Electrochemical removal of microalgae with an integrated electrolysis-microbial fuel cell closed-loop system

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Abstract

Uncontrolled algal growth in water systems causes a number of serious issues that range from unpleasant odours and tastes to eutrophication. In this work, we propose for the first time to integrate an electrolysis process with the microbial fuel cell (MFC) technology as a sustainable way to treat algal contamination in water systems.

Removal of chlorophyll-*a* by electrolysis was investigated in a fixed bed electrochemical reactor. The effect that operative parameters, such as current density and hydrodynamics, have on the process was analysed by using *Chlorella vulgaris* as a model of microalgae. Based on these results, a combined closed-loop system was developed in which the electrolysis unit was coupled with a cascade of miniature single chambered air-cathode MFCs. The electrolysis of *C. vulgaris* was performed under an applied current density of 25 A m^{-2} and for Reynolds equal to 13. The treated water was fed into a cascade of MFCs for further treatment and energy generation. The effect of the electrode surface area and of the number of MFCs in the cascade on both algae removal efficiency and power output was investigated. It resulted that the greater the active area of the electrodes in the MFCs, and the larger the number of fuel cells, the better the performance of the stack. The integrated system led to a 20% of reduction on the electrical energy requirement of the electrochemical reactor, giving the best results when the electrode surface area of the MFCs in the cascade was 0.32 cm^2.

Our approach provides a sustainable alternative to current algal removal systems that not only is chemical-free but also aims to be energy-neutral, thus reducing the large amount of energy that current water treatments require.

Keywords: *Chlorella vulgaris*; electrolysis; microbial fuel cells; algae blooms; algae removal.

1. Introduction

The release of waste from domestic, agricultural and industrial activities in water systems can lead to extremely high concentrations of nutrients and cause explosive proliferations of plants and algae (eutrophication). The uncontrolled growth of algae (blooms) affects the taste and odour of drinking water and can lead to the generation of toxins that critically compromise its safety. Algae blooms also pose a series of issues in wastewater treatment plants by interfering with both physical and chemical water purification processes. Costly dredging and disposal processes are consequently required [1].

To remove microalgae several processes have been proposed [2], including sand filtration and coagulation [3,4]. These techniques are, however, not efficient enough due to the small size of the microalgae cells (2-10 μm), which causes clogging and fouling of filters [4,5]. Consequently, to improve their effectiveness, different water treatments are usually coupled with pre-oxidative steps in which disinfectant agents, such as hypochlorite, chlorine and ozone, are commonly generated *via* electrolysis [6,7]. An efficient alternative is direct electrolysis (DE), which has proved to successfully inactivate different types of algae [8–10]. The main drawback of DE is, however, associated to its energy requirement, which would add to the great amount of energy that water treatments require. In the United States only, approximately 3–4% of the average daily electricity consumption is used for the treatment of wastewaters (WWs), which in turn results in the emissions of more than 45 million tons of greenhouse gases annually and a cost of approximately $4 billion (US EPA). Nonetheless, the of DE microalgae leads to the release of intracellular matter, such as lipids [11], which could be exploited as fuel in microbial fuel cells (MFCs) to produce useful electricity.

MFCs have attracted a lot of attention in the past decade as innovative renewable and carbon-neutral bio-electrochemical devices, capable of generating energy from WW effluents through the action of electroactive microorganisms [12–14]. In this particular type of fuel cell, microorganisms at the anode break down organic matter into carbon dioxide, protons (H^+) and electrons (e^-). The electrons flow from the anode to the cathode generating an electrical current, while the protons flow across a proton exchange membrane to combine at the cathode with the electrons and an electron acceptor, usually oxygen, to form water.

MFCs have been proposed as an attractive means to treat wastewaters while generating electricity [13]. Contrary to anaerobic digesters, the energy conversion in MFCs is direct and, therefore, the

theoretical energy efficiency of MFCs is much higher. One of the biggest limitations of this technology that still prevents practical applications is, however, associated with the difficulty in the scaling-up [15]. The miniaturisation of the fuel cell design and the arrangement of multiple miniature units in stack is currently considered one of the most viable approach to overcome this limitation [16]. A wide variety of organic matter, originating from any sort of WW, has been tested as fuel in MFCs, and performance varied according to the biodegradability and bioavailability of the organic substrate [17]. Recently, algae have been considered as a new organic source for the anodic bacteria [18–20]. To improve the anaerobic biodegradability of microalgae biomass, pre-treatment techniques have been proposed to dissolve or disrupt the algae cell membrane and favour the accessibility of the bacteria to the organic matter [6,21–23].

In this work, we have integrated for the first time a DE system with the MFC technology with the aim of reducing the energy consumption of the electrolysis process and, therefore, the operating costs. An integrated closed-loop system is in particular proposed, in which a fixed bed electrochemical reactor with three-dimensional electrodes for the microalgae electrolysis is coupled with a cascade of miniature air-cathode MFCs. *Chlorella vulgaris* was used as the model microalgae. The configuration of the DE system has been designed to minimise the presence of long life oxidants in the outlet of the DE unit (the feed of the MFCs), to prevent any damage to the anodic biofilm inside the MFCs [24]. In particular, since active chlorine species are the most persistent among the oxidants electro-generated in DE, boron-doped diamond (BDD) was used as anode material. BDD combines in fact high effectiveness in electrochemical treatments with relatively low catalytic activity towards active chlorine formation [25,26]. We also investigated the effect that increasing the electrode surface area, as well as the number of single units in the cascade, had on the overall algae removal efficiency and on the power generated by the MFC stack.

2. Materials and methods

2.1. Algae culture

Chlorella vulgaris green algae was kindly provided by the Department of Biology and Biochemistry, University of Bath (UK). Wastewater (WW) from the Wessex Water treatment plant in Somerton (UK) was used as the grown media for *C. vulgaris*. The characteristics of the Somerton WW are reported in Table 1. The WW was ozonised and oxygenated prior to use. *C. vulgaris* was cultivated in 1 L flasks under continuous fluorescent light at 25 ± 1°C and at 43% of humidity. All experiments

were carried out when *C. vulgaris* was in the log-growth stage, which corresponded to an algal concentration of 10 x 10^6 - 20 x 10^6 cells ml^{-1} and to a COD value of about 35 ± 7 mg L^{-1}.

Table 1. Characteristics of the Somerton wastewater.

