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Article

Hybrid Microbial-Photosynthetic Biofuel Cells for Simultaneous Bacterial Glycerol Biotransformation and Algal Carbon Dioxide Capture

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A geração de potencial em bioânodos de *Pseudomonas aeruginosa* a partir da bio transformação de glicerol, foi acoplada à captura de dióxido de carbono em biocátodos de *Chlorella vulgaris* em uma célula a combustível híbrida fotossintética (HPSBC). Parâmetros bioquímicos tais como crescimento microbiano, consumo de substrato, produção de pigmentos bacterianos e a captura de CO_2 foram estudados. Igualmente, parâmetros eletroquímicos de densidades de correntes máximas (Id_{max}), geração de densidade potencia máxima (Pd_{max}) e eficiências coulômbicas (C_E), foram estudados. Inicialmente, ambos os sistemas foram avaliados em separado contra os correspondentes pares redox $Fe^{3+}|Fe^{2+}$. No sistema bacteriano, resultados importantes em termos de Id_{max} de 42 ± 2,1 μA cm^{-2}, C_E de 48 ± 2,4% e Pd_{max} de 350 ± 17,5 mW cm^{-2} foram alcançados. Igualmente, para o sistema catódico algal isolado, valores de Id_{max} de 93 ± 4,65 μA cm^{-2}, C_E de 56 ± 2,8% e Pd_{max} de 3,2 ± 0,16 mW cm^{-2}, foram atingidos. Em contraste, quando os dois sistemas foram acoplados, um valor menor de Id_{max} de 48,5 ± 2,42 μA cm^{-2} foi observado. Finalmente, as condições bioeletroquímicas foram melhoradas com base no consumo de substrato, a geração de produtos eletrogênicos, o transporte de cátions e os sistemas para transporte de elétrons empregados. Assim, maiores valores médios para Id_{max} de 80 ± 4,0 μA cm^{-2}, para C_E de 71,5 ± 3,57% e para Pd_{max} de 650 ± 32,5 mW cm^{-2} foram obtidos.

Power generation at bioanodes of *Pseudomonas aeruginosa* for glycerol biotransformation was coupled to the carbon dioxide capture in biocathodes of *Chlorella vulgaris* in hybrid photosynthetic biofuel cells (HPSBC). Biochemical parameters such as microbial growth, substrate consumption, production of bacterial pigments and CO_2 capture were studied. Also electrochemical parameters of maxima current densities (Id_{max}), power output (Pd_{max}) and coulombic efficiencies (C_E) were studied. Initially, both systems were evaluated in separate against the corresponding $Fe^{3+}|Fe^{2+}$ redox pair. In bacterial systems, important results in terms of Id_{max} of 42 ± 2.1 μA cm^{-2}, C_E of 48 ± 2.4% and Pd_{max} of 350 ± 17.5 mW cm^{-2} were achieved. Likewise, for isolated algal cathode systems, Id_{max} of 93 ± 4.65 μA cm^{-2}, C_E of 56 ± 2.8% and Pd_{max} of 3.2 ± 0.16 mW cm^{-2}, were achieved. In contrast, when both systems were coupled, a lower Id_{max} of 48.5 ± 2.42 μA cm^{-2} was observed. Finally, bioelectrochemical conditions were improved based on substrate consumption, electrogenic products, cation transport and mediated electron transfer systems. Thus, higher average values for Id_{max} of 80 ± 4.0 μA cm^{-2}, C_E of 71.5 ± 3.57% and Pd_{max} of 650 ± 32.5 mW cm^{-2} were obtained.

Keywords: *Pseudomonas aeruginosa*, *Chlorella vulgaris*, microbial fuel cells, bioelectrodes, glycerol biotransformation, carbon dioxide capture, electron shuttles

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Introduction

The search for alternative sources of renewable energy with low environmental impact has been the major concern in recent years due to the availability of sources with higher energy densities, which, nowadays are obtained mainly from fossil fuels. Microbial fuel cells (MFC) are a relatively new and promising option for renewable and clean energy production. However, the power densities and efficiencies achieved up to this date are still low, demanding research to provide solutions for their implementation and scaling-up. Efforts have been concentrated to overcome some major problems involving high costs caused by: the use of metallic catalysts; losses associated with energy and mass transfers; to increase the rates of microbial metabolism,[1-3] and finally, the use of ferri- or ferrocyanide that have been widely used in lab scale MFC systems, although not as expensive as metallic catalysts, require replenishment and are toxic to the environment.[4] MFC are electrochemical devices able to convert chemical energy to electricity from reactions catalyzed by microorganisms. This energy harvesting from organic matter can be achieved either from pure substrates, residues, domestic or industrial wastewaters, greenhouse gases or any other source with variable levels of complexity or purity. In this context, glycerol and carbon dioxide can be targets for their use as fuels in bioelectrochemical devices.

Despite glycerol being initially considered as a very desirable by-product, since it can be used as intermediate in food and cosmetic processing, it has now become a great market problem due to its accumulation and to the rapid growth of the very profitable biodiesel industry. The use of glycerol in bio and chemical transformation processes to obtain high-priced by-products has already been successfully achieved.[5,6] Glycerol as substrate in MFC has also been explored to produce new substances that act as final electron donors or acceptors, or electron shuttles which can be oxidized or reduced at the electrodes' surfaces, resulting in indirect electron transfer mechanisms, which in most cases can be reversibly oxidized or reduced, making the entire electron shuttle system recyclable.[7-9] Among these electron shuttles, pyocyanin or 1-hydroxy-5-methyl-phenazine, also known as PCN, is produced using *Pseudomonas aeruginosa*. The optimization of culture conditions for PCN production have achieved improvements that allow its application in the construction of bioanodes for MFC prototypes using glycerol as biofuel.[9]

In contrast, biofuel production by algae can be advantageous if we keep in mind the impressive productivity that these microorganisms have, their non-competitiveness with agriculture activities, their flexibility in terms of water quality and specially the possibility of coupling the sequestration, fixation and biotransformation of CO_2 throughout the photosynthetic process to the generation of algae by-product with industrial interest,[10] including the energy conversion at biocathodes in photosynthetic fuel cells (PSFC).[4,11] Conventionally, in fuel cells electrons from the anodic compartment flow through an external circuit where its energy can be used; meanwhile protons are transferred to the electrolyte and transported through suitable means to the cathodic compartment. At the cathode, protons and electrons are used to reduce air oxygen on the surface of metallic catalyst, closing the cycle. A wide variety of studies have been already achieved in terms of the biocatalyzed oxygen reduction using microbial anodic half cells,[12-15] coupled to electrochemical half cells, and more recently microbial cathodes,[16-20] as well as photosynthetic microbial cathodic half-cells,[11,21] employing the microalgae species as direct electron acceptors.

