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Photosynthetic microbial fuel cell with polybenzimidazole membrane: synergy between bacteria and algae for wastewater removal and biorefinery

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Abstract

Here, we demonstrate a very efficient simultaneous approach of bioenergy generation from wastewater and added-value compounds production by using a photosynthetic microalgae microbial fuel cells (PMFC), based on polybenzimidazole (PBI) composite membrane as separator. The use of PBI was proved to be very promising, even more convenient than Nafion™ in terms of energy performances as well as cost and sustainability. This polymer is also easily autoclavable, so allowing a re-use of the separator with a consequent beneficial cost effect. Two PMFCs were investigated: 1) Pt electrocatalysed and 2) Pt-free. They were operated as microbial carbon capture (MCC) device under continuous illumination, by using a domestic wastewater as anolyte and *Scenedesmus acutus* strain in the catholyte. The Pt-based cell allowed to generate higher volumetric power density ($\sim$400 mW m^{-3}) after more than 100 operating days. This resulted in an improved wastewater treatment efficiency, determined

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in terms of normalised energy recovery ($NER > 0.19$ kWh kg_{COD}^{-1} in case of Pt). The CO_2 fixation of the PMFC-grown microalgae leaded to a high accumulation of added-value products, namely pigments and fatty acids. A significant quantity of lutein was observed as well as a relevant amount of other valuable carotenoids, as violaxanthin, astaxanthin and cantaxanthin. The lipids were even excellently accumulated ($49\%_{dw}$). Their profile was mainly composed by fatty acids in the range $C_{16\text{-}18}$, which are particularly indicated for the biofuel production. These results demonstrate the feasibility and the implemented sustainability of such PMFCs as a great potential technology for the wastewater treatment and the simultaneous production of valuable products.

Keyword: Energy

1. Introduction

Bioenergy is renewable energy made available from materials derived from biological resources (e.g. forest, agricultural and algae-derived biomass, biogenic fraction of municipal and industrial waste), which can be converted into biofuels, as well as directly into bioelectricity [1].

Nowadays, biofuels production seems to offer new opportunities to reduce greenhouse gases (GHG) emissions and to replace the conventional fossil fuels for uses in conventional engines [2]. Biofuel is typically produced by food, plants or animal oil, and/or by agricultural residues. Recently, a great deal of attention has been devoted to the production of biodiesel from microalgae [3, 4, 5], which seem to be an important source of bioenergy, sustainable both in terms of economy, because they use free CO_2 and free sunlight, and of environment, because they consume flue gas or CO_2 from the atmosphere and from industry. Microalgae possess an excellent photosynthetic efficiency and require less water than terrestrial crops to be cultivated. Moreover, some algal strain can have a very high lipid content (up to 50% on the dried system) [4]; ii) and in general, according to the kind of strain and to the culture conditions in proper photobioreactors, microalgae can accumulate several important secondary metabolites with health and nutritional implications. In addition to lipids and fatty acids, in fact, other co-products with relevant commercial value may be obtained as proteins, sugars, or carotenoids, animal feed, super-food, advanced plastics for smart packaging. Exploiting of algae, therefore, has a dual benefit: it serves as biomass for the biofuels production and it has potential for the concurrent extraction of valuable co-products with a wide range of applications [4, 6].

Currently, there are numerous technologies for microalgae biomass production, which can be based on photoautotrophic or heterotrophic growth [3]. Among them, microalgae production based on the integration with microbial fuel cells

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(MFCs) seems to be a very promising synergistic strategy, since phototropic organisms act as *in-situ* O_2 generators, which promote the bioelectrochemical reactions in MFCs. Basically, in a microbial fuel cell, CO_2 is produced at the anode as the oxidised product of an organic substrate (for instance wastewater), whereas O_2 is required at the cathode to accept protons coming from the anodic chamber and free electrons from the external circuits. In presence of microalgae and of light, photosynthesis occurs at the cathode, where CO_2 is metabolised by such microorganisms with the help of light to produce biomass. The general flow chain reaction may be stated from the following steps: 1) half reaction @anode (oxidation): Organics $\rightarrow CO_2 + H^+ + e^-$; 2) half reaction @cathode (reduction): $2O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$; 3) overall electrochemical reaction: i) Organics $+ O_2 \rightarrow CO_2 + H_2O +$ external power; ii) $CO_2 + H_2O +$ light $\rightarrow$ biomass $+ O_2$ [7, 8].

Electricity may be produced and harvested at the expenses of organic substrate decomposition, with algae growth in the cathodic chamber, and subsequent oxygen generation by the photosynthetic process. For this reason, these systems are commonly known as photosynthetic MFC (PMFCs) [9]. Several geometries were discussed in literature, as for instance tubular, coupled PMFCs, single-chamber PMFCs, sediment PMFCs, dual-chamber PMFCs, depending on the presence of algae at anode or cathode, the need of a chemical or biological mediator to allow the electron shuttling, the use of an ion exchange membrane (IEM), the coupling with a photo-bioreactor [8]. Among them, dual-chamber PMFCs with algae biocathode are particularly interesting, because they offer several advantages: i) the treatment and purification of urban or industrial wastewaters, ii) the growth of functional microalgae, iii) the harvesting of bioelectricity. The presence of microalgae at the bio-cathodes also makes this technology more sustainable in terms of costs, because it helps to replace the mechanical aeration methods. In addition, it reduces also the CO_2 generated from bacterial metabolisms and respiration [8].

PMFCs properties depend on several parameters: the dark/light regime, the geometry of electrode (e.g. brush or planar), the electrode distance, and the family of the microalgae (*Scenedesmus, Chlorella vulgaris, Chlamydomonas reinharditi*, etc.) [7, 10, 11, 12, 13, 14]. Oxygen production is normally sufficient for the optimum operation of PMFCs, even if the electricity generation may be not constant due to fluctuations of diluted O_2 concentration, which is dependent on the illumination of the chamber. Furthermore, the use of electrode without precious metals leads to good performances and, in particular, carbon brush cathodes allow reaching power density 6.5 times higher than plain electrodes (30 mW m^{-2} vs 4.6 mW m^{-2}). Key-factors as light, nutrients, and pH are also important parameters contributing to the biomass growth and composition. Under stressing limitation conditions, in fact, microalgae increase the production of lipids, carbohydrates and pigments [11, 15].

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Despite these promising results, some bottlenecks are still present, concerning the understanding of biologic aspects related to algae growth mechanisms, but also PMFCs performances, which gradually worsen due to issues related to the biocathode, as the cell start-up time, the sensibility of pH-splitting, biofouling, substance crossover across the membrane, catalysts poisoning and adjustment of CO_2, light and nutrients supply to microalgae, in addition to the MFC costs [7, 8]. All these aspects may be improved by optimising the cell design and materials, namely ion exchange membrane (IEM) and electrodes, in order to increase the power output and to reduce the cost of the whole device. Regarding the IEM, which plays a fundamental role to avoid contamination between bacteria and algae [16], the research should find materials cheaper and electrochemically more performing as MFCs separators [17]. Recently, we described a microbial fuel cell with a pyridine-PBI composite as proton exchange membrane (PEM) for the treatment of municipal wastewater. Such device resulted extraordinarily promising in terms of membrane cost/power generation ratio [18].

This work aims at evaluating the potential of a PBI-based photosynthetic MFCs with algae-assisted biocathode for the simultaneous wastewater bioremediation and biomass growth, necessary for the production and subsequent extraction of products with relevant commercial value, such as lipids, fatty acids and carotenoids. A pure culture of *Scenedesmus acutus* microalga as the biocathode, and a returned sludge from a domestic wastewater treatment plant as the anode were used. The power generation of the cell was investigated under continuous illumination. The content of valuable products as oils, lutein and other carotenoids produced by microalgae through such bioelectrochemical route was evaluated. The aim was to verify how the stress conditions occurring in the MFC reactor affect the algae metabolism and in particular the accumulation of important secondary metabolites with nutritional and health properties.

