina* biomass production rates were observed in the P-MFC and the open-circuit control PM culture (Fig. 3c). Average PM growth rates of duplicate experiments, considering two representative cycles, resulted in around $0.1 \text{ g}_{\text{DM}} \text{ L}^{-1} \text{ d}^{-1}$, in both P-MFC and the control PM culture. This is a typical growth rate for *Spirulina* cultivation under similar conditions of day/night illumination (Chen and Zhang, 1997).

sCOD in the cathodic chamber of P-MFC resulted significantly higher ($P < 0.01$) than the control W-MFC, while the difference with the control PM culture was not significant (Table 2). Therefore, the leakage of organics to the cathodic chamber was negligible. Details of 2 PM growth cycles are reported in Fig. 3e: sCOD was increasing at the end of each cycle by nearly a factor of 2, with respect to the beginning of the cycle. This happened in both the P-MFC and in the PM culture. The consistent presence of sCOD in the cathodic compartment of P-MFC has to be ascribed mainly to the PM culture itself: PM are known to produce extracellular substances (Filali Mouhim et al., 1993).

3.4 DO and pH trends in the bulk and at anode-cathode interface

The PM culture conditions and growth in P-MFCs were monitored with respect to both W-MFCs and the control PM culture (not assisted by MFC). Fig. 3b reports the trend of bulk DO over time in P-MFC and W-MFC (cathodic compartments). DO in cathodic chamber of W-MFCs was always lower than DO at saturation, measured in water at identical conditions (7.8 mg/L): it ranged between 2 and $6 \text{ mg}_{\text{O}_2}/\text{L}$. In the bulk P-MFC catholyte, DO concentration increased up to $30 \text{ mg}_{\text{O}_2}/\text{L}$ at the beginning of each PM growth cycle and always remained higher than saturation (around $18 \text{ mg}_{\text{O}_2}/\text{L}$ as average). Comparable DO trends along significant cycles were measured in both P-MFCs and control culture, in the range 12–25 $\text{mg}_{\text{O}_2}/\text{L}$ (Fig. 3d).

Fig. 4a reports the DO profiles recorded at anode-cathode interface. DO profiles in the anodic compartment were very similar and completely overlapped for all MFCs. The curves exhibited almost flat profiles approaching to the cathode surface, where DO

content was approximately 0.7 mg_{O₂}/L. Evidently, differences in DO concentrations in the cathodic compartment did not affect the anodic compartment. According to previous findings, biofilm formation on the microporous layer interface acted as a barrier to the incoming of oxygen into the anodic compartment (Grattieri et al., 2015).

DO profiles at the interface in the cathodic compartment exhibited very different behaviors (Fig. 4a). Both for P-MFC and W-MFC, a decrease of DO occurred moving from the bulk towards the cathode surface. In W-MFCs, DO at the cathode interface was as low as 2.3 mg_{O₂}/L. In the case of P-MFCs, DO linearly decreased from 11.5 mg_{O₂}/L (at 9 mm distance) to 8.8 mg_{O₂}/L at the cathode surface, i.e. still higher than saturation. This decrease can be imputed to continuous DO consumption by ORR with simultaneous diffusion-limited mass transport from the bulk.

In P-MFC, the linear decrease in DO profile at cathode interface (from 12 to 8 mg_{O₂}/L, Fig. 4a), compared to the bulk phase (up to 30 mg_{O₂}/L), highlighted the relevance of ORR rates in consuming DO. However, oxygen was solubilized by photosynthetic activity at comparable and even higher rates. This is evident from the fact that DO was never lower than saturation concentration (Fig. 3b), not even at cathode interface (Fig. 4a). This profile suggests that P-MFC could act as 'degassing' devices to control DO concentrations. This feature of P-MFC might be useful, especially for dense PM cultivations. pH increased almost linearly in both P-MFC and the control (Fig. 3f), in line with PM growth. In microalgae cultivation, alkalization of growth medium is known to be related to photosynthetic activity (Markou and Georgakakis, 2011). However, in this experiment pH was always half-unit higher in P-MFC, as compared to the control PM culture. This was evidently ascribable to the presence of the cathodic ORR, with a net concentration of OH⁻, as well-known phenomenon in MFCs (Guerrini et al., 2013; Winfield et al., 2016).

Fig. 4b reports the pH profiles recorded at anode-cathode interface. In the anodic compartment, a slight increase of pH was observed for all the systems approaching the cathode. For P-MFC, the pH increased slowly from 9.5 to about 10. For W-MFC and A-MFC, slightly higher values of pH were measured. In the cathodic compartment, pH profiles showed a marked increase with respect to anodic compartment, especially for P-MFC. pH was about 11 at the cathode interface, showing a progressive decrease of half-unit in the bulk medium. These pH profiles at cathode interface in P-MFC confirm the prevalence of partial ORR with accumulation of hydroxyl radicals. Maintaining higher pH might be an advantage for several PM cultures. *Spirulina*, for example, is known to be an alkaliphilic microorganism and high pH (10–12) could favor selective conditions against culture contamination (Markou and Georgakakis, 2011). The marked pH gradient induced by the ORR at cathode interface might also help in preventing bacterial contaminations from organic-rich wastewaters. Indeed, excessive contamination by bacteria can compromise optimized conditions for PM growth and favor pathogens (Acién et al., 2016). To better understand these aspects, microbiological analyses would be needed in future experiments.