This work is focused on the utilization of a previously optimized bacterial anodic system for glycerol biotransformation using *P. aeruginosa* in MFC,[9] now coupled to the bioelectrochemical sequestration and conversion of CO_2 by algal biocathodes using the microalgae *C. vulgaris.*

Experimental

Microorganisms

Pseudomonas aeruginosa ATCC 27853 was donated by the Group of Microbial Biotechnology from the University of São Paulo, São Carlos, Brazil. *Chlorella vulgaris* wild type was donated by the Group of Research in Fuels and Energy from the University of Pernambuco, Recife, Brazil.

Other materials

Pyocyanin (PCN) ≥ 98% HPLC grade was purchased from Sigma-Aldrich (USA). Ammonium hydroxide, absolute ethanol, isopropanol (99%), sodium monobasic and sodium dibasic phosphates, hydrogen peroxide (30 vol.), potassium chloride and sodium hydroxide were obtained from J.T. Baker Co. (USA). Potassium hexacyanoferrate(II) trihydrate (ACS reagent, 98.5-102.0%) and potassium hexacyanoferrate(III) (ACS reagent, ≥ 99.0%) were purchased from Sigma-Aldrich All aqueous solutions were prepared with MilliQ grade purified water. Bromocresol green (BCG, ACS reagent, dye content 95%), and methylene blue (MB, dye content ≥ 82%), were purchased from Sigma- Aldrich.

Carbonaceous materials

Carbon felt Type A (0.35 mm thickness) from E-TEK Inc. (USA); carbon felts were pre-treated with ammonia prior to their use according to previous studies.[9,22] Vulcan XC-72R® carbon black (bulk density 96.11 g L^{-1}) was obtained from Cabot Carbon Corp. (USA) and Vulcan 0.5% platinized carbon black was donated by the Group of Electrochemistry, IQSC, University of São Paulo, São Carlos, Brazil.

Bacterial growth, PCN production and glycerol consumption

For the bacterium adaptation, plates containing King A solid medium (KA) were inoculated and incubated at 37 ºC for 48 h. For the immersed culture, an optimized King A broth medium (OKA) was used, contained 25 g L^{-1} of glycerol purchased from Vetec, (Brazil), 20 g L^{-1} of soybean peptone purchased from Bacto®Peptone-Difco Lab. (Brazil), dibasic potassium phosphate 1.5 g L^{-1} and 1.4 g L^{-1} of $MgCl_2$ all purchased from Synth (Brazil), and 2.0 g L^{-1} tetrahydrate ferric sulfate, purchased from Vetec. All experiments were performed during 120 h and pH 7.4, at 37 ºC. Pre-inoculum was adjusted to a cellular concentration of 10^9 colony forming units (CFU) by optical density (OD) at $\lambda = 610$ nm. An inoculum of 24 h was used for the in-cell experiments to inoculate the anodic compartment at the fuel cell. Duplicated samples of 2 mL were taken every 12 h and centrifuged at 4500 rpm for 10 min. Supernatants were used for PCN quantification using UV-Vis spectrophotometry according to the methodology described below and, for glycerol consumption, a colorimetric enzymatic kit purchased from Bioclin® (Brazil) was used for triglyceride-glycerol determination and the concentration was expressed in mg L^{-1}. Bacterial growth was followed by OD at $\lambda = 610$ nm using the re-suspended cells in normal saline solution. All spectrophotometric determinations were performed employing a Biochrome Libra S32 UV-Vis spectrophotometer.

Total PCN concentration quantification

For quantification of the total PCN concentration, a liquid-liquid extraction was performed using consecutive addition of two volumes of chloroform to one volume of cell free culture supernatant and stirred for 5 min. PCN was then extracted from the chloroform phase into 0.2 mol L^{-1} HCl aqueous phase. To this deep red acid solution 0.4 mol L^{-1} borate-NaOH buffer (pH 10) was added until complete color change into blue. The concentration of extracted PCN was estimated by measuring the absorbance at 520 nm and comparing this to a standard solution of 0.4 mol L^{-1} PCN in borate-NaOH buffer (pH 7), and is expressed as mmol L^{-1} according to its molar extinction coefficient ($\varepsilon_{520\,nm} = 4310$ mmol^{-1} L cm^{-1} at pH 7 or 2460 mmol^{-1} L cm^{-1}, in 0.2 mmol L^{-1} HCl).[23]

Algae growth and CO_2 sequestration

Chlorella vulgaris was maintained in sterilized tap water, pre-adapted and cultivated in a modified medium according to the standard ISO 8692.[24] The algal experiments were conducted also at 25 ºC, for 120 h. A cellular concentration of 3×10^3 cells mL^{-1} was used as the starting point in all experiments. Algae cultures were left to grow for an adaptation period equivalent of 3 logs using the mineral medium already described. Dilutions up to the desirable cell concentration of 3×10^6 cells mL^{-1} were made using fresh medium. Hence the microalgae were left to be acclimatized during 24 h until their use. After inoculation, the cell concentration was adjusted, and the growth media were pre-aerated using pure CO_2 bubbling or air (containing approximately 0.038% of CO_2 *per* volume or 380 ppm) through fritted glass bubblers at a total flow rate of 200 mL min^{-1} for 1 h at 25 ºC. During algal cultivation, aeration to the cathode was stopped. For lighting, 18 W fluorescent lamps (3500 to 4000 lux) purchased from Philips Co (USA) were used. Samples were then taken in duplicate from the culture vessel using a hypodermic syringe to measure the cell growth with time by the increase in absorbance at 648 nm compared with a standard concentration curve of ABS *vs.* number of cells *per* milliliter. CO_2 consumption was quantified by titration to a phenolphthalein end point at pH 8.3 with a sodium hydroxide standard solution according to previous reported methodology,[25] since the analysis for dissolved carbon dioxide in water is similar to that for acidity. The total amount of CO_2 consumed (CO_{2C}) was expressed in terms of percentage of titrated dissolved carbonate, according to equation 1:

$$CO_{2C} = CO_{2D} - CO_{2F} - CO_{2R} \quad (1)$$

where CO_{2D} is the initial CO_2 concentration, solubilized as carbonate at the beginning of the experiment; CO_{2F} is the final CO_2 concentration solubilized as carbonate that remained at the end of the experiment and CO_{2R} is the amount of CO_2 released from the medium that was not consumed nor solubilized as carbonate. CO_2 and O_2 concentrations were also determined from the cell up-stream exits using an on-line GEM2000® gas analyzer purchased from LandTec (England) and results were expressed as ppm.