2. Materials and methods

2.1. Microalgae inoculum growth conditions

The microalga used in this study was *Scenedesmus acutus PVUW12*, which was isolated from urban wastewater of Pavia, Italy [19]. A modified version of Setlik Simmer medium was used to grow the pure culture of *Scenedesmus acutus PVUW12*. The medium consisted of (g L^{-1}): KNO_3, 2.02; KH_2PO_4; 0.34; $MgSO_4.7H_2O$; 0.988; $CaCl_2$, 0.015; microelement solution 0.5 mL L^{-1} at pH 7.4, (namely g L^{-1} EDTA sale disodico, 50; H_3BO_3, 11.4; $ZnSO_4 * 7H_2O$, 2.2; $MnCl_2 * 4H_2O$, 5.06; $FeSO_4 * 7H_2O$, 4.99; $CoCl_2 * 6H_2O$, 1.61; $CuSO_4 * 5H_2O$, 1.57; $(NH_4)_6MO_7O_{24} * 4H_2O$, 1.1).

Microalgae were grown in flasks at 25 °C on an orbital shaker (130 rpm) under continuous light at 80 μmol photons m^{-2} s^{-1}, as reported in Fig. 1.

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Fig. 1. Image of the pristine photobioreactor.

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An inoculum (4×10^6 cells mL^{-1}) from this culture was first diluted up to 10^6 cells mL^{-1} and then suspended in the PMFC reactor without mechanical shaking.

2.2. MFC assembly and operation

The photosynthetical MFC used in this study is sketched in Fig. 2. It is a double chamber (H-type) reactor, consisting of anodic and cathodic chambers with equal volumes (75 mL). The separator is a proton exchange composite membrane (PEM) (thickness 150 μm), based on Poly [2,2′-(2,6-pyridine)-5,5′-bibenzimidazole] (Py-PBI) filled with 30 wt% of sulphonated mesoporous silica, whose preparation was already described in detail elsewhere [18, 20].

Prior to the use in the cell, the membrane was treated with HCl at 80 °C for 2 h and then repeatedly washed with distilled water. The separator was clamped between the hollow tubes (15 mm diameter) connecting the chambers. The anode consisted of pure carbon cloth (SGL carbon), and two different cathodes were investigated: i) pure carbon cloth without any precious metal coating, and ii) a Pt-based gas diffusion electrode. In the latter case, the cathode was fabricated by spraying a dispersion of platinum and carbon black, 40% Pt on Vulcan XC72 (Pt/C Sigma-Aldrich), onto a gas diffusion layer (SGL carbon − Sigracet 34bc) in order to obtain a Pt loading of 0.5 mg cm^{-2}. The ink was composed by 22.5 mg of Pt/C in 9 mL of DMA, added to 0.15 mL of the starting ionomer solution (Py-PBI in DMA, $3\%_{w/w}$). The working area of the electrodes and the membrane was 56 cm^2 and 3 cm^2, respectively.

The anode chamber was inoculated by using returned sludge taken from an activated sludge process (Milan Domestic Wastewater Treatment Plant, Italy) whereas the cathodic side was inoculated with a suspension of microalgae (*Scenedesmus acutus*). The two chambers were connected to a silicon pipe, in order to capture the carbon dioxide, produced at the anode, as the nutrient for algae. All MFC reactors were

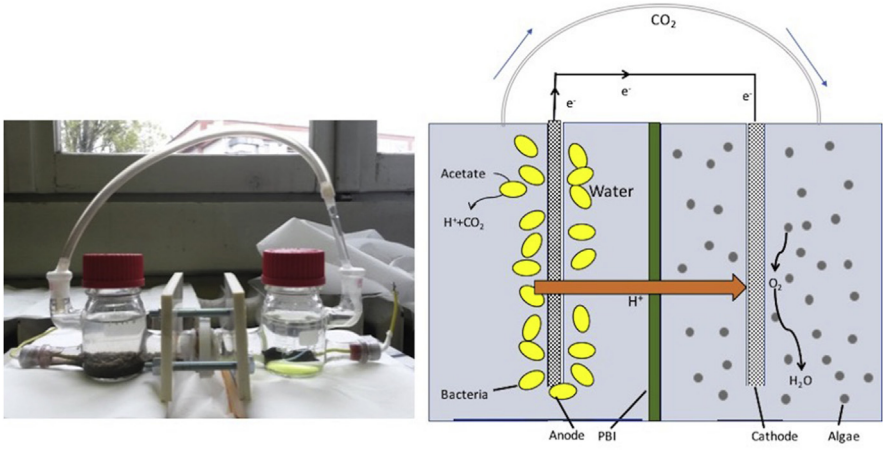

Fig. 2. PBI-based photosynthetic microbial fuel cell (PMFC).

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operated at room temperature in a fed-batch mode and kept under an external load of 100 Ω before each measurement until a stable voltage (OCV) was obtained. The starting COD was about 1800 mg L^{-1}. The electrochemical experiments were carried out in duplicate to check the system reproducibility.

The culture medium for the catholyte *Scenedesmus acutus* was sterilized and buffered by a phosphate buffer (PBS) at pH = 6.8. The microalgae were grown in the PMFC under continuous illumination of 1000 lm. The algae were fed with the carbon dioxide produced by the bacterial metabolism in the anodic compartment, as stated above. The cultures were grown in batch mode without any stirring. When the population started to decrease, the algae were harvested, separated from water by centrifugation (10 min−5000 rpm) and a new medium was inoculated in the cathode compartment. The obtained biomass was a green/yellow solid. It was dried in oven at 90 °C for 24 h and used for the chemical analyses.

2.3. Growth kinetics

The microalgae density was measured at defined times by settling the culture on a Burker chambers and counting the cells by means of an optical microscope at 500X (Zeiss Axioplan). The growth rate of *Scenedesmus acutus* was determined by the following equation [21]:

$$\mu = \frac{log_2 A_f - log_2 A_0}{t_f - t_0} \quad (1)$$

where μ is the growth rate, A_f is the cell number at time t_f and A_0 the cell number at time 0. $1/\mu$ is the doubling time, t_d, of the culture. The equation was used by considering only the data in the exponential phase of the growth.

2.4. Lipid and pigments extraction

All algal samples were centrifuged at 3000 rpm for 10 minutes and the resulting pellets were dried at 50 °C for 48 h. Dry biomass was weighed and ground to a fine powdery flour using liquid nitrogen in a mortar; the samples were extracted by adding 3.5 mL of chloroform/methanol/diethyl ether solution (2:1:0.5, v/v/v), shaken for 5 minutes and then sonicated for 10 minutes. The obtained solution was transferred to separating funnels and 3 mL of chloroform/methanol (2:1, v/v) mixture was added as an extracting agent of lipids [22]. Three ml of sodium chloride solution (5% v/v) were added to the mixture and the separating funnel was shaken vigorously before leaving for 15 minutes the phases to separate. The lower layer was collected in a test tube and evaporated to dryness by using a speed vacuum. The dry sample was suspended with 1 ml of hexane for the further analysis.

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2.5. Quantification of chlorophyll and photosynthetic activity

Quantification of chlorophyll content in *Scenedesmus acutus* was performed by spectrophotometric analysis at 645 nm and 663 nm according to the Ritchie's equation and following the procedure described elsewhere in detail [23]:

$$C\left(\mu g\ mL^{-1}\right) = (20.2 \times A_{645} + 8.02 \times A_{663})/2 \quad (2)$$

The photosynthetic activity was determined by means of measurements of the oxygen dissolved in the catholyte. To this aim, the cathodic compartment was first kept under dark conditions for 24 hours, then exposed to illumination. The oxygen consequently produced and dissolved in the catholyte was finally measured during time, until a plateau was reached.

2.6. Characterization methods

The surface morphology of the membranes before and after the experiments was observed by using a scanning electron microscope (Zeiss MA10). The samples were directly placed onto the proper stub and previously gold-sputtered. The images were collected by mapping the sample at different magnitudes.

The electrochemical tests were performed throughout the whole experimental period (>100 days). The polarization curves were collected at room temperature by means of an electrochemical interface (Solartron 1287) scanning the potential from Open Circuit Voltage (OCV) to 0 V, at 0.1 mV s^{-1}.