Parameter	Value
pH	7 ± 0.5
Conductivity (µS)	810 ± 50
COD (mg L^{-1})	35 ± 7
Phosphate (mg L^{-1})	3.2 ± 0.9
Nitrate (mg L^{-1})	21.3 ± 3
Total Nitrogen (mg L^{-1})	27 ± 5
Total Suspended Solids (mg L^{-1})	1

2.2. Electrochemical system

DE experiments were performed with the fixed bed electrochemical reactor previously developed [24]. The cell was made of Plexiglas with a cylindrical shape constituted of a single compartment with an inner diameter of 5 cm and a height of 15 cm (Figure 1A). The 3D electrodes consisted of: six discs of niobium grids coated with conductive diamond (BDD) in the case of the anode; and six discs of titanium grids coated with platinum in the case of the cathode. Details of a typical grid are shown in Figure 1B. The solid surface of both electrodes was 100 cm^2. The anode packing was placed 6.5 cm far from the inlet section, and the inter-electrode gap was approximately 0.5 cm wide. The resulting reactor was filled with glass spheres (d_p = 0.2 cm) leading to a bed void fraction (ε) of 0.36, and a liquid volume of 100 cm^3. Sampling ports were located at the bottom and the top of the reactor.

The cell was used in batch recirculated mode (Figure 1C). The electrolyte was recirculated from the cell to the reservoir in a closed-loop at flow rates within the range 33 - 200 ml min^{-1}, which corresponded to Reynolds values of 13 - 80. The DE cell was operated under a fixed current density (range: 25 - 60 A m^{-2}) and no chloride ions were added in the electrolyte. The volume of the system was 200 cm^3.

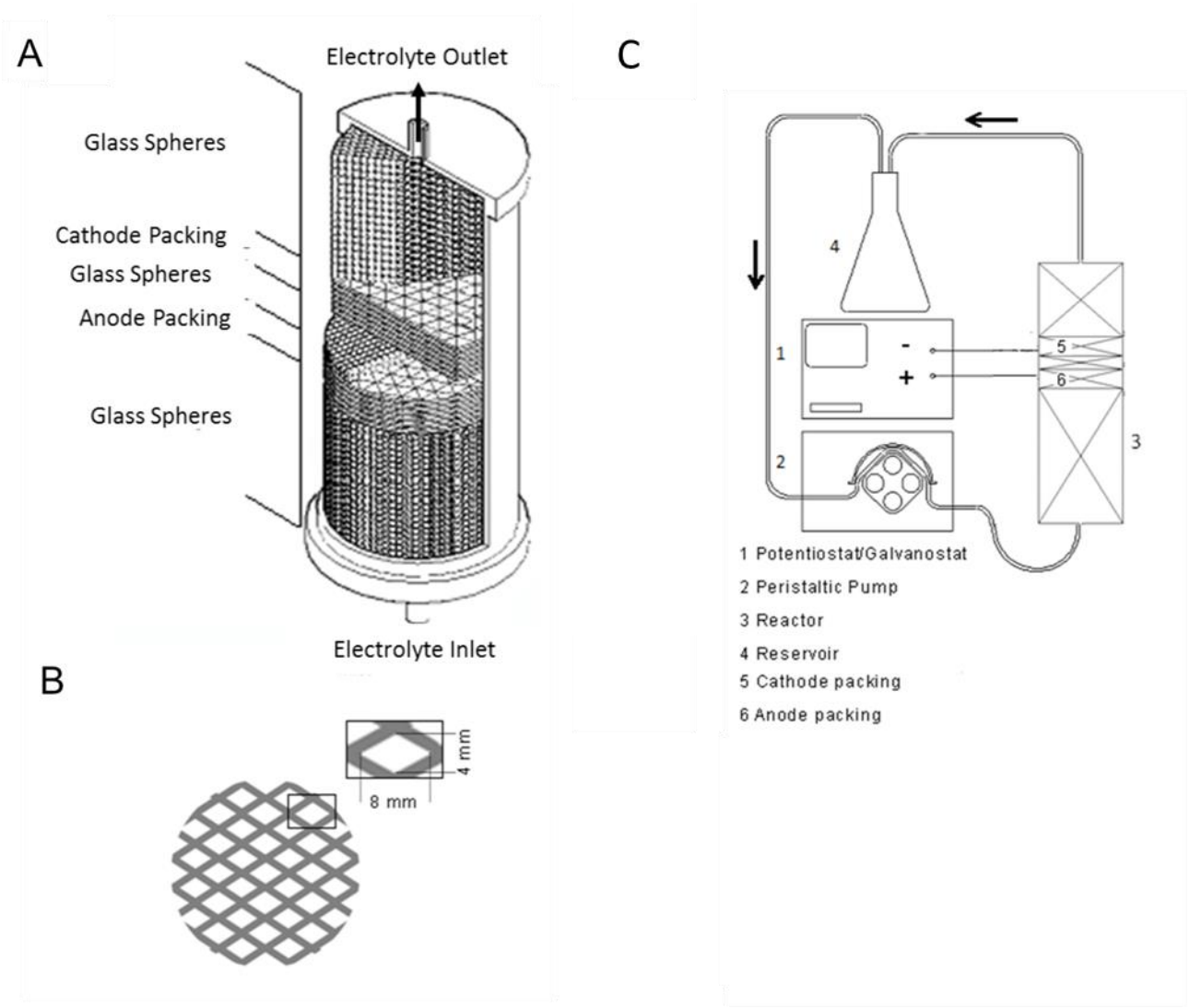

Figure 1. Experimental apparatus for the DE. (A): axonometric sketch of the electrolysis reactor, showing the stacks of grids that constitute the anode and cathode packing, the inert filling (glass spheres) and the inlet and outlet. (B): details of a grid. (C): hydraulic scheme of the system in batch recirculated mode configuration.

2.3. Microbial fuel cell design

Two designs of miniature air–cathode microbial fuel cells were used in this study, fabricated as previously reported [16]. The anodic channel was made of polydimethylsiloxane silicon (PDMS, Ells Worth Adhesives) with a 10:1 ratio. Carbon cloth (untreated carbon cloth type B, E-Tek, USA) was used as both the anode and cathode material. Nafion® (115, Sigma-aldrich) was used as the proton exchange membrane, and was hot pressed to the cathode by applying a pressure of 3 bar for 90 s at 130°C. Figure 2 shows the general schematic and a picture of the two devices used. Two cell lengths were considered: 4 and 8 mm. As a result, the electrode nominal surface area was of 0.16

and 0.32 cm^2, corresponding to anodic volumes of 0.048 and 0.096 cm^3, respectively. The inter-electrode gap was of 0.3 cm. Titanium wire (Advent Research Materials, diameter 0.2 mm) was used as electrical contacts.