Fuel cell configuration

In general, the hybrid photosynthetic fuel cells (HPSFC) consisted in cylindrical acrylic reactors with working volumes of 100 mL in each compartment. Bacterial anodes (BA) were composed by bacteria cell suspension and as electrodic material an immersed carbon cloth electrode pre-treated with ammonia was used. Algal cathodes (AC) were composed of algae cell suspension and as electrodic material a Pt-Black 10% (m/m) dispersed on a carbon felt with carbon black powder electrode was used. Initially, both BC and AC systems were evaluated in separate against the corresponding $Fe^{2+}|Fe^{3+}$ redox pair using immersed carbon felt electrodes in a 10 mL volume of 20 mmol L^{-1} potassium ferricyanide or potassium ferrocyanide solutions, for the cathodic or anodic compartment, respectively. Lastly, both MFC approaches were put together in a hybrid system using BA and AC at the same time. In all cases, the electrodes had surface areas of 19.6 cm^2, connected to an external resistance of 1000 Ω. Saline bridges (SB) of potassium chloride saturated agar gel of 5 cm length and 0.5 cm diameter were used as cation transport system between compartments. Additionally, based on previous reports,[11,26] bromocresol green (BCG) and methylene blue (MB) were evaluated as electron shuttles as a way to enhance the AC coulombic efficiencies.

The acrylic apparatus and the nickel wires were sanitized using 70% ethanol and sterilized under UV light during 30 minutes. Other fuel cell components such as electrodes, magnetic bars, and saline bridges were sterilized in autoclave for 20 minutes. The MFCs were operated in a temperature-controlled room at 25 °C. The bioanolytes and biocatholytes, both with external volumes of 200 mL, were continuously circulated inside the respective fuel cell compartment by peristaltic pumps at a 2 mL min^{-1} rate.

Electrochemical analysis

Chronovoltammetric analyses were performed using the bicompartmented microbial fuel cells described above, connected to a digital Fluke 8808A Multimeter coupled to a FlukeView® data acquisition system (Fluke Corporation, USA). All experiments were allowed to stabilize for about 30 minutes before each measurement, and the *in-situ* microbial cultivation was performed for up to 120 h at 25 °C. The obtained voltage values were then converted to current density (μA cm^{-2}) and power density (mW cm^{-2}) according to Ohm's law. The coulombic efficiency (%) determination was calculated from the expression in equation 2 adapted from Logan *et al.*:[14]

$$C_E = \mathrm{M}\int_0^{tf} Id\,dt\,A / \mathrm{F_Z V_{AC/CC}}\,\Delta\mathrm{S} \quad (2)$$

where M (g mol^{-1}) is the molecular mass of the substrate used (PCN = 210.23, O_2 = 16, CO_2 = 44.01 and glycerol = 92.09 g mol^{-1}), I is the integrated current density (A cm^{-2}), t is the time (s), A is the electrode area (cm^{-2}), z is the number of electrons transferred *per* mole of substrate (O_2 = $4e^-$, glycerol = 12 e^- and CO_2 = $12e^-$); F is Faraday's constant; $V_{AC/CC}$ is the volume (L) at the electrode compartments or the volumetric influent liquid flow rate inside the electrode compartment (L min^{-1}), and ΔS is the change in substrate concentration (g L^{-1}). Polarization curves and power output curves were also obtained from steady-state experiments, using a PalmSens® potentiometer coupled to a PSTrace 4.1® software, using an Ag|AgCl electrode as reference and scan rates of 0.05 V s^{-1}. The potential ranges were established from the open circuit voltage (OCV) observed in each case.

Statistical analysis

Each bacterial or algal growth experiment lasted 120 hours and was repeated in triplicate to determine the standard deviations for the growth rates, the PCN production and the glycerol or the CO_2 consumptions. Each bacterial fuel cell (BFC) condition was also performed in triplicate and since the optimized substrate concentration did not affect previous fuel cell runs, two HPSFC runs were combined to demonstrate the reproducibility of the transient chronovoltammetric curves. The standard errors and 95% confidence limits, regression analysis relating the maximum power densities and the microbial growth rates were statistically analyzed by variance analysis (ANOVA) using Origin® software from OriginLab Corp. (USA).

Results and Discussion

Bacterial growth, PCN production and glycerol consumption

Despite the minor differences, all bacterial experiments showed high reproducibility and similar profiles of growth, glycerol consumption and PCN production. An example of the bacterial growth profiles obtained can be observed in Figure 1. As demonstrated in previous results,[9] no significant differences were observed when a catholyte of ferricyanide and KCl saline bridge was used compared with Pt-Black cathodes and Nafion® as proton exchange system. Although a low detrimental effect caused by ferricyanide and the use of saline bridges was observed, this system continues to be the best choice, since it represents an economical alternative rather than using the highly priced proton exchange membrane (PEM)

systems and expensive electrodes based on rare metals, such as platinum.

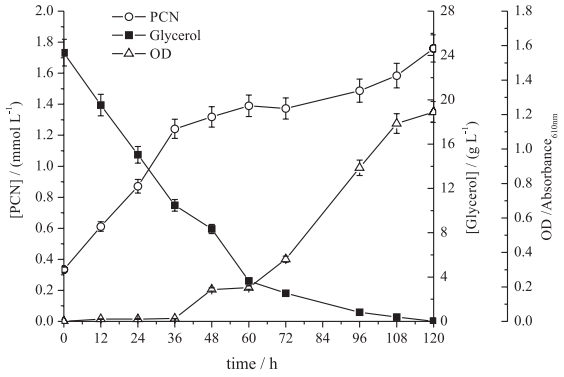

Figure 1. Bacterial growth, PCN production and glycerol consumption observed in *P. aeruginosa* from 25 g L^{-1} glycerol, at 37 °C for 120 h. OD: optical density.

The same profiles were observed for *P. aeruginosa* when it was grown in Bacterial Anodes (BA) against cathodes containing of potassium ferricyanide 20 mmol L^{-1}. It was also observed that glycerol consumption reached a maximum after 60 h, following the maximum growth rates observed close to the first 40 h according to the curve-fitting analysis by ANOVA. As was also observed, PCN production under the studied conditions was almost 15 times higher (x-bar 1.333 ± 0.066 mmol L^{-1}) if we compare it to previous reports where less than 0.1 mmol L^{-1} PCN was obtained from *P. aeruginosa* cultures using 2.5 to 10 g L^{-1} glucose or glycerol, respectively.[15,27,28] Previous observations also demonstrated that solvent extracted total PCN concentrations from free cell media ranged almost twice the concentration of PCN measured directly (x-bar 2.411 ± 0.1205 mmol L^{-1}). In terms of cell growth rates, this reached the highest values of 1.1023×10^{-4} CFU h^{-1} at 42.3 h.