COD measurements were carried out by means of HACH COD analyzer, using a ISO-15705 Kit.

The normalized energy recovery, *NER*, was used as parameter to described the energy produced in a time *t* by the investigated PMFCs. It was calculated by means of the following equation [24]:

$$NER = \frac{PD \times t}{\Delta COD_t} \quad (3)$$

where *PD* is the power density normalized with respect to the anode chamber volume, *t* is the operation time and ΔCOD_t is the COD removed during time *t*.

The photosynthetic production of oxygen dissolved in the medium was measured by means of a dissolved oxygen meter (Delta OHM, HD 2109.1).

The fatty acids were analysed by GC/MS (Gas Chromatography − Mass Spectrometry). Analyses were carried out on a *ThermoFisher Scientific DSQII* GC/MS system (TraceDSQII mass spectrometer, TraceGCUltra gascromatograph), Xcalibur MS Software Version 2.1 (including NIST Mass Spectral Library (NIST 08) and Wiley

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Registry of Mass Spectral Data 8th Edition for assignment of chemical structures to chromatographic peaks). The sample preparation was performed through a trans-esterification reaction, converting the fatty acids into methylated fatty acids (FAMEs). The reference standard *Marine Oil FAME Mix* from Restek (cat. 35066) was used to identify and quantify the fatty acids. The multianalyte standard solution was 1000 μg mL^{-1} (total FAMEs) in hexane. GC conditions were set up as in the following: *i)* Column: Restek capillary column, Rtx-5MS 30 m × 0.25 mm × 0.25 μm film thickness*; ii)* Oven temperature: 45 °C × 4 min, 13 °C min^{-1} up to 175 °C × 27 min, 4 °C min^{-1} up to 215 °C × 35 min*; iii)* Injector PTV: CT split 250 °C, split flow 10 mL min^{-1}*; iv)* Transfer line: 250 °C*;* v) Carrier gas: He, 1 mL min^{-1} constant flow. The MS operative protocol used an Ion source at 250 °C, the EI ionization mode of 70 eV, a Scan mode in full scan, a Mass range 35−650 Da and a Scan rate of 803.7 amu sec^{-1}.

The HPLC instrument used for the pigments analyses was a Kontron Instrument 420 system, equipped with a C18 column (Kinetex 5 μ XB-C18 100 A, 250 mm × 4.6 mm). Methanol/ammonium acetate 1 M (80/20, v/v) was used as mobile phase A and methanol/acetone (80/20, v/v) as mobile phase B. The flow rate was 1.0 mL min^{-1}. Under these conditions, the retention time of lutein was 12.3 min. All measurements were conducted in triplicate.

Pre-coated silica gel ALGRUM® SIL G/UV_{254} for thin layer chromatography plates 5 × 10 cm (MACHEREY-NAGEL) and a solvent system of hexane: diethyl ether: acetic acid (170:30:2, v/v/v) [25] (Alonzo, 1999) was used for the separation of lipids. The layer was made by 0.20 mm silica gel 60 with fluorescent indicator UV_{254}.

3. Results and discussion

3.1. Performance of the photosynthetic microbial fuel cells

3.1.1. PMFC operation

As already described before, the aim of this work is the investigation of PMFCs with microalgae-assisted biocathode, which can produce O_2, namely the electron acceptor for electricity generation, by means of photosynthesis, without any need of aeration. In particular, these devices behave like microbial carbon capture cells (MCCs), because under light illumination the microalgae in the cathode chamber can use CO_2 coming from the wastewater degradation at the anode as a carbon source for photosynthesis and, consequently, for the oxygen production.

Two PMFC reactors were simultaneously studied, both of them consisting of similar bioanode, inoculated by domestic wastewater, and biocathode, inoculated by the same algae culture (*Scenedesmus acutus PVUW12*). The two compartments were externally

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connected to pipe CO_2. In one case, the cathodic gas diffusion electrode (GDE) was Pt-free, in the other case, a Pt-coated electrode was used (Pt loading = 0.5 mg cm^{-2}). The proton exchange membrane was a PBI-based composite, which is significantly promising as separator for MFCs for what concerns functional performances as well as cost. In our recent study, in fact, we showed that such a system allows a strong increase of power density, durability and wastewater efficiency with respect to Nafion™ [18]. Moreover, this kind of membrane does resist against sterilization processes in autoclave at 120 °C under water, without any mechanical/structural degradation, so allowing a re-use of the separator. This is certainly a beneficial aspect in terms of reduced cost and implemented sustainability of the MFC technology.

The functional performances of the PBI-based PMFCs were investigated by means of long-term operation experiments over about 100 days under continuous illumination. Both the cells were kept under the same conditions of substrate, fed-batch cycles and algal inoculum regeneration, necessary when the culture stopped to grow (after ~3 weeks, as better described in the following sections). The electrochemical characterization was performed by collecting polarization curves every 3 days, after that the microbial acclimation was complete (typically when the open circuit voltage, *OCV*, did not change more than 5−7% during one day). The polarization was carried out at low voltage scan rate (0.1 mV s^{-1}) in order to avoid power overestimation [26].

Fig. 3a−b shows, as an example, the voltage and power density (PD_v) plots vs. the current density for the Pt-based and Pt-free PMFCs, after 25 and 62 operation days, respectively, which correspond to the obtained maximum values during time operation (see below). In order to rule out variations of electrode configuration and size (e.g. surface area), volumetric power density (mW m^{-3}) was used as the parameter to describe the MFC performances [24]. Both the devices did not evidence any curve distortion, and this is an indication of a stable biofilm deposited onto the anode during the anode-enrichment period and the fed-batch cycles. However, the figures show different MFC performances. As expected, the presence of an electrocatalyst

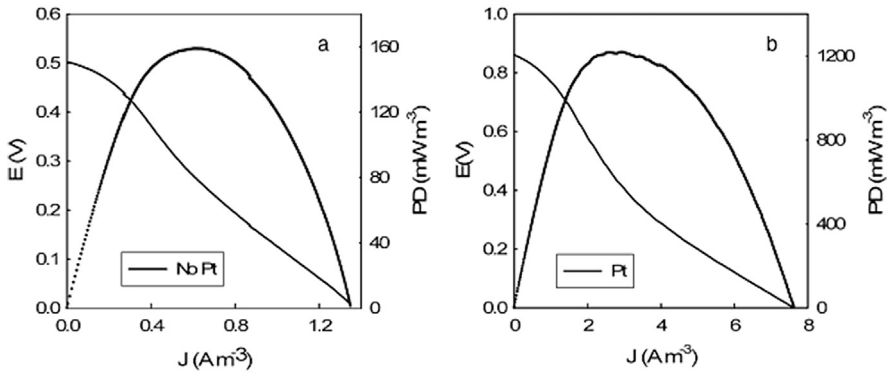

Fig. 3. Polarization and power density curves of the investigated *Scenedesmus acutus* PMFCs. (a): Pt-free biocathode; (b): Pt-biocathode.

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Table 1. Operation parameters (*OCV*; power density normalized vs. the cathode surface, $PD_{c/a}$ and vs. the cell volume, PD_v), and treatment efficiency (*COD* removal rate, *COD r.r.*; normalized energy recovery, *NER;* CO_2 production) of the two investigated PMFCs. As stated in the experimental details, the electrochemical measurements were carried out in duplicate. Error on PD data lower than 10%.

