Algal biomass as fuel for stacked-MFCs for profitable, sustainable and carbon neutral bioenergy generation.

Y. Asensio^a, C.M. Fernandez-Marchante^a, J. Villaseñor^b, J. Lobato^a, P. Cañizares^a,
M.A. Rodrigo^{a,*}

^a Department of Chemical Engineering. Faculty of Chemical Sciences & Technologies.
Universidad de Castilla La Mancha. Campus Universitario s/n 13071 Ciudad Real. Spain.

^b Department of Chemical Engineering. ITQUIMA. Universidad de Castilla La Mancha.
Department. Avenida Camilo José Cela S/N. 13071 Ciudad Real Spain

Abstract

BACKGROUND: This work compares the performance of three stacked Microbial Fuel Cells constructed with different number of single-MFC (MFC₁ with two stacked-MFCs, MFC₂ with ten stacked-MFCs and MFC₃ with twenty stacked-MFCs), and operated under the same conditions for one month.

RESULTS: According to results, algae suspensions can be used as fuel of MFC-stacks, although current efficiencies obtained are low. In comparing the effect of number of cells stacked on the performance of the stacks, it was found that the higher the number of cells stacked, the higher was the energy harvested from algae. However, because of the very efficient consumption of COD in the first MFC of the stacks (not only by electrogenic but also by non-electrogenic microorganisms) and the sequential circulation of the fuel through the different cells of the stack, in all cases the systems were run out of fuel and this was reflected in a lower production of electricity, as compared to that expected taking

into account the number of cells stacked. Results obtained from the polarization curves and the cathodic oxygen consumption also support this explanation.

CONCLUSIONS: Results demonstrate that algal biomass is a suitable fuel for energy generation using MFC technology and provides microorganisms not only of a carbon source but also with the required nutrients. However, the low coulombic efficiencies obtained in the three stacks indicate that feeding algae to MFC also promotes the formation of an important amount of non-electrogenic microorganisms that compete successfully with bioelectrogenic microorganisms for the substrate provided.

Keywords

Stack; microbial fuel cell; carbon felt anode; wastewater; algae

Highlights

- Algal biomass is suitable as fuel for MFCs, providing not only carbon source but also of nutrients
- Under fuel limitations energy generation does not increase linearly with the number of MFC stacked.
- Algae fuel leads to low current efficiencies because of the promotion of non-bioelectrogenic cultures of microorganisms
- Cathodic oxygen consumption also informs about energy production limitations cause by the run out of fuel

*author to whom all correspondence should be addressed: manuel.rodrido@uclm.es

1 Introduction

Microbial fuel cells (MFC) are bio-electrochemical devices, which can directly transform the chemical energy contained in the organic matter, into valuable electric current, with the aid of biocatalytic reactions carried out by electroactive bacteria¹⁻⁴. The sustainable energy generation provided by these bio-reactors has drawn the attention of the scientific community in the last decades⁵⁻⁷, because the further development and improvement of this technology could be a partial solution for the increasing levels of greenhouse gases in the atmosphere⁸. However, the obtainable output energy by MFC technology is still very low for practical applications, considering that only a maximum theoretical value of 1.1 V could be obtained by a single MFC system⁹⁻¹¹. Because of this, the development of stacked-MFCs is needed for larger amount of output energy produced by this biotechnology¹²⁻¹⁴.

In the last years, MFC and algae technology have been combined among them, attending not only to the carbon-neutral electric power generation but also to the possibility of obtaining a self-sustainable source of energy¹⁵⁻¹⁸. The ability of algae cultures, to produce biomass only from the Sun and CO₂, and to release oxygen by photosynthetic process, make them very interesting for being used as fuel of MFCs^{19, 20}. The released oxygen could be used as electron acceptor in the cathode compartment, increasing the sustainability of the technology, and decreasing the operational costs because no mechanical aeration would be required²¹⁻²³. At the same time, the produced algal biomass could be used as organic fuel for the microorganism cultures hosted in the anode compartment^{21, 24}. Attending to both possibilities, different approaches to self-sustainable MFCs have been studied by different authors²⁵⁻²⁷. Nevertheless, algae biomass is a complex substrate with a composition mostly based on oils, pigments and

carbohydrates with poor biodegradability, which would yield lower total energy generation as compared to the most used synthetic fuels fed in the anode compartment of MFCs (f.i. glucose or acetate). Another important drawback of using algae biomass as fuel is that they may lead to accelerate membrane and electrode biofouling, due to the occurrence of particulate organic compounds, which may gradually increase the internal ohmic resistance of the bio-device and the mass transfer limitation between the biofuel and the attached anode biofilm. Different solutions should be developed to mitigate this undesirable effect, either by preventing its appearance, or by combating it once it occurs. They are necessary for the design and long-term operations of stacked-MFC and the implementation to real applications.