Algal growth and CO_2 consumption

Previous studies have shown us how air or pure CO_2 supplementation can cause well-differentiated algal growth profiles. As expected, the algal growth was notoriously lower when the culture was only supplemented with air. Nevertheless, the continuous supplementation of an oxidant (such as oxygen) present in the air stream supply to the cathode is essential to maintain the potential and current of a PSFC.[29] Since the rates of oxygen generation from the algae cell are lower than the rates for CO_2 intake, the presence of alternative sources of O_2 at the cathodic compartment are mandatory. From Figure 2, it remains remarkable that the lowest algal growth was observed when algal cultures were supplemented only with air instead of CO_2. In both cases, the growth curves were normalized from the initial cell concentration at t_0. Thus, only the increase in OD was plotted. Table 1 shows the average values observed for the growth of *C. vulgaris* in the biocathodes (AC) under the two evaluated conditions of aeration.

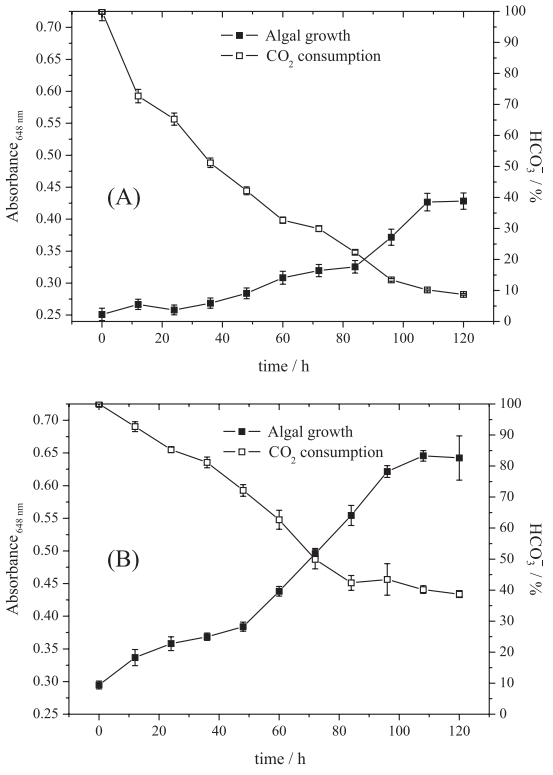

Figure 2. Effect of the gas supplementation on algal growth and carbon dioxide consumption observed for *C. vulgaris* cathodes. (A): Air 380 ppm; (B): pure CO_2.

There is a clear consistency between our observations and previous reports on AC using the same microorganism, where a substantial increase in biomass generation was related to the source of CO_2.[11,21] In this case, it was observed a two log increase on the microbial growth rate when pure CO_2 was supplied. In contrast, only approximately 61% of

Table 1. Growth profiles for *Chlorella vulgaris* cathodes using air or pure CO_2 supplementation against 20 mmol L^{-1} ferrocyanide anodes, at 37 °C for 120 h

Supplied with	μ_{max} / (Δabs_{648} h^{-1})	Initial solubilized CO_2 / ppm	Final CO_2 / ppm	Approximate CO_2 consumption / %	Real CO_2 consumption / %
Air	$0.0001 \pm 3.146 \times 10^{-4}$	380 ± 11.4	33.2 ± 0.9	91.2	88.7
Pure CO_2	$0.0130 \pm 4.638 \times 10^{-4}$	1500 ± 45.0	582.0 ± 17.5	61.2	55.8

the initial CO_2 concentration was consumed when pure gas was used, compared with the almost depletion of the CO_2 (91.2%) when the medium was supplied with ar. Control experiments were performed to estimate the release of CO_2 from the media during the whole experimental time, and this led us to estimate the real algae carbon sequestration. As such, gas traps were attached to the up-stream exits of the cathodic compartments, and the trapped CO_2 was then quantified. The results showed that in average only less than 5 and 1.5% of the solubilized CO_2 was released back from the AC during the experiments using enriched media with pure CO_2 or air, respectively. According to this, to reach the optimum growth rate of *C. vulgaris* cathodes, it is important to increase the dissolved CO_2 concentration. Some reports have shown that the increase of dissolved carbon dioxide must be simultaneous to the increase in radiant flux, since both clearly are limiting factors for growth rate.[11,21,30,31] However, in this study, only one radian flux condition was studied. On the other hand, the yield of biomass produced in the case of AC agreed with previous values reported for *C. vulgaris* cultures,[11,32] in which it was found that the CO_2 consumption follows a biomass yield coefficient of 0.5 mg biomass *per* mg CO_2.

Half microbial cell using bioelectrodes and redox counter-electrodes

Both sets of bioelectrodes, bacterial as well as algal, were evaluated independently against the redox pair $Fe^{2+}|Fe^{3+}$, prior to their use together within the hybrid system. Initially, aeration was performed directly into the cell compartments, however the mass transfer from the gas source into the liquid cultures varied the experiment performance since the gas remained on top of the culture media. The use of external vessels where the aeration can be made alternatively by bubbling into the media directly through fritted glass bubblers solved this problem, and improved the final performance of the PSFC. The effect of this change on mass transfer rate was determined by measuring the rate of the dissolved CO_2 of build up as described earlier.[33] As related on previous reports based on this approach, the bubblers improved the gas transport rate into the culture media by a factor of three and four in the case of CO_2 and O_2, respectively.[11,21]

Examples of the chronoamperometric profiles obtained for each half fuel cell can be observed in Figure 3. Experiments involving the algal cathodes with air supplementation showed to be very stable along the evaluation period (Figure 3A). In this case, the apparent stability for the observed current densities (Id) was related to the highest concentration of oxygen present in the air stream, which can contain up to 23% of O_2, and only 0.046% (m/m) of CO_2. As previous reports have shown, under illumination, and assuming that algae in the cathode compartment generate oxygen, the rate of current generation of a PSFC will be proportional to the algal oxygen producing activity, if oxygen is rate-limiting.[11,21] The O_2 concentration could easily reach 40 mg L^{-1} when solubilized at room temperature, while the measured CO_2 reached a maximum concentration of 380 mg L^{-1}, which corresponds to almost 10 times the O_2 concentration. Consequently, the low concentration of solubilized O_2 may compensate the low rates of O_2 generated by the algae and hence maintains the Id relatively constant between 3.5 and 4.0 μA cm^{-2}.