PMFC	OCV (V)	$PD_{c/a}$ (mW m^{-2})	PDv (mW m^{-3})	COD r.r. (g m^{-3} h^{-1})	NER (kWhkg$_{COD}^{-1}$)	CO_2 production (g m^{-3} h^{-1})
Pt-free	0.50	1.3	98	3.3	0.196	3.3
Pt	0.64	5.3	400	3.4	0.014	3.3

(Pt) at the biocathode was beneficial for the cathodic oxygen reduction reaction (ORR) and, consequently, in terms of generated power density. Table 1 compares the MFCs properties observed in the cells, showing better performances when Pt was used as a catalyst. Indeed, the maximum power density generated by the Pt-based devices exceeded 1200 mW m^{-3} (PD_{MAX} = 1217 ± 58 mW m^{-3}), which is almost 10 times higher than that one observed in case of a Pt-free GDE, where PD_{MAX} of 170 ± 16 mW m^{-3} was obtained. These values are quite in line with what reported in literature for PMFCs with microalgae at the cathode [8 and refs. therein cited], even if a thorough comparison is challenging because the functional performances of such devices may strongly depend on several parameters, including MFC architecture, light intensity, illumination period (continuous or dark cycles), nature of wastewater and microalgae strain. However, the obtained values of power density confirmed the capability of the algae to produce enough oxygen for optimal MFC operation.

In order to check the durability of the two PMFCs (Pt-free and Pt-coated cells), long-term experiments were carried out, by monitoring functional parameters as the generated maximum power density, open circuit voltage (*OCV*) and the amount of dissolved oxygen (*DO*), which is produced at the cathode compartment by algae photosynthesis. Fig. 4a–c shows the behavior of volumetric PD_{MAX}, *OCV* and *DO* vs. the operating time, respectively. In the Pt-free PMFC reactor the power density initially increased up to a maximum of about 170 mW m^{-3} after ~60 days, then it gradually decreased to a value of 108 ± 10 mW m^{-3} that remained constant until the end of experiment (Fig. 4a). The Pt-based cell showed a comparable trend but, in this case, a remarkably higher power density was generated throughout the entire experiment. Indeed, after at least 100 days PD_v of 400 ± 31 mW m^{-3} was obtained, which is 4 times higher than that observed in the Pt-free device.

Similarly, the Pt-MFC open circuit voltage was significantly higher than that one measured in the Pt-free cell. Fig. 4b shows the *OCV* behavior vs. the operating time for both the systems. Average values ranging between 0.8 V and 0.6 V were found with the cathode containing Pt, whereas values gradually increasing up from 0.35 V to 0.5 V were detected in case of Pt-free cell, in agreement with the voltages

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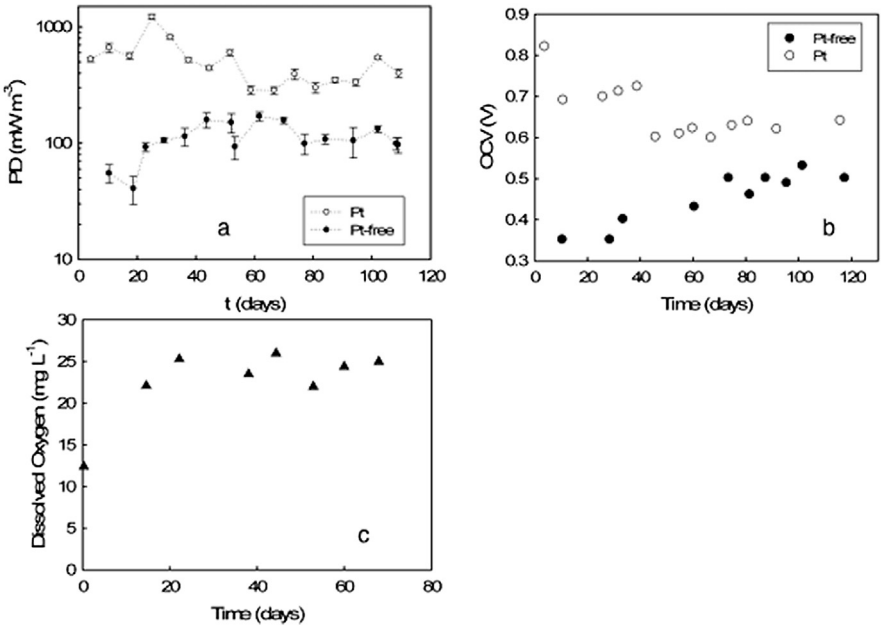

Fig. 4. Long-term performances of the investigated PMFCs. (a) volumetric power density; (b) OCV; (c) dissolved oxygen. In part (b) and (c) the error is inside the symbol dimensions.

generally reported in the literature, which are typically below 0.6 V [26]. The behavior of the actual cell voltage against the operating time is a quite complex phenomenon. Indeed, it depends on the contribution of several factors, as for instance the charge transfer process, mass transport and in particular the bacteria metabolism [26].

Basically, the open circuit voltages remained stable for several days. When the voltage generation decreased below 0.3 V, the anolyte containing the wastewater substrate was regenerated [14].

The better performances of the Pt cell seemed to be exclusively due to the Pt catalytic effect on the ORR reaction at the biocathode. In fact, with respect to other critical aspects, such as membrane biofouling and oxygen production, the two cells showed a totally overlapping response. Regarding the separator biofouling, for instance, the PBI-composite did not show any evidence of microbe adhesion on the membrane surface, as we already reported in our recent paper [18]. Furthermore, similar reproducible cycles of O_2 production at the algae-assisted cathode compartment were generated by both the system. A maximum oxygen amount of about 25 mg L^{-1} was always produced by MFC-grown *Scenedesmus ac.* that remained stable within the overall algae growth cycle, namely 3 weeks before the addition of a fresh inoculum (Fig. 4c).

Despite the higher power density, Pt-based MFC suffered higher performance loss during the operating time with respect to the Pt-free reactor. Such a result may be likely attributed to a significant presence of crystals or agglomerates, produced during the electrochemical process, which were deposited onto the biocathode, so

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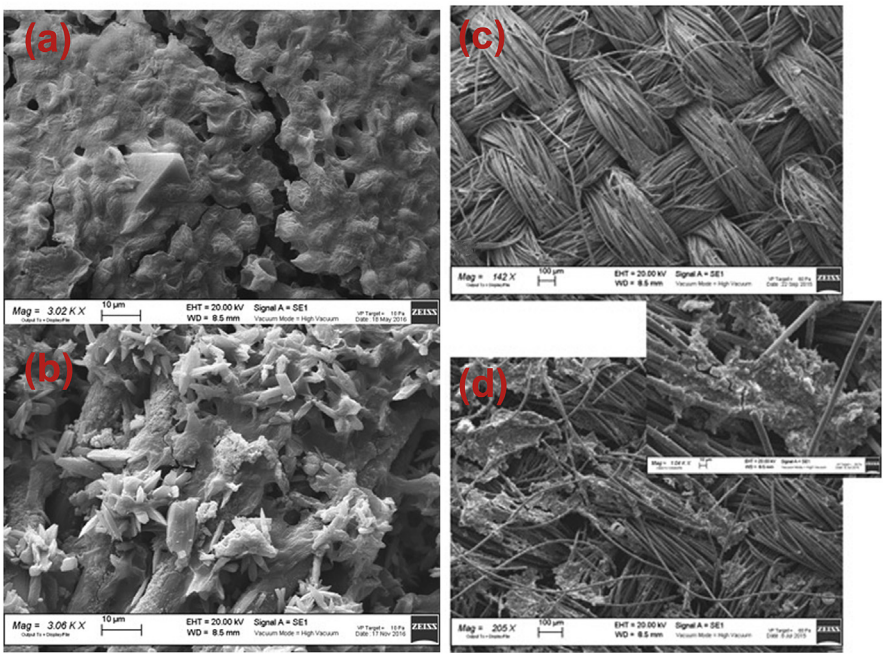

Fig. 5. SEM analysis of the biocathode surface (membrane side). (a): Pt-free PMFC; (b) Pt-PMFC and of the pristine biocathode (before MFC functional tests). (c) Pt-free GDE; (d) Pt-based GDE.

covering the electroactive sites of its surface. This is well evident by comparing the results of scanning electron microscopy carried out on the two devices. Fig. 5a–d reports the SEM images of the Pt-free GDE (a), Pt-GDE (b) of the tested PMFC reactors, and those corresponding to the pristine biocathode @t = 0 (before MFC functional tests), (c) Pt-free GDE and (d) Pt-based GDE, respectively. The images show several large particles (>10 μm) that are copiously distributed along the surface only in case of the electro-catalyzed cathode.