The obtained output energy using algal biomass is still low^{21, 24, 28, 29}. This means that more basic research should be carried out to observe the behavior of single MFCs and small-scale stacked-MFCs fueled by algal biomass, identifying the critical steps and seeking for solutions. The aim of this work is to evaluate the performance of three stacked-MFC systems, MFC₁ with two cells, MFC₂ with ten cells and MFC₃ with twenty cells, fed with a raw algae culture of “*Chlorella vulgaris*” and connected in parallel to avoid voltage reversal and maximizing the output electric current of the different stacked-MFCs. Cells in MFC₁ and MFC₂ were fully hydraulically connected, whereas MFC₃ was hydraulically divided in two semi-stacked-MFCs of ten modules each (MFC₃-A and MFC₃-B) in order to decrease the internal pressure of the system, as shown in Figure 1.

Figure 1. Details of the hydraulic and electric connections in MFC₃.

The stacks were operated for more than one month, period in which they reach a steady state in all parameters monitored and values of the different parameters were compared. In addition, polarization curves and electrochemical impedance spectroscopies (IES) were carried out during the long-term operation to monitor the most relevant electrochemical parameters.

2. Materials and Methods.

2.1. Experimental set-up.

The setups used in this research consisted of two stacked-MFCs (MFC₁), ten stacked-MFCs (MFC₂), and twenty stacked-MFCs (MFC₃). All the single-MFCs that formed all the different stacked-MFCs consisted of two chambers (4 cm³ volume each one) separated by a proton exchange membrane, PEM (Nafion-117, DuPont). MFC is formed by two phenolic resin plates, due to its chemical resistance, and two silicon plates to improve the mechanical properties and avoid liquid losses. Carbon felts (KFA10, SGL Carbon Group[®]) were used as electrodes in both chambers (7 cm² each one). The space between electrodes was minimized in order to reduce as much as possible the internal electrical losses from the system. The electrodes were parallelly connected during the

experiments and an external resistance (R_{ext}) of $120\ \Omega$ was used for each stacked-MFC. The stacked-MFCs studied in this work were operated simultaneously in semi-continuous mode and at room temperature ($23 \pm 2^\circ\text{C}$). The cathode compartments were connected to a water reservoir of $100\ \text{cm}^3$ and a peristaltic pump was used to circulate an HCl solution (pH 3) from the reservoir to cathode compartment to avoid low concentration of protons that could decrease the electrical efficiency of the biological reactors. A fishery compressor was placed in the cathode reservoir to provide oxygen to the solution, the flow rate was $1.6\ \text{L min}^{-1}$. The anode compartments of each of the stacks were connected to $100\ \text{cm}^3$ algae reservoirs by peristaltic pumps. Everyday, $33\ \text{cm}^3$ of the mixed liquor of these tanks were replaced by fresh algae.

2.2. Inoculum and substrate.

2.2.1. Activated sludge.

Activated sludge from wastewater treatment plant (Ciudad Real, Spain) was used as inoculum for the anodic compartment. The activated sludge was preconcentrated by sedimentation and then, it was placed in the anodic compartment in a 1:2 ratio ($33\ \text{cm}^3$ of preconcentrated activated sludge and $66\ \text{cm}^3$ of raw wastewater) in anaerobic conditions to favor the growth of electroactive bacteria. During these days, no organic fuel was added to the microorganisms culture.

2.2.2. Algal biomass production.

Chlorella Vulgaris, purchased to Spanish bank of algae (Spain), was used as initial seed, and a 2 liter tank was used to produce the raw alga suspension. It was prepared in Bold Modified Basal freshwater nutrient solution ($0.220\ \text{g L}^{-1}\ \text{NaHCO}_3$, $0.075\ \text{g L}^{-1}\ \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.175\ \text{g L}^{-1}\ \text{K}_2\text{HPO}_4$, $0.125\ \text{g L}^{-1}\ \text{NaNO}_3$, $0.125\ \text{g L}^{-1}\ \text{NH}_4\text{Cl}$, $0.075\ \text{g L}^{-1}$