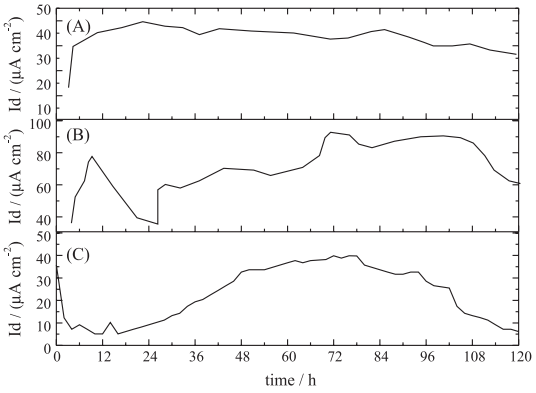

Figure 3. Chronoamperometric profiles observed for the algal cathode, supplemented with air (A) or pure CO_2 (B) and for bacterial anode (C).

In Figure 3B, it was observed a dramatic decay in Id, after 12 h. This behavior was observed several times when the culture medium was supplemented with pure CO_2 instead of air. This is related to the decrease in the pH value caused by the solubilized CO_2 in the form of HCO_3^-, which caused a cytotoxic effect over *C. vulgaris*, observing that the average measured concentration of solubilized CO_2 reached 1500 mg L^{-1}, when pure CO_2 was used. After 24 h, the Id values reached higher levels that were not observed in the case of the medium supplemented with air. In addition, profiles showed to be less stable than the previously observed for air supplementation; however, the highest Id peak at 93 μA cm^{-2} at 72 h was obtained when pure CO_2 was used. After this point a small decrease on the Id values was observed, and then Id values maintained a relatively constant and slow increase for the next 30 h ranging between 8.0 and 9.0 μA cm^{-2}. After 100 h of process, the CO_2 depletion caused a rapid decrease in Id. Figure 4 shows the control experiments that were performed to determine the effect that the illuminated algal cultures caused on the chronoamperometric profiles without aeration and the effect of free cell media supplemented

with air or pure CO_2. At first, when the cell free media were evaluated, it was demonstrated the contribution of the O_2 present in the air stream to the Id generation since higher values were observed when compared to the pure CO_2 supplementation. Since there were no algae responsible for the conversion of CO_2 to O_2, the lowest Id profiles were observed. In both cases, very stable profiles were obtained since the very beginning, as such plateaux were obtained with Id values ranging from 0.5 to 0.8 μA cm^{-2} and 2.0 to 2.5 μA cm^{-2}, for the free cell media (abiotic control) supplemented with CO_2 or air, respectively. In general, the major contribution to the Id values was caused by the algal culture. As such, in this process, a slow increase in the Id values was observed during the first 40 h followed by a fast increase until 100 h, where the highest peak of Id of approximately 3.5 μA cm^{-2} was observed. After this point, a slow decrease in Id values was observed following the O_2 depletion and the cellular death. This indicates that even without the gas supplementation the algae were able to generate O_2 from other processes that did not involve CO_2 capture and biotransformation. These three profiles were in accordance with previous reports in terms of behavior.[11,21] However, lower Id values were observed in our control experiments. In general, the results obtained for the AC Id_{max} were clearly consistent with the previous reports where similar approaches were used, such as: algae species, ferrocyanide as counter-electrode and saline bridges as cation exchange system.[4,11,21]

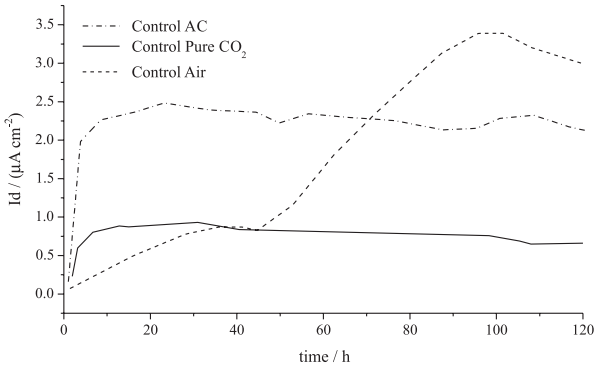

Figure 4. Chronoamperometric profiles obtained for control experiments using algae cathodes without aeration (AC) and with air or CO_2 supplementations.

Finally, Figure 3C shows the chronoamperometric profile for the bacterial anode. As previous results have demonstrated (data not shown) the use of a saline bridge promotes more stable profiles for Id generation than other cation exchange system, such as Nafion membranes. This is related with the inherent deficiencies during the proton exchange through the membrane towards the cathodic compartment. PEMs such as Nafion, when used in microbial cultures at pH values above 7.0, tend to be especially affected.[1] In this case, the Id values increase rapidly during the first 48 hours following the substrate consumption, however, after the 48 h the Id generation enters a steady-state plateau until the 96 h of process, with Id maxima values ranging between 40 to 45 μA cm^{-2}. This maximum Id plateau is closely related to the accumulation of PCN in the medium. As previous studies have shown,[9,33] the electron generation in anodes of *P. aeruginosa* is dependent on PCN production and especially limited only to the protonated species of PCN ($PCNH^+$), which is actually responsible for the electron transportation between the bacterium cell wall and the electrode surface. PCN and other phenazine-like compounds could also play an important respiratory role in other conditions of high bacterial densities, poor mixing, and oxygen limitation. How these parameters affect PCN production and electron transportation has been already described.[9,34] Nevertheless, it is interesting to mention that during the production of PCN, this electron shuttle could be present inside the culture media in three different forms: PCN zwitterion (PCN^{+-}), dihydro form ($PCNH_2$) or protonated form ($PCNH^+$) (due to factors such as: pH value, oxygen concentration), and other bio or electrochemical conditions or even recycling.[9,34,35]

Despite the minor differences, all bacterial cultures showed similar profiles of growth, glycerol consumption and PCN production. At this point, we can assume that with Fe+SB or Pt+PEM systems, we can achieve good results and reproducibility in terms of current densities, coulombic efficiencies and power outputs, so we could choose either system for future works. Although there are some detrimental effects, ferricyanide and the use of saline bridges continue to be the best system, since it represents an economical alternative to highly priced PEM systems and expensive electrodes based on rare metals, such as platinum.[9] The current tendency is to avoid PEM systems as well as saline bridges and work in membraneless devices where phase separation can be achieved by other means. Nevertheless, crossover problems can be observed in terms of oxygen and carbon source migration to one compartment (phase) to another.[36]