3.1.2. Efficiency of the wastewater treatment

The PMFCs efficiency in the treatment of the domestic wastewater was investigated by measuring the chemical oxygen demand (*COD*) during the operation time, and the normalized energy recovery (*NER*), defined as the energy production in terms of kilowatt-hour per kilogram of removed *COD* [24]. Fig. 6 shows the *COD* removal as a function of time after the cell stabilization, calculated as the ratio between the removed and influent chemical oxygen demand values. This parameter reveals the amount of the organic substrate, converted into bioelectricity by the MFC reactor. In both cases, the substrate degradation is efficient. Indeed, a COD removal exceeding 87% was obtained during the first 19 days of the long-term experiment. This is in very nice agreement with what already found in our recent work on PBI-based open-to-air-cathode MFC for wastewater treatment [18]. After this time the anolyte was re-feed in order to continue the investigation of the cell durability.

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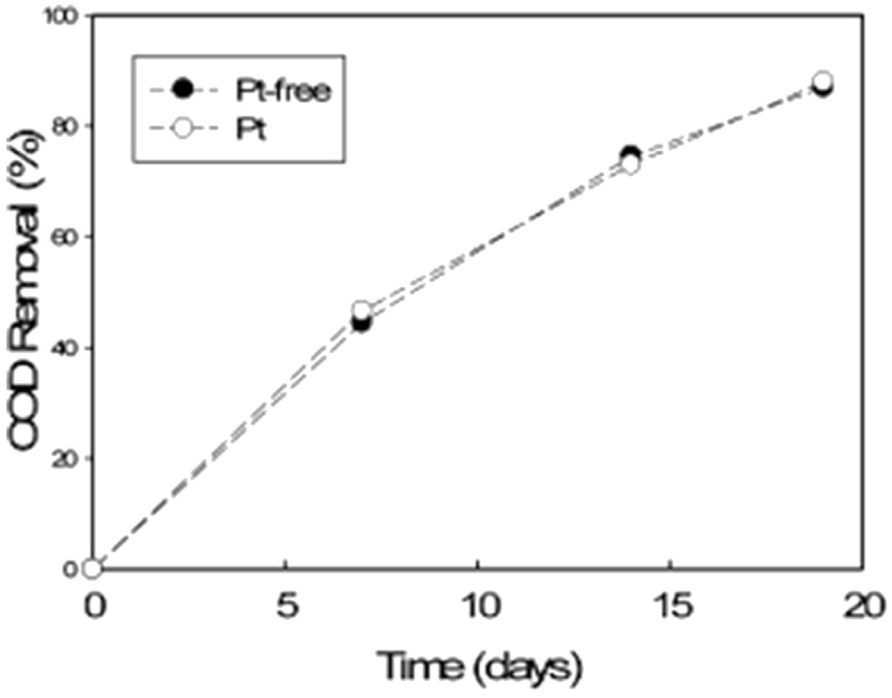

Fig. 6. Efficiency of the Chemical Oxygen Demand, *COD*, removal for the investigated PMFCs. The error is inside the symbol dimensions.

As introduced before, the *COD* removal is used to determine the normalized energy recovery, *NER*, which is a convenient way to describe MFC energy production in kWh m^{-3}, taking into account the wastewater treatment capacity. Recently, it was discussed that *NER* allows a better understanding of the actual organic substrate conversion into energy with respect to the net power density or the coulombic efficiency, ε_c. It also makes possible a more appropriate cross-comparison of the several MFCs proposed in the literature, which is still a critical issue when only power density is used in the evaluation of the wastewater treatment efficiency [24]. *NER* parameters were calculated both in case of Pt-free and Pt-PMFCs by using Eq. (2), as reported in the Experimental Section. As reported in Table 1, NER = 0.196 kW h kg_{COD}^{-1} and 0.014 kW h kg_{COD}^{-1} was the energy production determined in the two reactors, respectively. This result suggests a remarkably more efficient treatment in presence of Pt at the biocathode (more than a factor of 10), which could likely mitigate the cost issue due to the use of a precious metal as the electrocatalyst.

3.2. Microalgae biomass production: efficiency of the synergistic strategy and biorefinery

Investigation on some important aspects, such as i) cell growth rate; ii) chlorophyll content; iii) composition of some carotenoids, and iv) total triglycerides content and their identification, was carried out on the *Scenedesmus acutus* culture grown in the MFC reactors. These data were necessary to assess the efficiency of such an

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integrated system to produce energy, treat wastewater and, in the same time, to accumulate several important secondary metabolites. Regarding these properties, it was observed that the presence of the Pt electrocatalyst at the biocathodes does not affect in any way the growth kinetics and the production of valuable products. Similar photosynthetic activity and pigments/lipids composition were, in fact, obtained in both the tested PMFCs.

3.2.1. Microalgae growth kinetics

Fig. 7 shows an optical microscopy image (500X) of the *Scenedesmus acutus* population cultivated in the PMFC reactor. As already described before, the cell density was measured by settling the microalgae in a Burker chamber and counting them via microscopic route.

Fig. 8 describes the growth of *Scenedesmus* in the PMFC determined as described by Damiani et al. [21]. The initial cell concentration and biomass content were 10^6 cells mL^{-1} and 0.02 g L^{-1}, respectively. Basically, the growth process follows a quasi-exponential behavior. The algae needed few days (about 4 days) of adaptation before enhancing their biomass. The concentration increased of one order of magnitude, up $1.4 \pm 0.1 \times 10^7$ cells mL^{-1}, then a growth recovery could be observed after 22 days. Subsequently, the algal cell number began to decrease, as expected, because of the environmental stressing conditions typical of the PMFC, namely exhaustion of nutrients in cultural medium and oxidative stress. The cell growth rate, μ, determined

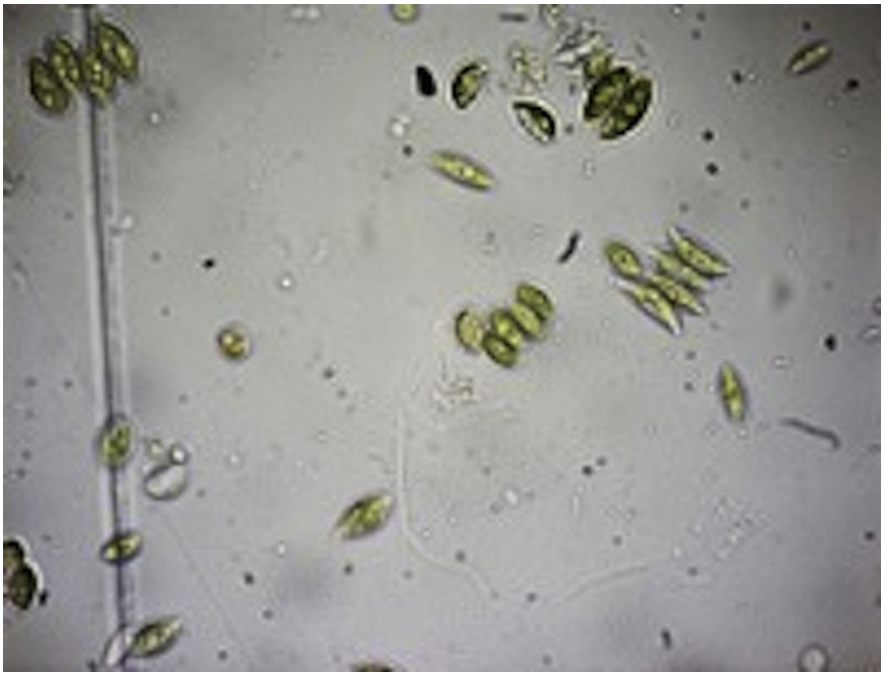

Fig. 7. Optical microscopy image (500X) of the *Scenedesmus acutus* strain, grown in the PMFC.