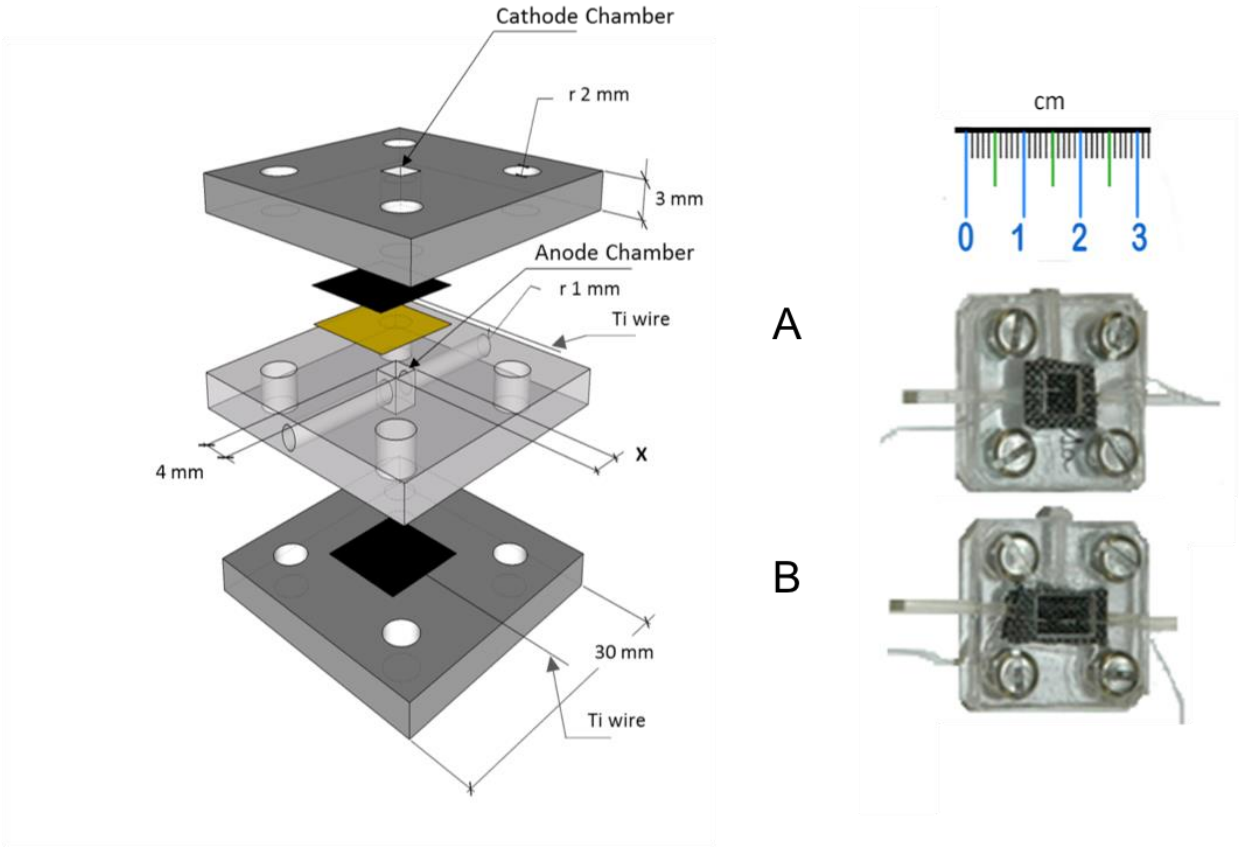

Figure 2. Schematic and photograph of the fuel cell devices used in this study. Electrode surface area: 0.16 cm^2 (A) and 0.32 cm^2 (B).

2.4 Fuel cell operation

The anode and the cathode electrodes of each air-cathode MFCs were connected through a fixed external resistor (R_{ext}) and to a data acquisition system (PicoLog 1012), and the cell voltage of each MFC was recorded at intervals of 10 seconds.

The maturing of the electrochemically anaerobic active bacteria at the anode was performed by feeding the fuel cell (batch-recirculating mode at a flow rate of 0.4 ml min^{-1}) with artificial WW (AWW), containing 2% (v/v) of anaerobic sludge (Wessex Water, Scientific Laboratory in Saltford, UK). AWW was prepared by adding to distilled water: $(NH_4)_2SO_4$ (0.269 g L^{-1}); $MgSO_4 \cdot 5H_2O$ (0.059 g L^{-1}); $MnSO_4 \cdot H_2O$ (0.006 g L^{-1}); $NaHCO_3$ (0.130 g L^{-1}); $FeCl_3$ (0.003 g L^{-1}); $MgCl_2 \cdot 6H_2O$ (0.007 g L^{-1}). Potassium acetate at a concentration of 9.810 g L^{-1} was used as the carbon source. The resulting

COD value was 2,200 ± 200 mg L^{-1}, and the pH of the solution was adjusted to 7 ± 0.5 with 0.1 M HNO_3. The solution, replaced on a daily basis, was sterilised and purged with N_2 prior to its use. After approximately one week of operation, the output voltage reached a stable value and the enrichment phase was considered concluded. The MFCs were therefore hydraulically assembled in series, while still electrically independent from each other, and fed with electrolysed algae under a continuously recirculated flow rate of 0.4 ml min^{-1} (see Figure 3). Under this flow, the hydraulic retention time (HRT) of each MFC was of 7.2 or 14.4 seconds, according to the length of the anodic chamber.

Polarisation experiments were performed by connecting the MFCs to a series of external loads, varying from 10 Ω to 1000 kΩ, controlled by an external variable resistor (RS-200 Resistance substitute, IET Labs Inc., USA), and by measuring the pseudo steady state output potential after 10 minutes. Before the test, the MFC was left under open circuit for no more than 2 hours to allow a steady state open circuit voltage (OCV) to develop. Ohm's law was used to determine the corresponding current (*I*) at each external load value (*I = V/R,* where *V*, and *R* are the cell voltage and resistance respectively).

The volumetric power density, P, (*W* m^{-3}) generated by each MFC was calculated as:

$$P = \frac{V_{cell}\, I}{V} = \frac{\left(\frac{{V_{cell}}^2}{R_{ext}}\right)}{V} \quad (1)$$

where, V_{cell} is the cell voltage (*V*), *I* the current (*A*), R_{ext} the fixed external load (*Ω*) and *V* the system volume (m^3).

The energy (E, *Wh* m^{-3}) produced by each MFC, over the time t, was calculated as:

$$E = \int_0^t \frac{V_{cell}\, I}{V}\, dt \quad (2)$$

The overall energy generated by the MFC stack was calculated as the sum of energy produced by each single MFC.