Table 2 condenses the results achieved in terms of coulombic efficiencies (C_E) for each bioelectrodic system. Since good biotransformation into PCN was achieved for *P. aeruginosa* using the optimized medium containing glycerol at 25 g L^{-1}, that was also reflected on a good C_E value around 47%. In previous studies we have observed that specific C_E for PCN exceeded the one observed for glycerol.[9] This could indicate that PCN, like other previously described electronic shuttles, is suffering re-cycling during the bioelectrochemical process.[15,37]

Table 2. Electrochemical results for the half-biofuel cells using bacterial anode (BA) and algal cathode (AC) against counter-electrodes containing 20 mmol L^{-1} of $Fe^{+2}|Fe^{+3}$

	Substrate	C_E / %	I_d max / (μA cm^{-2})
BA	glycerol	46.71 ± 2.33	39.90 ± 1.99
AC + air	CO_2	56.77 ± 2.83	4.96 ± 0.24
AC + pure CO_2	CO_2	29.41 ± 1.47	9.23 ± 0.46
Control cell free + air	CO_2	24.33 ± 1.21	2.48 ± 0.12
Control cell free + pure CO_2	CO_2	6.03 ± 0.30	0.90 ± 0.04
Control algae without aeration	O_2	*16.85 ± 0.84	3.38 ± 0.17

BA: bacterial anode; AC: algal cathode; *calculated as oxygen released from algal biomass.

Having this in mind, we can estimate a turnover number (TON) for the total amount for the PCN produced from glycerol in 120 h, of approximately 50.3 for the half-biofuel cell system studied. In general, the C_E values could be even higher if the internal electronic and mass transfer losses are avoided as well as other biological processes that can reduce the concentration of available PCN inside the medium, as complexation or degradation.

Comparing both the bacterial and the algal electrodes, it was noticeable that the highest Id values were observed for the algal cathodes when pure CO_2 was supplemented instead of air. Nevertheless, higher C_E were observed when air was used instead of pure CO_2. As cited before, the presence of O_2 in the air stream was the main cause for the differences in terms of C_E and current densities observed for the algal cathodes. The electrochemical availability of O_2 is higher within the air stream than the one coming from the capture and biotransformation process due to the algae. This fact was confirmed throughout the observations made from the control experiments, where half of the C_E observed for the AC supplemented with air corresponded only to the air stream and 1/5 when AC was supplemented with pure CO_2. Another interesting observation was made from the contribution that algal biomass had over the total C_E in AC. In that case, C_E obtained from algal biomass corresponded to 1/3 and 1/2 of the total C_E value when algal cathodes were supplemented with air or pure CO_2, respectively.

Use of mediated electron system for C_E in ACs

Although several electron mediators or electron shuttles have been studied to improve the capture and transport of electrons, recent research has shown that mediator-less anodic cells are feasible, especially when the cells grow as a film coating on the anodic electrode. Nevertheless, due to the type of carbonaceous materials used as electrodes in this study, the formation of such bio-structures has not been accomplished successfully, making the addition of suitable electron shuttles mandatory to improve electron transfer between the electroactive species at the electrode, the solution and the biological component. The concentrations of the chosen electron shuttles were optimized in previous studies (data not shown), ensuring: maximum Id production, improved C_E and minor toxic effects over the algal cells. According to this, maximum concentrations of 0.1 and 0.2 mmol L^{-1} of MB and BCG were used, respectively. Although these mediators have proven electron transport capacity, with excellent regeneration and recycling features as previous studies have shown,[11,38,39] they have been employed mostly in bioanodes. Both mediators have well differentiated mechanisms for electron transportation. As such, MB or 3,7-bis(dimethylamino)-phenothiazin-5-ium chloride corresponds to a phenothiazine dye which transports electrons on a protonable nitrogen atom present at the heterocyclic ring. In the case of BCG or 3,3',5,5'-tetrabromo-*m*-cresolsulfonphthalein, it is a triphenylmethane dye which can transport electron throughout the formation of quinone-like derivatives.[26,38]

Chronoamperometric profiles for AC hemi-cells were obtained in separate experiments evaluating both electron shuttles to understand the effect that these compounds really caused on the current profiles and consequently on the CE values during the algae growth using the previous studied gas supplementation. It can be observed in Figure 5 how noticeable the increments on CE were when both electron shuttles are employed. The real CE values were recalculated using the CE observed in control experiments, allowing the determination of the real efficiency that the electron shuttles caused on the overall current values. In general, the best results were observed when BCG was employed using air supplementation, achieving efficiencies 3 times higher than when no mediator was used. When CO_2 was supplemented along with the mediators, no significant CE differences were observed among them. However, increases in the CE values were higher when MB was used instead of BCG in the case of the supplementation with pure CO_2. It

must be considered that both electron shuttles were chosen due to their recycling properties. Having this in mind, it was considered that the observation of such high CE values is closely associated to the occurrence of recycling processes which are responsible for the high turnover numbers already observed for both mediators.[26,38]

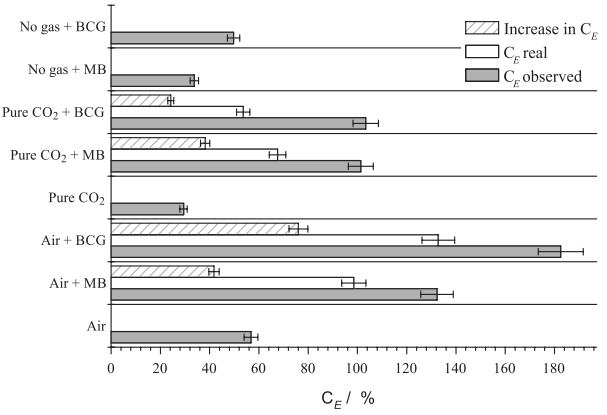

Figure 5. Increase percentage in the C_E values for AC using as electron shuttles methylene blue (MB) and bromocresol green (BCG).