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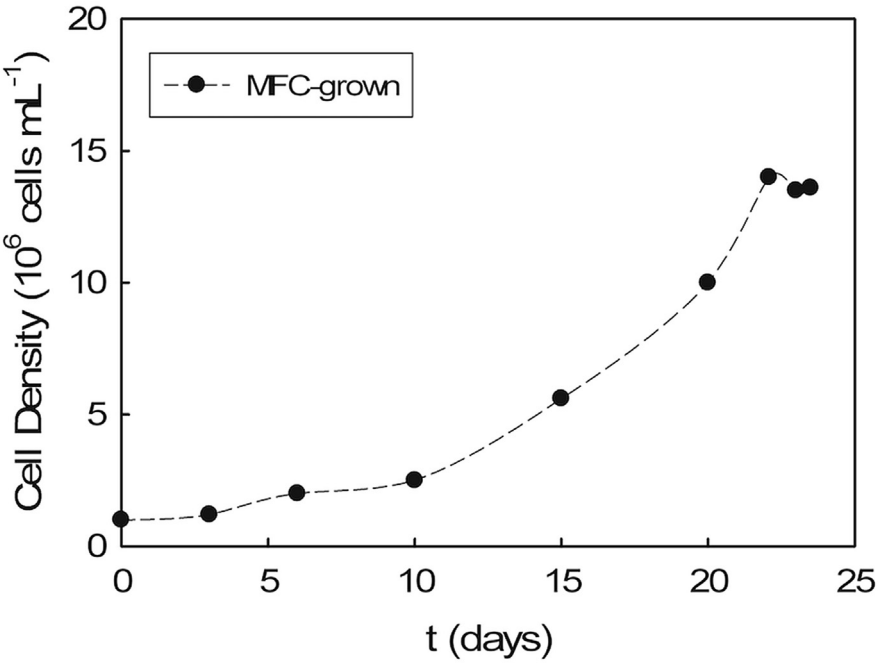

Fig. 8. Growth rate of the *Scenedesmus acutus* strain, μ, grown at the PMFC biocathode.

only in the exponential phase, is 0.17 cell mL^{-1} day^{-1} with a doubling time, t_D, of ~6 days. The biomass collected from the culture grown in MFC reactor was 0.29 ± 0.2 g L^{-1} after 22 days of cultivation. The good growth rate accompanied by the great biomass production, registered in algal population cultivated in the MFC reactor, was essentially due to enough CO_2 produced by bacteria at the anode of the MFC reactor during the electrochemical wastewater degradation of the substrate. The quantity of produced carbon dioxide was ~3 g m^{-3} h^{-1} in both the PEMFC reactors (Pt and Pt-free). It is well known in literature the positive effect of CO_2 on the algal biomass concentration and cell growth, which also depends on factors as light intensity, algae family, gas flow rate etc. [27]. This result is an index that the MFC electrochemical processes properly run in terms of bioenergy production as well as harvested CO_2, whose concentration seems to be enough to stimulate the cell growth and to fix gas into added value products.

3.2.2. Migroalgae photosynthetic activity

The chlorophyll concentration of *Scenedesmus acutus* was measured by means of spectrophotometric methods, as described in the experimental section. The value obtained in case of MFC-grown culture was 106 ± 3 μg mL^{-1}. This value is satisfying and suggests that the environmental stress of the MFC reactor was not so high to inhibit the photosynthetic activity of algae. In order to further check the cell vitality, oxygen production measurements were carried out on the *Scenedesmus acutus*, cultivated in the PMFC reactor. Fig. 9 shows the amount of oxygen produced by

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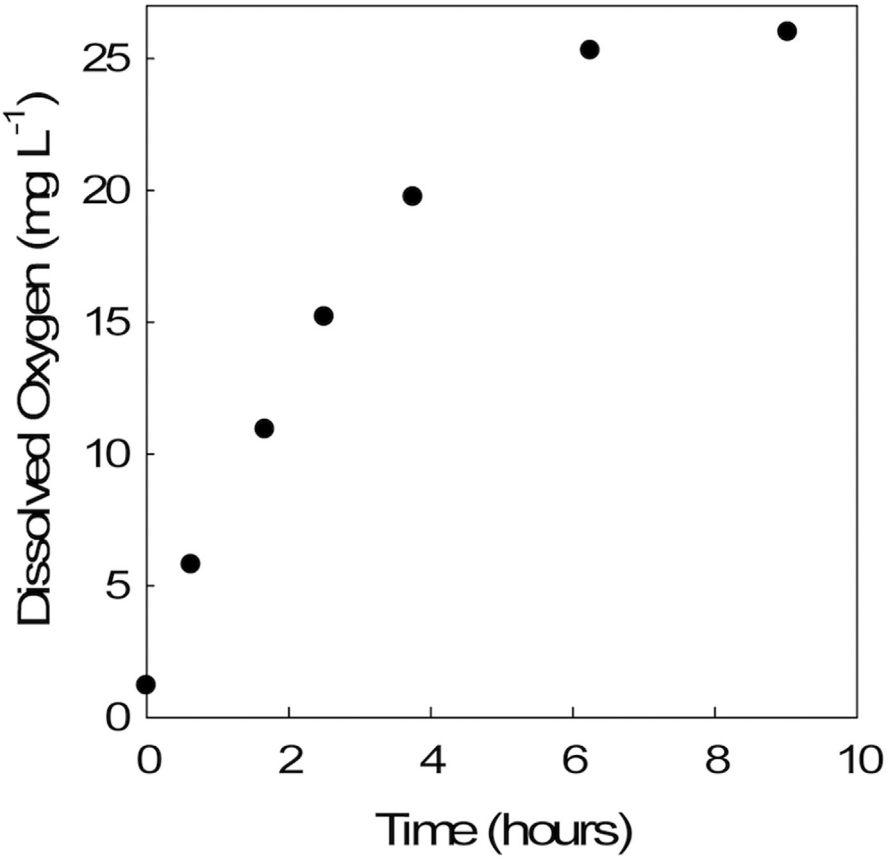

Fig. 9. Photosynthetic activity of the PMFC-grown *Scenedesmus acutus* culture, measured in terms of O_2 production in the reactor. The error is inside the symbols dimensions.

microalgae, arranged under illumination after 16 hours dark phase (see Fig. 7). The time-dependent behavior of the O_2 production was similar for both types of PMFCs (with- and without Pt at the biocathode). The oxygen dissolved in the catholyte increases exponentially up to $\sim$25 mg L^{-1} within 7 hours, before reaching a plateau. This content, whose value is in fair agree with what recently reported in literature [28], seems to be enough for the electricity generation of MFC and the microbial growth inoculated in the anodic chamber.

3.2.3. Production of added-value products: carotenoids and fatty acids

Scenedesmus acutus is considered an efficient strain for CO_2 mitigation and for pigments and lipids accumulation [6, 23]. In order to evaluate the efficiency of the algae-assisted PMFC device to produce carotenoids and fatty acids, qualitative/quantitative analyses were carried out on the culture grown in the MFC during a long-term functional experiment. The results were compared with the pigments and total lipid amount found in the culture grown in a flask at the same conditions

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of light, temperature and pH of the MFC reactor. The carotenoids composition was investigated by HPLC analysis, which revealed a similar total content of pigments for both types of tested fuel cells (Pt-free and Pt), namely 3.7 ± 0.5 and 3.9 ± 0.7 mg g_{dw}^{-1}, respectively. In terms of composition, data showed a very relevant level of lutein and a significant level of other carotenoids, as astaxanthin, violaxanthin and cantaxanthin. Fig. 10 reports the content of these pigments, expressed as mg per gram of dried biomass, in *Scenedesmus* culture cultivated in the PMFC reactor.

The lutein content in *Scenedesmus* culture in PMFC was more than 15 times higher than the average level of other carotenoids, and almost the double with respect to that one found in flask (1.85 mg g_{dw}^{-1}), whereas the lowest level was obtained for the cantaxanthin (0.16 mg g_{dw}^{-1}), namely 1.4 times lower than the average value of astaxanthin and violaxanthin. In contrast, no other carotenoids, besides lutein, were observed in the flask culture.