2.5 Set-up and operation of the closed-loop integrated system

Figure 3 shows the set-up of the integrated system. The electrolysis unit (2) was inserted into a batch recirculating hydraulic circuit and pumped with a *C. vulgaris* algae solution (35 mg COD L^{-1}, volume: 200 cm^3) (1) at Re = 13. An electric field, parallel to the fluid flow, was applied by setting a current density of 2.5 mA cm^{-2} with a power supply (3), leading to a cell voltage of 12 V. The

electrolyte (1) was also fed into the cascade of MFCs (4) at a flow rate of 0.4 ml min^{-1} with a multichannel peristaltic pump equipped with 2-stop tubing. The electrolysis unit was operated for one hour, the solution in reservoir 1 was, however, recirculated to the cascade of MFCs for the following three days.

To test the effect of numbering up the fuel cell in the cascade on the algal inactivation and energy generation, the stack (4) was made up of either three or five MFCs. The MFCs in the stack were electrically independent from each other to monitor individually their performance.

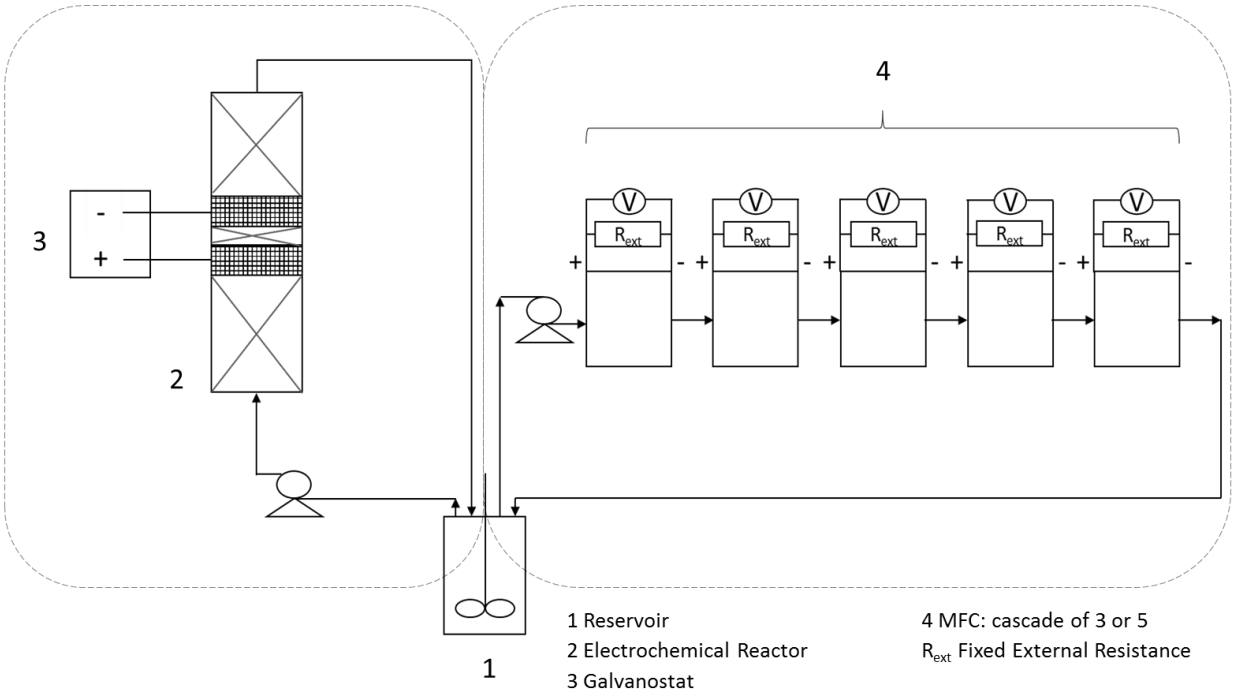

Figure 3. Set-up Scheme of the combined system. The algae solution (1) is recirculated into the electrolysis unit (2) as well as into the cascade of MFCs (4). The cascade consisted of either three or five microbial fuel cells.

2.5 Analysis

The inactivation of *C. vulgaris* was monitored by measuring the absorbance at 680 nm (corresponding to the maximum absorbance of chlorophyll-*a*), with a spectrophotometer (VARIAN-50). The cell density was also analysed through counting under microscope (Olympus) at x40 magnification, with the use of a Thoma counting chamber as a comparison. There was a linear dependence between the algal concentration and the absorbance in all the performed experiments.

The concentration of total oxidising species was determined by using the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method. DPD is oxidized to form a red-violet product, the concentration of which is determined reflectometrically (ASTM 4500 G). Each sample was analysed three times, high repeatability was observed, with differences within 5% in all cases.

The electrical energy (E_r) required by the DE system, for a given algae removal R (between 0-1), was obtained by combining the Ohm's law with the kinetic equation:

$$E_r = \frac{I\,\Delta E_{cell}\,t}{V} = -\frac{I\,\Delta E_{cell}}{V}\frac{\log(1-R)}{k_{App}} \quad (3)$$

Where: I (A) is the applied current; ΔE_{cell} (V) is the cell voltage; t (h) is the time and V (m^3) the volume of the reservoir.

3. Results and Discussion

Figure 4 shows the semi-logarithmic trend with time of the normalised chlorophyll-*a* inactivation during electrolyses under several applied current densities and flow rates. The statistics of the linear regression of data are reported in Table 2, where F is the ratio between-groups variance divided by within-groups variance and P is the statistical significance. Values of $P < 0.05$ indicate a significant relationship.