Hybrid biofuel cells using bacterial anodes and algal cathodes

The hybrid photosynthetic biofuel cell (HPSBC) combined best results were from isolated experiments for both algal and bacterial bioelectrodes in a novel single device. Initially, when both approaches were set together it was expected to obtain close profiles to the ones previously observed, however, the results clearly showed the dominance of the bacterial anodic profile over the algal one. From the initial observations some adjustments were made in a way to improve the performance and efficiency of this device. At first, HPSBC was supplemented with a glycerol concentration of 25 g L^{-1} for the BA and air for the AC. The chronoamperometric profiles were similar to the ones observed for the bacterial half-fuel cell, where an inverse parabolic curve was obtained rather than the sigmoidal curved observed for the algal half-cell. It must be mentioned that the Id maxima values observed at the plateau presented values between 35 and 40 μA cm^{-2} lower than those previously observed. Similarly, the C_E value obtained was just 34%. Some explanations are now identified, and they are mostly related with charge and mass transportation problems. Ion generation and transportation is faster when chemical electrolytes are used as observed during the half-cell approaches. As such, in BA when PCN is oxidized at the anode, protons are released and their migration to the cathodic chamber is expected, where they will be consumed during the processes of O_2 reduction. However, since protons cannot migrate at a sufficient speed from the anode to cathode, the pH decreases rapidly due to their accumulation. In this case, the electrolyte balance was maintained by K^+ coming in the opposite direction from the cathodic compartment as a product of the reduction of ferricyanide, as shown in equation 3.

$$\text{BA half-cell: } PCNH^+ \rightarrow PCN^{+-} + H^+ \mid K_4[Fe(CN)_6] \rightarrow K_3[Fe(CN)_6] + K^+ \quad (3)$$

An antagonistic process was observed during the O_2 reduction in AC, as shown in equation 4. In this case, an increase of pH was expected, due to the consumption of protons during the O_2 oxidation without the corresponding replacement from an external source.

$$\text{AC half-cell: } K_3[Fe(CN)_6] + K^+ \rightarrow K_4[Fe(CN)_6] \mid O_2 + H^+ \rightarrow H_2O \quad (4)$$

The resistance depends on the salt bridge construction: its length, diameter and the type and concentrations of dissolved ionic species. Since the redox pair $Fe^{+2}|Fe^{+3}$ was no longer employed, we decided to replace the KCl bridge with other cation exchange systems. Even when proton exchange membranes such as CMI-7000® or Nafion 117® are expected to be the best choice, our previous experiences with bacterial anodes showed us that similar results can be achieved using cheaper materials. For the HPSBC device we adapted the saline bridge replacing the saturated KCl agar gel for saturated K_2HPO_4 agar and this will lead the cation transportation not just of K^+ ions but also protons.

$$\text{HPSFC: BA: } PCNH^+ \rightarrow PCN^{+-} + H^+ \mid \text{AC: } O_2 + H^+ \rightarrow H_2O \quad (5)$$

On the other hand, PCN production rate was much faster than the O_2 generation from the algae. This was also related with the initial concentration of substrates that are quite different, 25 g L^{-1} of glycerol (BA) and 0.38 or 1.5 g L^{-1} of CO_2 (AC). PCN, like other previously described electronic shuttles, can suffer re-cycling during the bioelectrochemical process.[26] Hence, it can be estimated a turnover number (TON) for the total amount of the PCN produced from glycerol during 120 h, of approximately 50.3, which corresponds to a maximum concentration of electrogenic by-product of approximately 0.5 g L^{-1} (or 2.5 mmol L^{-1}). According to equation 6, the maximum O_2 concentration can be estimated by the O_2 produced from algal biomass. Thus, if one considers that during the photosynthesis 2 moles of CO_2 generate 2 mole of O_2, an average O_2 concentration of 59.4 mmol L^{-1} can be obtained.

The obtained values indicated a concentration of O_2 at the AC at least 20 times higher in comparison with the expected H^+ generation at the BA if the relation between PCN and H^+ is also equimolar. Nevertheless, in electrochemical terms and according to equation 7, the amount of O_2 generated from algal cathodes are half of the CO_2 moles consumed. Therefore, the concentration of O_2 is only 10 times higher than the expected proton concentration coming from the BA compartment.

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2 \quad (6)$$

$$6CO_2 + 12H^+ \rightarrow 12e^- + C_6H_{12}O_6 + 3O_2 \quad (7)$$

Figure 6 shows the chronoamperometric profiles obtained for the HPSBC approaches using glycerol and either air or pure CO2 as substrates for the BA or the AC, respectively, in the presence of these two different electron shuttles. In general, profiles present consistent patterns that are similar to those observed in the case of bacterial anodes when cultures were supplemented with air instead of CO_2. As such, an initial current lost during the lag-time of the microbial growth during the first 24 h was observed; followed by a fast increase in Id rate values during the next 20 to 40 h when a plateau of Id was reached, in which the current values have not changed significantly, lasting in average 40h. And finally, a rapid decrease on Id values due to the substrate starvation and cellular death.

It can be observed, that when both electron shuttles were used with air supplementation, peaks of higher Id values were obtained earlier, within 50 and 56 h, differently than the usual 72 h when no electron shuttle was added. In the case of cultures supplemented with CO_2, earlier Id peaks were obtained when only MB was added at 45 h. From the

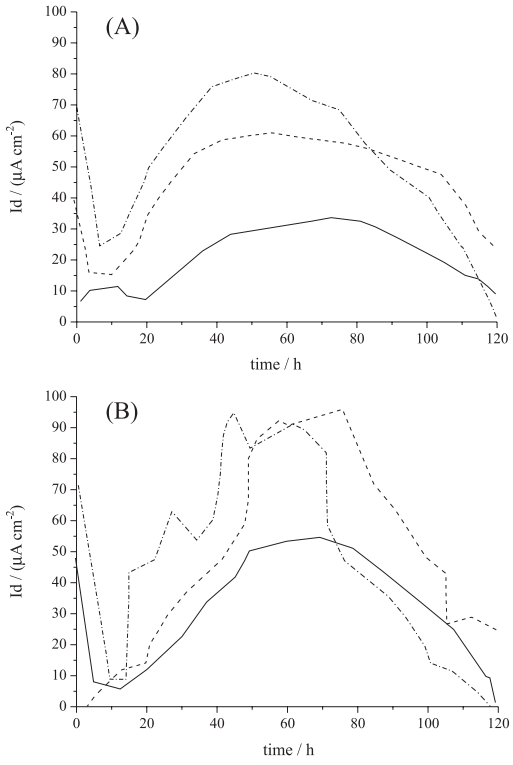

Figure 6. Effect of electron shuttles on the chronoamperometric profiles for HPSFC. No mediator (line); MB (dash); and BCG (dash-dot).