¹ KH_2PO_4 , 0.025 g L^{-1} $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05 g L^{-1} EDTA, 0.025 g L^{-1} NaCl , 0.031 g L^{-1} KOH , 0.01142 g L^{-1} H_3BO_3 , 0.00498 g L^{-1} $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.00882 g L^{-1} $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.00176 g L^{-1} $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.00157 g L^{-1} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). This bioreactor was operated during all the experiment in semi continuous mode, being fed once a day with 117 mL of Bold's basal medium. In addition, this bioreactor was under a light/dark cycle of 12/12 h, to maximized the biomass production rate and enriched the biomass concentration. Light was applied using Light Emitting Diodes (LED) to ensure that algae cells were under illumination during the light cycle. Algae culture was stirred to prevent sedimentation of the algae and to ensure that all cells of the population were exposed to the light and nutrients and to improve the gas exchange between the culture medium and the air.

2.3. Experimental procedure.

A digital multimeter (Keithley 2000 multimeter) was connected to the external electric circuit to monitor continuously the cell voltage of each stacked-MFCs at the value of the external resistance (120Ω). Chemical oxygen demand (COD) in the output effluent was evaluated using a Velp ECO-16 digester and a Pharo100 Merck spectrophotometer analyzer. Total Suspended Solids (TSS) were estimated gravimetrically, by evaluating the difference in weight of $0.45 \mu\text{m}$ glass microfiber dried filters (Prat dumas, France), before and after filtering a volume of 10 mL of re-suspended anodic reservoir bulk solution. Filters were dried for 1 day at 105°C . Total Organic carbons were measured along the experiments with a multi N/C 3100 (Alitik Jena) analyzer. The analysis have been done to the filtered samples. The pH, conductivity, and dissolved oxygen were measured with a GLP22 Crison pH meter, a Crison Cm 35 conductivity meter, and an Oxi538 WTW oxy meter, respectively. Polarization curves were carried out in all the stacked-MFC. Three important parameters were evaluated, open circuit voltage (OCV),

the maximum intensity and the maximum power density of all the stacked-MFCs. Electrochemical impedance spectroscopy (EIS) were performed using frequencies between 10 mHz and 10 KHz at open circuit conditions. Individual resistances were obtained by equivalent circuit fitting analysis of the resulting data, using NOVA 1.1 software integrated in the potentiostat. All electrochemical measurements, polarization curves and EIS experiments, were carried out on an Autolab potentiostat/galvanostat (PGSTAT-302N).

3. Results and discussion.

3.1 Treatment capacity of the MFCs

In studying the behavior of MFCs, it is important not to focus only on the production of energy, but also to monitor other parameters, which can give valuable information. Figure 2 shows the changes in concentration of total suspended solids, pH and conductivity in the tanks connected to the anode compartments of the three stacks studied in this work. In the case of the largest stack, there were two tanks instead of only one, each of them connected to a series of ten MFCs. As it can be observed, there are no significant differences between the changes obtained in the different setups. SST decreases down to a value of nearly 3.0 g L^{-1} , while conductivity increases up to 1400 mS cm^{-1} . This means that electrode surface (directly related to the number of MFC modules) does not influence on both volumetric parameters, which seems to be only affected by the fixed hydraulic residence time (HRT).

Regarding pH, it can be seen that it increases slightly during operation time from 7.0 up to almost 8.5, which is slightly higher than the value obtained using other synthetic fuels, as acetate^{30, 31}. This value has to be explained in terms of the nature of the compounds contained in the algal biomass. However, no differences were found between the different

stacked-MFCs, indicating that number of cells contained in the stack has not a substantial influence on this parameter.

Figure 2. Changes in the TSS (part A), conductivity (part B) and pH (part C) of the anode compartment. Operational conditions: carbon felt as anode and cathode electrode material. (■) MFC₁ (▲) MFC₂, (●) MFC₃-A, (◆) MFC₃-B.

In evaluating the wastewater treatment capacity of a stacked-MFC, two parameters are worth to be monitored: TOC and COD concentration in the outlet of the stacked-MFC (effluent). Figure 3 shows the soluble carbon and the total COD fed to the three different stacks. From the very first moments, there is an almost total consumption of the TOC and COD in the bio-electrochemical systems, which does not depend on the number of cells stacked. Hence, all the stacks run out of fuel and this demonstrates that the algae suspension fed (more precisely the organic and biodegradable compounds contained in the algal biomass) are a good fuel for this type of biological reactors. In addition, this depletion indicates that the suspension contains all the nutrients required by the microorganisms contained in the MFCs for the oxidation of the organic matter. In addition, after twenty days of continuous operation, values of COD and TOC in the effluent were completely stabilized, with not very large fluctuations in their values.