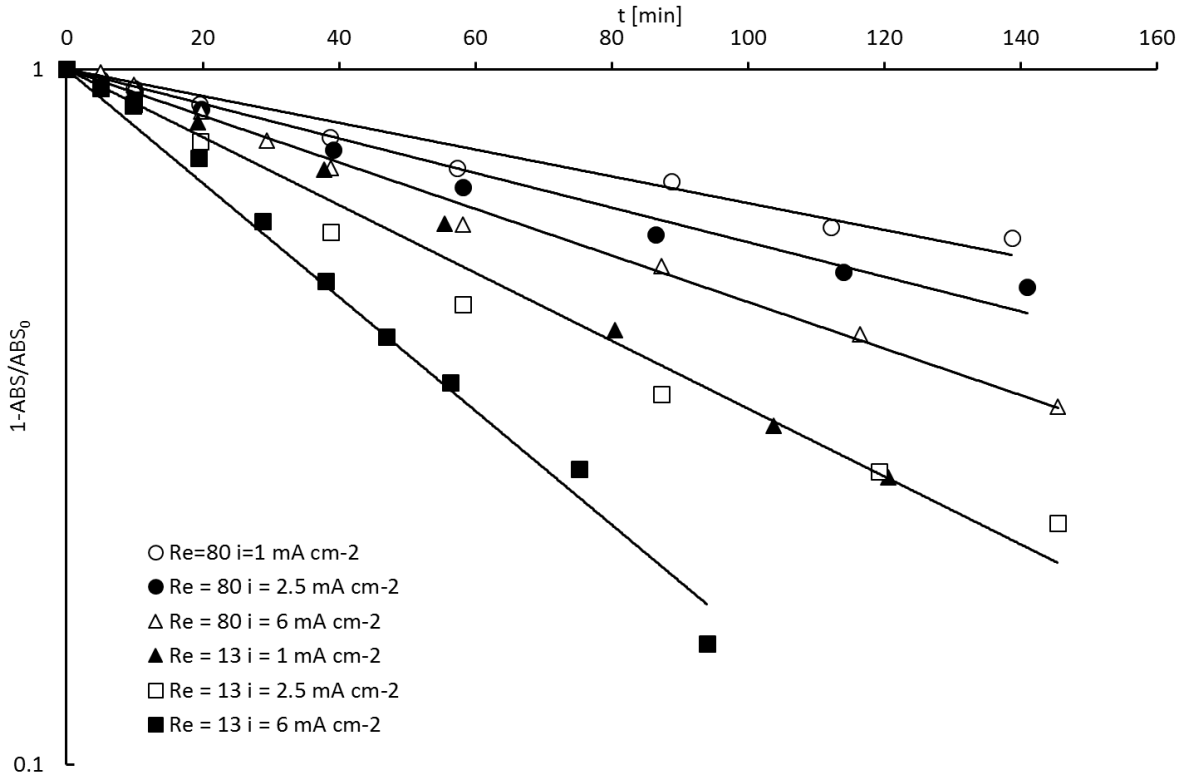

Figure 4. The semi-logarithmic trend with time of the normalised removal of chlorophyll-*a* during electrolyses under different experimental conditions.

Table 2. Values of the apparent kinetic constant (k_{app}) in the relevant experimental conditions for the linear regression statistics for the semi-logarithm of the chlorophyll-*a* concentration $\ln(ABS/ABS_0)$ versus time.

			Regression Statistics			Regression line	
Re	i [A m^{-2}]	ΔE_{CELL} [V]	F	P	R^2	k_{app} x 10^4 (±5%) [s^{-1}]	standard error
80	10	7.5	513.24	8.3E-08	0.992	0.73	1.96E-04
80	25	12.0	654.62	2.3E-07	0.995	0.95	2.24E-04
80	60	21.7	3358.49	8.7E-12	0.999	1.28	1.33E-04
13	10	7.4	1501.11	2.0E-08	0.998	1.82	2.81E-04
13	25	11.9	813.88	1.2E-07	0.996	1.87	3.94E-04
13	60	20.4	1900.49	8.5E-11	0.998	3.13	4.32E-04

The data suggest that the removal rate can be interpreted by a pseudo-first order kinetics, where the apparent kinetic constant k_{app}, calculated from the slopes of the curves in Figure 4, depends on both flow rate and applied current density. As reported, at a fixed current density the increase of Re values from 13 to 80 caused a twofold decrease in k_{app}. On the other hand, Figure 4 and Table 2 show that, for a set value of Re, the applied current density has a significant effect on the removal process. In particular, when the current density ranged from 25 to 60 A m^{-2}, an increase of 33% in k_{app} is observed at Re = 80, whilst the k_{app} increases by 65% at the lowest Re value. These results can be explained by considering the mechanism of microalgae inactivation and the geometry of the adopted system. As previously reported, under similar operating conditions here used, the algal inactivation is mainly attributed to the disinfection actions of oxidants electrogenerated by the DE system [8,25,27]. Moreover, the applied potential gradient can promote the formation of transient or permanent pores in the membrane wall of the algal cells, thus facilitating the attack of the electrogenerated oxidising species inside the cell [28,29]. This mechanism becomes significant for electric field gradient in the order of tens 10 V cm^{-2} when electrodes with pore size 4 orders of magnitude larger than microorganisms are used [30].

Taking into account the geometry of the system, three different zones can be identified: the anodic and the cathodic reaction zones, which correspond to the two electrode areas in the DE system; and the bulk zone, which comprehends reservoir, tubing and inert packing of the DE system.

The chemical and electrochemical reactions occurring in the anode zone during the electrolysis of water containing sulphates, lead to the formation of different oxidants, according to the following reactions:

$$H_2O \rightarrow OH^{\cdot} + H^+ + e^- \quad \text{(R1)}$$

$$OH^- \rightarrow OH^{\cdot} + e^- \quad \text{(R2)}$$

$$OH^{\cdot} \rightarrow O^{\cdot} + H^+ + e^- \quad \text{(R3)}$$

$$2OH^{\cdot} \rightarrow H_2O_2 \quad \text{(R4)}$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad \text{(R5)}$$

$$2O^{\cdot} \rightarrow O_2 \quad \text{(R6)}$$

$$O_2 + O^{\cdot} \rightarrow O_3 \quad \text{(R7)}$$

$$2SO_4^{-2} + 2OH^{\cdot} \rightarrow S_2O_8^{-2} + 2OH^- \quad \text{(R8)}$$

The oxygenated radicals desorbed from the BDD electrode surface (R1-R3) [10] react with water either in proximity of the anode or in the bulk zone, to generate oxygen (R5,R6) and other reactive oxygen species (ROS) (R4,R7) [31,32]. In addition, peroxydisulfates can also be generated (R8) [33]. In the cathode zone, along with the hydrogen evolution reaction, the reduction of oxidants may occur [8].

The presence of oxidants in the bulk zone during the galvanostatic electrolysis of AWW in the presence and absence of *C. vulgaris* was monitored. The detection of oxidants with the DPD colorimetric method used was, however, possible only for current densities higher than 40 A m^{-2} and Re equal to 13. Figure 5 compares the trend of the concentration of oxidising species as a function of time for runs carried out at Re = 13 and i = 40 A m^{-2}.