chronoamperometric experiments using the HPSBC cells a new set of data in terms of C_E and Id_{max} are shown in Table 3. In both gas supplementation approaches, the best results in terms of overall coulumbic efficiency were obtained when electron shuttles were added. However, the increase in C_E was remarkable in the case of air-supplemented cultures in which the obtained values doubled the previous observations, when no electron shuttles were added. In the case of CO_2 supplementation, similar results were obtained

Table 3. General results under optimized conditions for HPSFC using bacterial anode and algal cathode

System	Substrate BA | AC	Overall C_E / %	MET		I_d max / (μA cm^{-2})	Pd / (mW cm^{-2})
			BA	AC		
BA	glycerol	46.71 ± 2.33	PCN	–	39.90 ± 1.99	350 ± 17.5
AC	air	56.77 ± 2.83	–	–	4.96 ± 0.25	28.9 ± 1.44
	pure CO_2	29.08 ± 1.45	–	–	9.23 ± 0.46	53.8 ± 2.69
HPSBC 1	glycerol + air	29.18 ± 1.46	PCN	–	33.66 ± 1.68	250 ± 12.5
HPSBC 2	glycerol + air	61.92 ± 3.09	PCN	MB	60.97 ± 3.04	343 ± 15.1
HPSBC 3	glycerol + air	71.56 ± 3.58	PCN	BCG	80.29 ± 4.01	387 ± 19.3
HPSBC 4	glycerol + pure CO_2	27.46 ± 1.37	PCN	–	54.61 ± 2.73	375 ± 18.7
HPSBC 5	glycerol + pure CO_2	37.00 ± 1.85	PCN	MB	94.80 ± 4.74	603 ± 30.1
HPSBC 6	glycerol + pure CO_2	39.00 ± 1.95	PCN	BCG	95.92 ± 4.80	655 ± 32.7

MET: mediated electron transport; BA: bacterial anode; AC: algal cathode; PCN: pyocyanin; HPSBC: hybrid photosynthetic bacterial cell; MB: methylene blue; BCG: bromocresol green; *calculated as oxygen released from algal biomass.

for both electron shuttles, but once more it remained clear that the absence of oxygen was directly related to the lower Id values observed. In general, the C_E values could be even higher if internal electronic and mass transfer losses are avoided as well as biological processes that can reduce the concentration of available mediators inside the culture media, by complexation or degradation. In general terms, best improvements were achieved when BCG was used compared with MB.

Also in Table 3 are shown the best results achieved for power outputs (Pd) in the BA, AC and the hybrid systems using electron shuttles and the two evaluated aeration modes. Figure 7 shows the results from the polarization curves obtained using a bicompartmented cell, where BA or AC were set in separate against $Fe^{+2}|Fe^{+3}$ redox pairs or together as the hybrid system. In all cases, microbial cultures were examined at 60 h of the process, where the Id_{max} plateaus were previously observed. As such, the results can be grouped into three blocks. The first one, the hemi-algal or bacterial fuel cells: power output values were noticeable higher in the case of BA in comparison with AC. As such, the Pd was at least 10 times higher when air was used and 6 times higher when pure CO_2 was used. The second one, in hybrid BA|AC cells, when only air supplementation was evaluated along either the absence and presence of mediated electron transfers (METs), similar results were observed when electron shuttles were employed, and also the Pd values were higher than the ones obtained when no mediator was used. Finally, the third approach, the hybrid BA|AC cells using supplementation of pure CO_2. The results showed the highest Pd peaks, ranging from 1.5 to 2 times higher than the values observed without mediators. Nevertheless, as previously discussed, stable Id and Pd profiles could be obtained when air was used instead CO_2. The obtained Pd values for the studied AC, when compared with the previously described algal-based cathodes, were shown to be up to 8 times higher when the non-modified algal cathode was used, and up to 20 times higher when either the algae/cellulose or the algae/wire electrodes were used.[4]

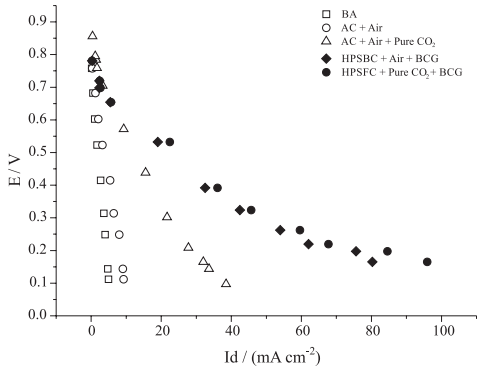

Figure 7. Polarization curves obtained for the evaluated systems at 60 h where the maxima current peaks were observed.

Conclusions

The use of a chemical catholyte or metal catalyst requires the physical separation of the anode and cathode chambers by an ion exchange membrane (IEM) to prevent substrate diffusion from the anode to the cathode that leads to a rapid deactivation of the cathode and deterioration of MFC performance IEM inhibits substrate diffusion but permits proton migration from the anode to the cathode. Yet, the application of IEM results in a high internal resistance and a retarded transfer of protons from the anode to the cathode, which leads to pH splitting and thus lowers the system stability and bioelectrochemical performance. In spite of the detrimental effect that ferro- and ferricyanide can cause on the microbial electrodes, their use together with the saline bridges continues to be the best choice, instead of using the highly priced PEM systems and expensive electrodes based on rare metals.[36] In general, C_E values at the BA could be even higher if internal electronic and mass transfer losses are avoided.[36] Under the evaluated experimental conditions, the average power output densities reached for BA systems were at least 4 times higher than the ones obtained in other MFCs based on the PCN production from glycerol and glucose.[14,15,36] The proposed hybrid photosynthetic fuel cell was shown to be viable when algae cathodes were used for CO_2 capture using either air or pure CO_2 streams. Clearly, higher Id peaks were achieved when external electron shuttles were added to the algal cathode. However, the best results in terms of Id and Pd were reached when the cathodic compartments were supplemented with pure CO_2 instead of air. Nevertheless, in terms of C_E the best results were achieved when air was used. In general terms, the best improvements can be achieved when BCG was used in comparison with MB when MET systems were used with algal cathodes. The HPSFC approach also was shown to be efficient when coupled to microbial anodes where glycerol biotransformation was already efficiently achieved by *P. aeruginosa*. This hybrid system could let us get rid of an inconvenient residue, such as glycerol, to produce energy, with the simultaneous production of bacterial and algal by-products, such as biomass, complex carbohydrates and lipids, which are all targets of the biofuel industries. Current works are exploring the use of raw glycerol as fuel for bacterial and mixotrophic algal cultures, which can also couple its biotransformation to the CO_2 sequestration and energy production in HPSFC.

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