Figure 3. Changes in the soluble carbon fractions (part A) and total COD (part B) in the anode compartment. Operational conditions: Carbon felt as anode and cathode electrode material. (■) MFC₁ (▲) MFC₂, (●) MFC₃-A, (◆) MFC₃-B, (x) Feed.

3.2 Production of electricity

The electricity produced increases with the number of cells contained in the stack and, hence, with the electrode surface area. This can be clearly seen in Figure 4, where the daily cycles of electricity production in each stack during a 30-day period are shown. As it can be observed, after the daily feeding of the stacks, current increases up to a maximum from which it decreases down to nearly 0. During the first operation days, these cycles were not observed and the acclimation time required in the three cases was almost the same: about 10 days. This acclimation period is in agreement with the values found in previous works of our group³².

In comparing the electricity produced in the three cells, it can be stated that maximum currents depend on the number of cells but not in a linear way. Thus, the stack with two cells produced a current peak of 3 mA, the stack with 10 cells of nearly 4 mA and the stack with 20 cells reached 5mA. This means that scale-up is not linear in terms of stacking and that complex processes are happening in the cells, which minimizes the expected additive effect of the stacking on the production of electricity. The total charge produced is plotted in the secondary y-axis of each graph. Maximum charge produced are

25 mA h in the stack with 20 cells, while the minimum value is for MFC₂ achieving a value of 15 mAh. This unexpected result is not caused by a bad performance of MFC₂ but to the faster starting up in the production of electricity in MFC₁, which started on day 5th instead of day 10th as it was the case in MFC₂ and MFC₃. If only data from day 10th on are considered, then the total amount of electricity (mAh) yielded by the three stacks increase in the sequence MFC₃>MFC₂>MFC₁, as expected according to the previous discussion.

Figure 4. Generation of electric current (♦) and total charge produced (■) by the stacked-MFCs. Operational conditions: Carbon felt as anode and cathode electrode material. MFC₁ (part A), MFC₂ (part B), MFC₃ (part B).

This point can be better seen in the polarization curves obtained at different times during the study. Figure 5 shows the changes during the operation time in the three characteristic parameters of these plots. As it can be seen, because of the connection in parallel, the Open Circuit Voltage (OCV) reached similar values in the three stacks studied regardless of the number of cells connected in each stack and it stabilizes in the nearness of 840 mV. The maximum intensity and power produced by each stack increases with the number of cells connected, although in a lower ratio than expected according to the number of cells. This lower increase indicates that it is not the number of cells which is limiting the production of energy, but the fuel fed into the MFC. It is the same observation made in Figure 3 with the electricity produced during operation.

256

257 **Figure 5.** Maximum open circuit potential (part A), maximum electric current (part B)
 258 and maximum power measured (part C) in the stacked-MFCs during tests. Operational
 259 conditions: Carbon felt as anode and cathode electrode material. (■) MFC₁ (▲) MFC₂,
 260 (♦) MFC₃.

261

262 The hydraulically connection of the anode and cathode tanks improves the wastewater
 263 treatment capacity of the stacked-MFCs, as seen in Figure 2 but, attending to the obtained
 264 results, it is not the right choice to improve the energy generation, due to the continuously
 265 decrease of the fuel concentration while pass through each single MFC modules that
 266 formed the different stacked-MFC. Hence, a higher number of cells allows to use more
 267 efficiently energy, but not to produce a linear increase in the energy yielded.

268 According to the COD consumed and to the electricity produced an efficiency can be
 269 calculated for each stack using equation 1.

$$270 \quad \eta = \frac{j \left(\frac{\text{mA}}{\text{m}^2} \right) \text{A (m}^2\text{)}}{r \left(\frac{\text{mg COD}}{\text{L d}} \right) \text{V (L)} \left(\frac{1 \text{ mmol COD}}{32 \text{ mg COD}} \right) \left(\frac{4 \text{ mmol e}^-}{1 \text{ mmol COD}} \right) \left(\frac{96500 \text{ mC}}{1 \text{ mmol e}^-} \right) \left(\frac{1 \text{ d}}{86400 \text{ s}} \right)} \quad [1]$$