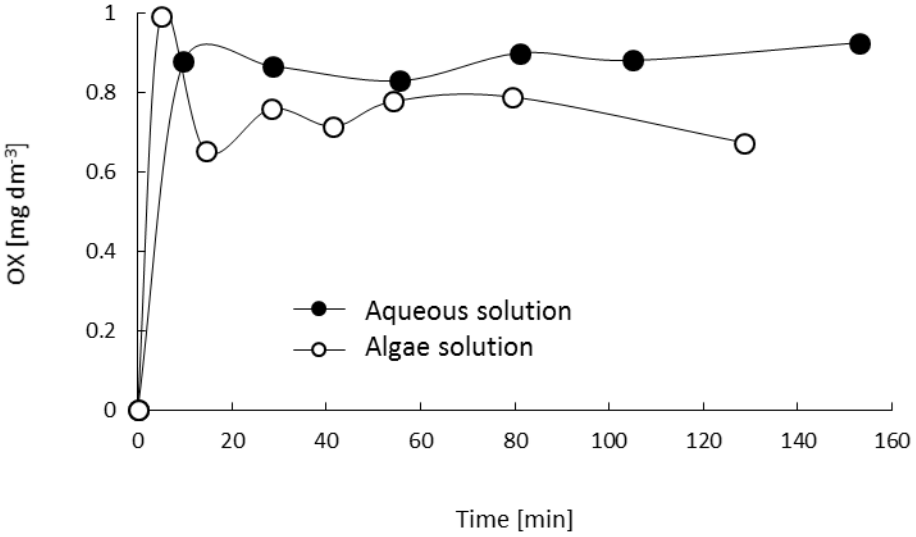

Figure 5. Trend with time of oxidants for batch-recirculating electrolysis at Re = 13 and i = 40 A m^{-2} in the presence and absence of *C. vulgaris* algae.

A pseudo-steady value in the concentration of oxidants was observed after few minutes of electrolysis. This pseudo-steady state is a result of the balance between anodic generation, cathodic reduction and spontaneous decay in the bulk solution. The concentration of oxidants is, however, very low, thus indicating that most of the oxidants generated are reduced in the cathode zone [34]. Since the difference in oxidant concentration in the presence and absence of algae was only marginal, we assume that the reaction of oxidants with *C. vulgaris* (i.e. the contribution of bulk disinfection to the removal process) was negligible in this case. Different results were obtained in a previous work where, with the same set-up but in the presence of 100 mg dm^{-3} of chlorides, nearly a threefold increase in the oxidants concentration was reached. Under these conditions the reaction with bulk oxidants, mainly constituted by active chlorine species, was the main responsible for the inactivation of *C. vulgaris* [10].

In a chloride-free electrolyte, the inactivation of microalgae is likely to occur in the anode zone, and it is due to a synergistic effect caused by the applied electric field and the very high concentration of oxidants in that area. As such, the value of k_{app} mainly depends on the electric potential, the concentration of oxidants, as well as on the hydraulic residence time in the cell.

Both the potential and concentration of oxidants within the anodic packed bed are controlled by the current density, i. Therefore, high values of i correspond to high values of the specific inactivation rate k_{app}, which increases with the residence time within the anodic zone, and therefore is inversely proportional to Re, as reported in Table 2.

Figure 6 reports the amount of energy, Er, required to remove 50% (Figure 6.A) and 75% (Fig. 6.B) of the initial concentration of *C. vulgaris*. Er was calculated by changing the value of R in Equation 3 and by using the values of cell potentials reported in Table 2 under different conditions of flow rate and current density.

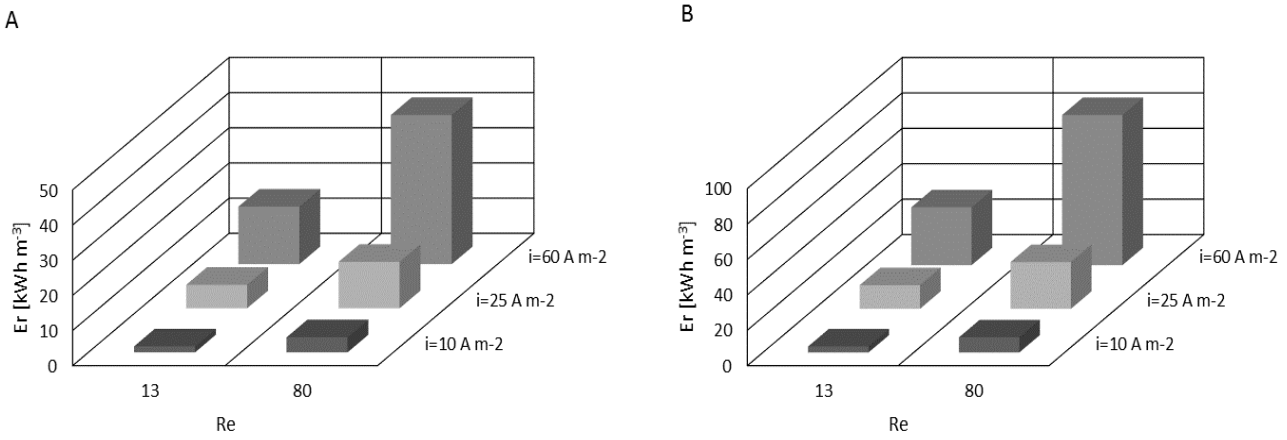

Figure 6. Electrical energy required by the DE cell for R= 0.5 (A) and R=0.75 (B) inactivation of the initial concentration (15 x 10^6 cells ml^{-1}) of *C. vulgaris*.

As it can be expected, the energy requirement is lower for Re = 13, where k_{app} reaches its maximum value, and at i = 10 and 25 A m^{-2}, characterised by relatively low cell potentials.

The applied current density and the flow rate chosen for the combined DE-MFC system were i = 25 A m^{-2} and Re = 13, corresponding to a 50% of the normalised removal of chlorophyll-*a* over an hour of operation. The outlet from the electrolysis unit was recirculated into a stack of MFCs hydraulically connected in series for a total of 3 days. The effect of two variables on the stack performance was investigated: the MFC characteristic length along the direction of the flow, which affects the surface area of both the anode and the cathode; and the number of MFC devices in the stack. In particular, two lengths were tested, 4 mm and 8 mm, leading to a surface area of both electrodes of 0.16 and 0.32 cm^2 respectively, and two different MFC stacks were investigated, one made up of three devices and the other of five.

The MFCs were enriched individually, with anaerobic sludge and AWW containing acetate as carbon source, and hydraulically connected in series after approximately one week when a steady state current was observed. Polarisation tests performed after one week of operation reveal a peak power output of 0.064 ± 0.003 µW cm^{-2}, for an external load of 250 kΩ, for the case of a surface area of 0.16 cm^2. For the fuel cells with an electrode surface area of 0.32 cm^2, the

maximum power was an order of magnitude higher, 0.293 ± 0.005 μW cm^{-2}, with an external load of 45 kΩ. The different value of optimal R_{ext} observed for the specific MFC design, was applied to the respective fuel cell prior to assembling them in a stack. Once integrated in the closed-loop circuit, the MFCs were fed with the algal solution from reservoir 1 (Figure 3). Although the DE unit was activated over the first hour of operation only, the cells were also run during the subsequent three days. After that, the MFCs were disassembled and a new batch of experiments was performed.