This obtained result demonstrated that the PMFCs operative conditions stimulate the biosynthesis of pigments. Therefore, the high amount of lutein and the presence of the other ketocarotenoids with a very important antioxidant effect in the algal cell grown in the MFC reactors are probably due to the environmental stress conditions able to induce in algal cells an accumulation of these xanthophylls that play a fundamental role in the protection of the photosystems against the oxidative stress. The production

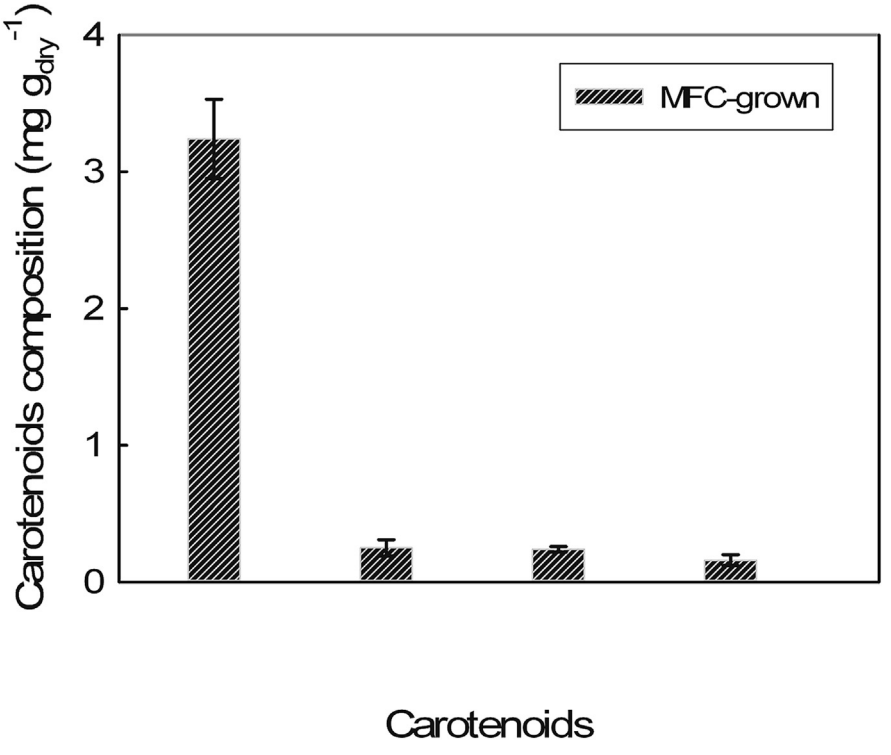

Fig. 10. Pigments extracted from the microalgae-grown PMFC. Data from HPLC analysis.

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of other carotenoids, besides lutein, in algae grown in a PMFC was previously described in literature, for instance in presence of *Chlorella vulgaris* and interpreted in terms of faster carotenogenesis favored by the fuel cell environment [29].

The lipid composition of the *Scenedesmus acutus* was preliminary investigated by TLC. This qualitative analysis method allowed to separate different kinds of lipids. Fig. 11 shows TLC runs for the lipids extracted from microalgae culture grown in MFC. Comparing the resulting spots with those reported in the literature at the same TLC conditions [30], it was possible to identify monoacyglicerols (MAG), diacylglicerols (DAG), free fatty acids (FFA) and triglycerides (TAG) spots. The different intensity of the spots showed a highest amount of monoacylglicerols and a lowest presence of diacylglicerols. In particular, the spot intensity of free fatty acids and triglycerides, which are the most interesting lipids for nutritional aims, resulted very well defined.

The effects of the electrochemical environment on the total lipids accumulation in *Scenedesmus acutus* were quantitatively evaluated by means of GC-MS analysis. In particular, fatty acids were identified and quantified after methyl esterification to form the corresponding fatty acid methyl esters (FAMEs).

The total content of neutral lipids accumulated in the MFC-grown microalgae was remarkably higher than that found for the culture grown in flask, namely 49% and 6% of the biomass dry weight, respectively. Such a high FAs concentration is particularly encouraging because the content of neutral lipids accumulated in PMFC-grown microalgae is typically lower than 40%. Recently, lipid productions ranging around 30−37% were observed in different microalgae strain (for instance *Golenkinia*, *Chlorella vulgaris*, *Scenedesmus SDEC-8* and *SDEC-13*), used as biocathode in a PMFC for kitchen waste effluent treatment [28]. In case of the *Scenedesmus* species, a total lipid content of about 30 wt% was found, despite of a faster growth process.

The fatty acids concentration in algal culture grown in MFC reactor described in this work is even higher that that obtained for the same strain (*Scenedesmus acutus PVUW12*) previously cultivated in a tubular photobioreactor placed outdoor, where microalgae were grown in nitrogen-free medium (N-stress). In this case, about 32% of total lipids were accumulated with a predominance of monounsaturated fatty acid (oleic acid) with respect to the saturated ones, namely palmitic and stearic acids [23].

Obtained results confirm how the stress conditions in the MFC lead to an accumulation of reserve substances that may ensure survival and recovery of the microalgae on long times. Nutrient depletion and oxidative stress might direct the algal metabolism towards the synthesis of reserve compounds and secondary metabolites such as lipids and protective pigments, rather than primary metabolites and cell division.

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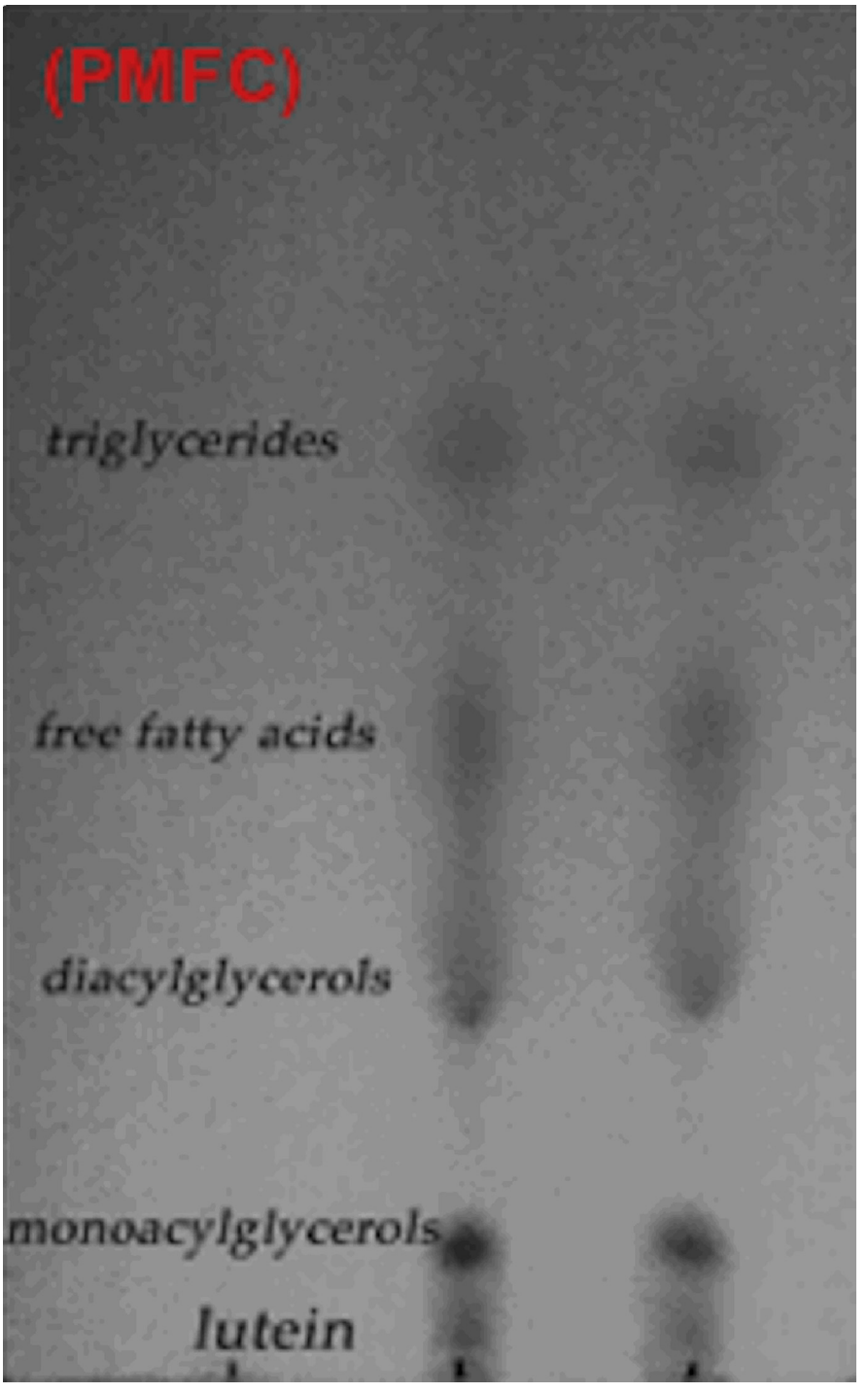

Fig. 11. TLC profiles of oil content in *Scenedesmus acutus* grown in PMFC.