271 This efficiency informs indirectly about the ratio between electrogenic and non
 272 electrogenic microorganisms, because only the electrogenic microorganisms can
 273 transform COD into electricity. To calculate this efficiency for the three stacks, data of
 274 the last two weeks of operation (fourteen days) were used, because in this case the system
 275 was operated at steady state and results were 0.01, 0.02 and 0.04% for MFC₁, MFC₂ and
 276 MFC₃, respectively. These low values point out the high COD consumption rate of the

stacked-MFCs compared to the achieved electric current of each stack and indirectly informs about the existence of large populations of non-bioelectrogenic microorganisms in the three stacks used. This means that although algae biomass can be used to produce electricity in MFC, it is not as good fuel as other organic substrate such as acetate, which allows to obtain current efficiencies more than 2-3 log-units higher.

Reaction in the cathode is also important when characterizing the behavior of single MFC, so it is also expected to be important for stacked-MFCs. To characterize it, the oxygen consumption rate was measured at different times during the study by stopping aeration and measuring the slope of the dissolved oxygen decay with time. As it was described in previous works^{33, 34}, the oxygen contained in the cathode compartment is consumed and transformed into water through the well-known equation 2:

Depletion rates of oxygen are shown in Figure 6 and they are in agreement with those shown in previous Figures. Differences between the stacks are not as large as they should be according to the different number of cells contained, which is in agreement with the limitation of the performance by the amount of fuel fed. Anyhow, there is a higher consumption of oxygen on the cathode, which means that electroactive microorganisms are performing better in the systems with larger electrode surface.

Figure 6. Oxygen consumption rate (part A) and pH (part B) splitting between the influent and the effluent in the cathode chamber of the stacked-MFCs. Operational conditions: Carbon felt as anode and cathode electrode material. (■) MFC₁ (▲) MFC₂, (♦) MFC₃.

Equation (2) also shows that there is an influence on the pH, as hydroxyl ions are consumed. This consumption lead during operation to an increase in the pH, which was corrected daily as it is shown in Figure 5b

Internal resistance directly affects energy generation by MFCs. Furthermore, this internal resistant became an important parameter to maximize the output energy due to the addition of the difference internal resistance of each module (as individual resistance due to activation kinetics, charge transfer, mass transport effects and ionic resistance) which conformed the whole stack³⁵. Figure 7 shows EIS carried out to each of the three stacked-MFCs in different days of the long-term tests.

Figure 7. Electrochemical impedance spectroscopy (EIS) measured in the stacked-MFCs during tests(■) day 9 (▲) day 17, (♦) day 32. Operational conditions: Carbon felt as anode and cathode electrode material. MFC₁ (part A), MFC₂(part B),MFC₃(part C).

As it can be observed, the electrolyte resistance was almost the same for each device (6.9 Ω for MFC₁, 6.6 Ω for MFC₂ and 6.27 Ω for MFC₃), and this can be easily explained because the same electrolyte was used in each stacked-MFC and, as already shown in this

discussion, not great changes are observed in the characteristics of the liquid recirculated through the three stacks. This is not the case of the charge transfer resistance, which shows a significant decrease over time in each stacked-MFC due to biofilm formation on the anode surface. This decrease indicates the catalytic role of this biofilm in the electron transfer. Nevertheless, higher charge transfer resistance were found for MFC₁ (32.5 Ω) than for MFC₂ and MFC₃ (18 Ω and 3.1 Ω , respectively), which should be explain in terms of the different fluid-dynamic conditions of the three stacks. Higher MFC modules increase the internal pressure of the bio-devices reducing the fuel flow through the following MFC modules. Thus, biofilm undergoes less turbulence stress leading to a better microorganism distribution on the electrode surface and this fact may explain the better values found for the charge transfer resistance.

4. Conclusions

From this work, it can be concluded that algae biomass could be used as fuel of MFCs, providing microorganisms not only of a carbon-source but also of the nutrients required for their metabolism. This fuel produces important amount of energy but the sequential feeding of the cells contained in each stack lead to its complete exhaustion under the operation conditions used. For this reason, although the energy harvested increases with the number of cells stacked, this increase is not linear because MFCs contained in the stack run out of fuel. Data obtained from the polarization curves and the cathodic oxygen consumption supports this explanation. On the other hand, the low coulombic efficiencies obtained in the three stacks indicate that feeding algae to MFC also promotes the formation of an important amount of non-electrogenic microorganisms that competes successfully with bioelectrogenic microorganisms for the substrate provided.

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