The performance of the MFCs stack in terms of electricity generation over three days was also investigated. Figure 7 shows the change in the power density with the time for both the three-MFC stack and five-MFC stack, with the electrodes surface area of 0.32 cm^2.

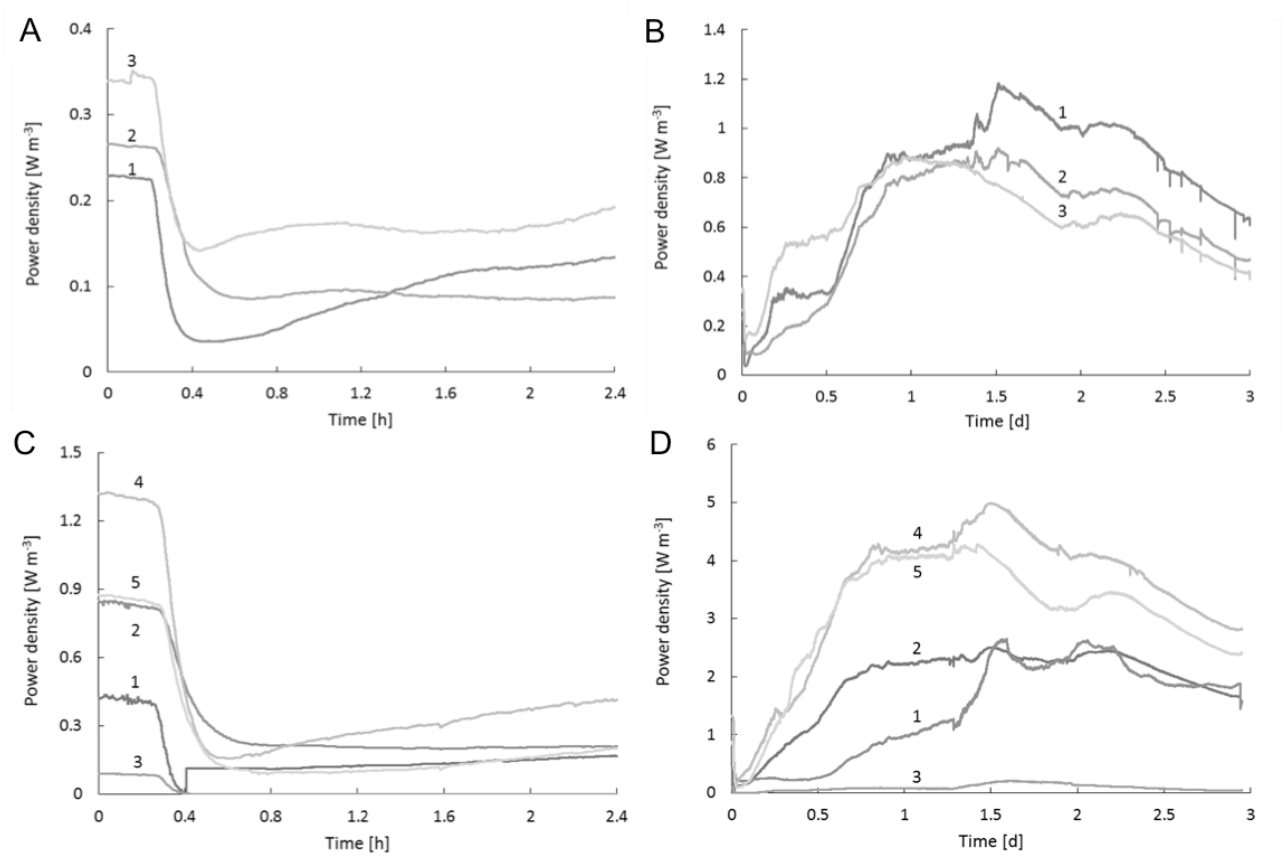

Figure 7. Power density output with time for each individual microbial fuel cell in the stack, over approximately the first 2.5 hours (A, C) and three days (B, D) of operation. Comparison between the case of the three-MFC stack (A, B) and the five-MFC stack (C, D). Numbers indicate the position of the MFC in the stack with 1 being the first MFC along the direction of the flow. The surface area of the electrodes was equal to 0.32 cm^2.

After the first 15 minutes of operation, a decrease in the power output was observed. This power drop was attributed to the drastic change in the COD value of the feeding solution, which decreased from value of 2200 mg L^{-1} (artificial wastewater containing acetate and anaerobic sludge) to only 35 mg L^{-1} (artificial wastewater with an initial concentration of *C. vulgaris* equal to 15 x 10^6 cells ml^{-1} in the DE unit inlet). On the other hand, the conductivity (810 ± 50 µS) and the pH (7 ± 0.5) were the same for both the AWW and the algae solution. Therefore, the power drop was associated only to the change in the concentration of organic carbon in the new feeding solution, and to the switch of fuel from acetate to the organics released from the broken cells, which might be complex molecules, more difficult to digest. Moreover, during this initial period, it is likely that the feed into the MFCs would be still characterised by a large amount of unbroken cells. On the one hand, this would mean a low concentration of organic carbon in the feed, on the other hand the living algal cells might inhibit the metabolism of the anodic bacteria, thus further decreasing the output power [35]. Power outputs close to zero have been previously observed when bacteria-enriched MFCs were suddenly fed with fresh algae cells, thus confirming this hypothesis [20].

Once the electrolysis unit was discarded (i.e. after 1 hour of operation), the power output started to increase, reaching a peak after approximately one day of operation, and then it slowly decreased.

For the case of the three-MFC stack (Figure 7A, and 7B), no marked difference in the performance of each fuel cell was observed. The peak power density was of 1 W m^{-3} with a 0.17% of variation. In the case of the five-MFC stack, the performance of the last fuel cells along the cascade, MFC4 and MFC5 (Figure 7C, and 7D) was different. The peak power in this case was of 5 and 4.11 W m^{-3} for MFC5 and MFC4, while for MFC1 and MFC2 it was respectively of 2.5 and 2.7 W m^{-3}. Note that the poor performance of MFC3 was caused by heavy leaking during the experiment.

Winfield et al. reported the effect that different organic loads have on the behaviour of the individual MFCs in a continuous-flow cascade system [36]. Usually, for easy-to-digest organics, the MFCs down the chain perform worse than the MFCs positioned at the beginning of the cascade, due to fuel depletion. On the other hand, the better performance of MFC4 and MFC5 in this case might be attributed to the fact that the first cells in the cascade help with the breaking down and release of organic molecules and relative metabolites from the algae cells, thus leading to an increased amount of available and easy-to-digest organic source to the last MFCs along the

cascade [21]. According to the results obtained, it seems that, when only three MFCs are used, this phenomena is not as marked as in the case of five cells.