3.5 Nutrients recovery from wastewater

In Fig. 5, the fate of nutrients contained in the wastewater at the beginning of the experiment (Table 1) in P-MFC was investigated, using W-MFC as control. The residual elements left in the anolyte solution are reported for both P-MFC and W-MFC (Fig. 5a), while the elements recovered in the catholyte are reported as trend over time for W-MFC (Fig. 5b). The migration of nutrients was feasible to monitor in W-MFC (water), while not in P-MFC due to the presence of the nutrient-rich growth medium.

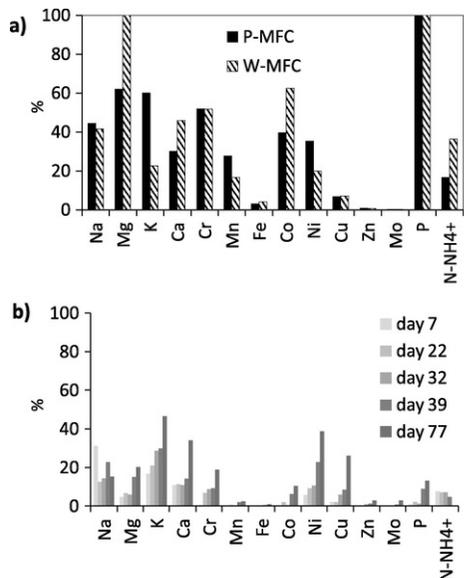


Fig. 5 a) Residual elements in anodic chamber at day 65, in both P-MFCs and W-MFCs. b) Trend of element recovery in the cathodic chamber of W-MFC, during the experiment. All data are reported as percentage of the total content in the wastewater at day 0.

At the end of experimentation (day 77), relevant fractions of most elements were removed from the anodic chamber, similarly in both P-MFC and W-MFC systems (Fig. 5a). The residual amount of Na, K, Ca, Cr and Co left in anodic chamber ranged between

40% and 60%. Lower percentages (about 20–30%) were measured for Mn, Ni and N-NH_4^+ . Negligible amounts of Fe, Cu, Zn and Mo were still present at day 77. Apparently, only Mg (in W-MFC) and P (in both systems) remained unchanged in the anodic chamber.

Simultaneously, most elements increased their concentrations over time in the cathodic chamber solution of W-MFCs (Fig. 5b). About 20% of Na, Mg, Cr and Cu initially present in the wastewater passed into cathodic compartment. K, Ca and Ni were recovered for about the 40%. The recovery of Co and N-NH_4^+ was lower (5–10%) and even lower for P, Mn, Fe, Zn and Mo.

Over simple diffusion, electro-migration of cations to the cathode of soluble compounds could contribute as factor for the observed macro- and micronutrients recovery from wastewater. Analyte dissociation phenomena are well known in membrane-based electrochemical systems and in MFCs (Gajda et al., 2014a). Like in electrodialysis studies, the electrical field applied over the separator induces ions migration to the oppositely-charged electrode (Zhu and Logan, 2014).

From Fig. 5, apparently conflicting data emerged. Most elements (N-NH_4^+ , Na, K, Ca, Cr, Mn, Co, Fe) were consistently removed and partially recovered in cathodic side. Only P and Mg were preferentially retained in the anodic chamber. For the majority of elements, the nutrient recovered in the catholyte was a consistent fraction of the amount removed from the anolyte. pH-related deposition/precipitation phenomena onto the cathode surface and/or in the anodic chamber might account for the lacking amounts (Santini et al., 2015).

For what concerns N-NH_4^+ removal and recovery, it is worthy to note that the 83% was removed from the anodic compartment in P-MFC and the 64% in W-MFC. The amount of N-NH_4^+ detected in cathodic compartment of W-MFC was only 5.5% of initial N-NH_4^+ . This gap in the N-NH_4^+ balance was probably linked to nitrification processes occurring at anode and anode-cathode interface. The same analysis in cathodic compartment of P-MFC, revealed undetectable N-NH_4^+ concentrations (data not shown), likely because of N-uptake by the PM culture. In this hypothesis, N uptake from PM in P-MFC would contribute to the drag from anodic compartment in parallel with electro-osmotic flux in W-MFC. A deeper look into the electro-migration mechanisms and on nitrification-denitrification processes should be addressed in future studies, to strike a more precise N balance.

4 Conclusions

MFCs can be an interesting option to assist PM cultivation in treating organic-rich wastewater. While the electrochemical system did not negatively affect *Spirulina* growth in comparison to control PM cultures, several positive effects were observed, e.g. consistent DO consumption and maintenance of alkaline conditions. Photosynthetic oxygen sustained cathodic reactions, ensuring electrochemical performances and COD removal in P-MFCs, at comparable extent to air-cathode MFCs. Most nutrients were recovered into the cathodic chamber, while only negligible COD leaked through the porous separator. Electro-migration, diffusion and PM-uptake phenomena deserve deeper studies, to optimize nutrients recovery with MFC-assisted PM cultivations.

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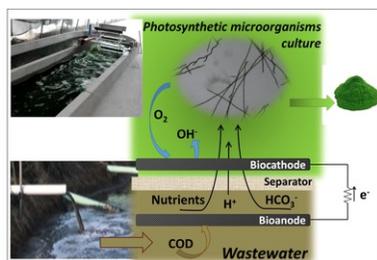
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Graphical abstract



Highlights

- MFC can assist photosynthetic microorganisms cultivation for wastewater treatment.
- Photosynthetic dissolved oxygen sustained cathodic reaction at comparable extent to air-cathode MFCs.
- Efficient removal from anodic chamber prevented COD leakage to the cathodic chamber.
- Cathode helped in consuming photosynthetic oxygen and increased pH in the bulk.
- Nutrients were recovered by electro-migration and diffusion to the cathodic chamber.

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