The FAMEs profile, obtained from the Total Ion Current (TIC) Chromatograms shown in Fig. 12, is reported in Table 2. Saturated lipids are mainly accumulated, even if some amounts of unsaturated systems are also present. The reason of such a result may be related to the low content of CO_2 coming from the anodic chamber

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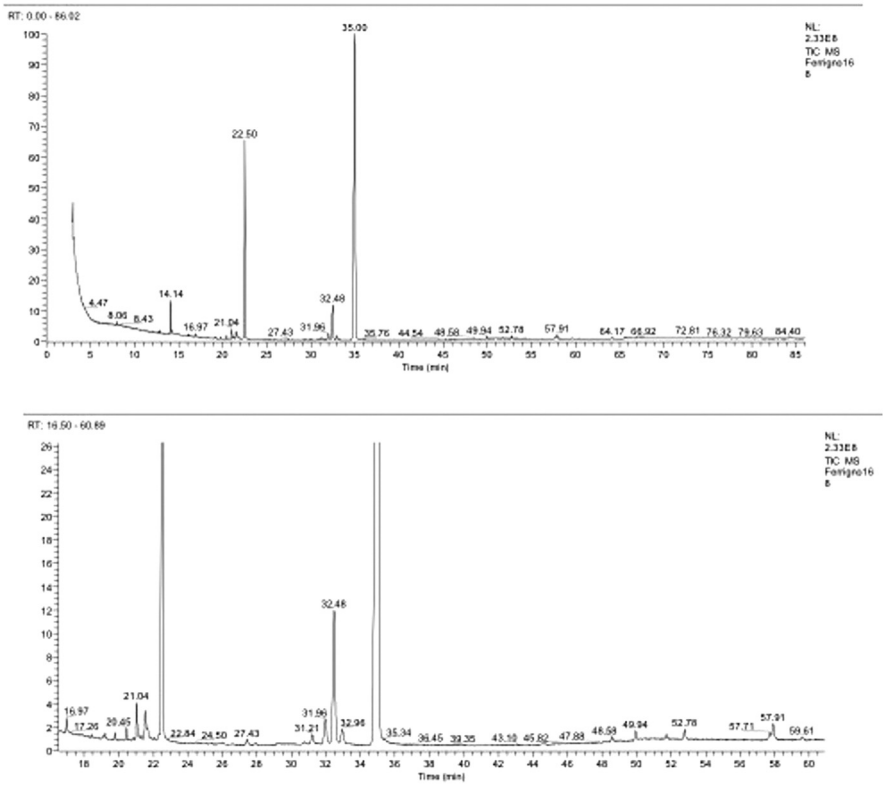

Fig. 12. Total Ion Current (TIC) chromatogram of the alga extract (up) and corresponding zoomed spectrum in the range 16−60 minutes.

to aerate the microalgal biocathode, which favors the accumulation of saturated fatty acid and short-chain fatty acids. It was described in literature, in fact, that a decrease of the feeding gas amount could lead to a O_2 increase with a negative effect on the enzymatic desaturation and a consequent enhancement of the saturated FAs [26 and refs. therein indicated].

The lipid composition in *Scenedesmus acutus*, grown in the MFC, is mainly in the C_{16}-C_{18} range, even if some amounts of C_{14} and C_{20}-C_{22} are present as well, similarly to what typically observed in plant oil [27]. This result is in fair agreement with what reported in literature for *Scenedesmus sp*., whose fatty acids profile is mainly represented by 14:0, 16:0, 16:4n1, and 18:3n3 along with minor levels of monounsaturated (16:1n7, 18:1n9) and polyunsaturated fatty acids (16: 3, 18:2 and 18:3n3) [31, 32].

In our strain grown in the fuel cell, a relevant production of methyl palmitate (palmitic acid, C16:0), methyl oleate (oleic acid, C18:1) and above all methyl stereate (stearic acid, C18:0) was observed, in the fraction of 25%, 6% and 59% of the total lipid content, respectively. The predominance of C_{16}-C_{18} lipids is an index of the potential use of *Scenedesmus acutus* cultivated in PMFC reactors in biodiesel feedstock production.

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Table 2. Identification and composition of the FAMEs, extracted from the *Scenedesmus acutus* microalgae grown in a PMFC reactor. Data from GC-MS analysis. (R_t = Retention time).

R_t (min)	FAMEs	MW (g mol^{-1})	FAME composition (mg g^{-1})
16.97	Methyl myristate	242	0.40
19.21	Methyl pentadecanoate	256	0.25
21.04	Methyl hexadecatetraenoate	262	0.71
21.54	Methyl palmitoleate or isomer	268	0.61
21.69	Methyl palmitoleate or isomer	268	<0.12
22.50	Methyl palmitate	270	12.3
27.43	Methyl margarate (heptadecanoate)	284	<0.12
31.21	Methyl stearidonate	290	0.31
31.96	Methyl linoleate	294	0.51
32.48	Methyl oleate	296	3.07
32.96	Methyl vaccenate	296	0.41
35.00	Methyl stearate	298	28.8
49.94	Methyl arachisate	326	0.29
57.91	Methyl erucate	352	0.44
59.61	Methyl behenate	354	<0.12

4. Conclusions

Here, we described the performances of two PBI-based photosynthetic algae MFCs, one with Pt-electrocatalysed biocathode, the other one Pt-free, as devices for a synergistic approach in the simultaneous wastewater treatment and biorefinery. The PMFCs were operated under continuous illumination, using a microbial community at the anode, inoculated by a domestic wastewater, and *Scenedesmus acutus* microalgae at the biocathode compartment. They worked as microbial carbon capture cells, because the microalgae in the catholyte use CO_2 coming from the wastewater degradation at the anode as a carbon sources for photosynthesis and for the consequent oxygen production.

The presence of an electrocatalyst greatly affects the electrochemical performances of the fuel cell, leading to higher power density, OCV and to a more efficient wastewater treatment. In contrast, no differences are observed for what concerns the microalgae properties, which are similar in both the reactors.

The efficiency of such integrated bioelectrochemical system resulted particularly encouraging. The algal photocatalytic activity was enough to guarantee high cell vitality, quite fast growth kinetics and a rich production of added-value compounds, as

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pigments and lipids. Such products were obtained with significant yields, due to the stressing conditions of the MFC, which are favorable to a secondary metabolism and to the accumulation of reserve products. Indeed, neutral lipids amount of 49% with respect the biomass dry weight were quantified, whose majority part is composed by fatty acids in the range $C_{16\text{-}18}$.

For this reason, the PBI-based PMFCs are potential electrochemical devices for a more sustainable simultaneous bioenergy generation, biofuel production and accumulation of added-value compounds.

Declarations

Author contribution statement

Eliana Quartarone: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Simone Angioni, Luca Millia, Enrico Doria, Marta Temporiti: Performed the experiments; Analyzed and interpreted the data.

Federica Corana, Barbara Mannucci, Piercarlo Mustarelli: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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