Overall, the power generated by the MFCs with the larger electrode surface area was higher. This might be due to the corresponding increase in the retention time (14.4 seconds versus 7.2) and, consequently, to a better digestion of the algal cells and in turn an increase the amount of ready-to-be oxidised organics for the anodic biofilm of the MFCs down the chain. The active surface area of the electrodes influences the performance of the MFCs [16,37–39]. For the three-MFC stack, when the electrode surface area was of 0.16 cm^2 the average power output was 82% lower than when the surface area was double. In the miniature MFC devices used in this study, the increase in the electrode surface area while keeping constant the cross sectional area produced a decrease of the diffusion resistance [16]. According to the results obtained, this improvement in the mass transport has a benefit on both the algae treatment and the energy production.

Figure 8 reports the average change in the COD value of the recirculating solution with the time. As shown, a peak of COD (92.5 ± 60.5 mg L^{-1}) was observed after the first hour of operation caused by the electrolysis of the algal cells by the DE unit. The COD then stabilised to 55 mg L^{-1} for approximately 1.5 days and then slowly increased. This increase with the time is probably caused by the release of organics and metabolites due to the bacterial action in the MFC. Although this trend in the COD concentration was highly reproducible for each MFC stack studied, the stack with the MFCs with the electrode surface area of 0.32 cm^2 led to COD values approximately 1.3 times higher (69 and 91 mg L^{-1} for the three- and five-MFC stack respectively).

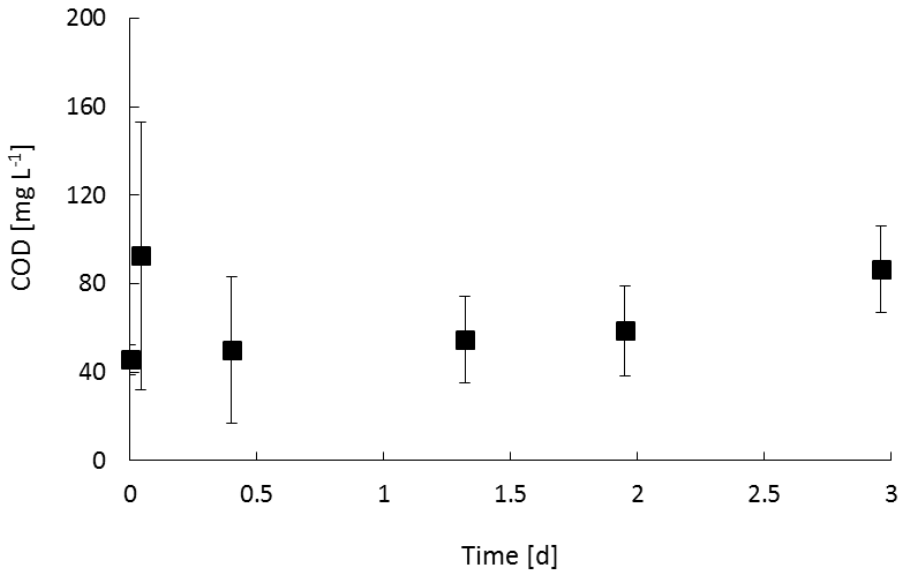

Figure 8. Average values of the COD for the recirculated electrolysed *C. vulgaris* algae solution in the stacks of three and five MFCs for both the electrode areas tested.

Figure 9 shows the total energy generated by the MFC stacks, calculated as the mathematical sum of the cumulative energy produced by each MFC unit in the specific stack configuration.

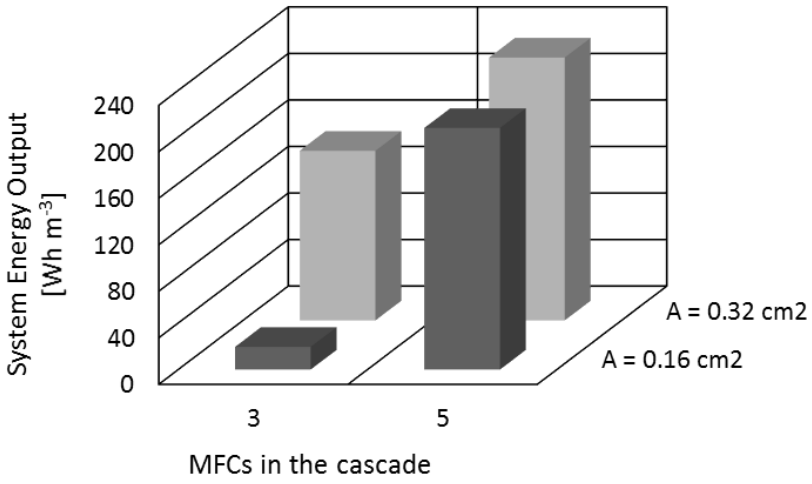

Figure 9. Total cumulative energy generated by the several MFC stacks investigated in this study.

As shown, the increase on the number of MFCs in the stack leads to higher power output levels for the case of the two anodic surface areas investigated. The use of sequentially positioned MFCs may maximise the oxidation of the organic matter [40] and in turn, an increase of the power production can be expected. The most significant increase (11 times the initial value) in the energy

output was observed when the smallest electrode area (0.16 cm^2) was employed. A much lower, but still a positive, effect of increasing the number of MFCs in the stack was also observed when the electrode surface area was 0.32 cm^2.

3. Conclusions

This study intends to provide a cost-effective and green solution to the treatment of algae contaminated water systems.

An innovative approach, based on the integration of a plug-flow electrochemical reactor with a stack of miniature air-cathode MFCs, is proposed. This integrated system allows the simultaneous treatment of algal biomass in wastewaters and energy generation. With our work, we not only demonstrate the effectiveness of such approach, but, with the aim of guiding on the design of such systems, we also investigate the effect that key features of the MFCs stack have on performance.

The lower energy demand of the integrated system leads to an energy cost of 0.9 € m^{-3}, which considering an energy price of 0.134 €kWh (Eurostat 2014), is 50% less than the operating cost of the single DE unit. Moreover, the generated cumulative power output of up to 226 Wh m^{-3}, when the MFCs were fed with the electrolysed algae over a period of three days, allowed to furtherly reduce the electrical energy consumed by DE reactor of about 20%.

These results are encouraging: by further improving the design of the MFC stack, a self-sustainable process could be obtained. Current water treatment systems are unsustainable, as they require consistent amounts of energy. This great energy demand not only has a high impact on the economy of our cities, but, considering that it is currently addressed by fossil fuels, it also has important environmental consequences. As such, our work can help to transform wastewater from an energy issue to an energy source.

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