Influence of the methodology of inoculation in the performance of air-breathing microbial fuel cells.

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Abstract

In this work, four air-breathing microbial fuel cells (AB-MFC) were operated for 1 month in order to determine if the methodology of inoculation affects the steady-state performance of this type of MFCs. For this purpose, anaerobic and aerobic sludge were fed to two identical AB-MFCs without any external carbon source into a tight sealed environment during the first three days of start-up. For comparison purposes, other two AB-MFCs were operated mixing the initial sludge and an amount of sodium acetate as substrate. Results point out that the inoculation procedure does not affect the steady-state treatment capacity of the cells but it affects very seriously the production of electricity. Only the system fed with concentrated aerobic sludge was able to develop an efficient culture of bioelectrogenic microorganisms, while the other three systems failed in this purpose. This result was confirmed in a second independent series of experiments and by a last operation test, which consists of a re-inoculation of a low active MFC with microorganisms obtained from the best-performing cell in order to evaluate how much the MFC performance can be enhanced.

Keywords

Air-breathing microbial fuel cells; start-up; inoculation; acclimation

Highlights

Steady-state operation conditions in AB-MFC can be reached in less than one month

Only aerobic sludge can be successfully used as inoculum of AB-MFC

For AB-MFC, the inoculation procedure does not influence the steady-state wastewater treatment capacity

For AB-MFC the inoculation procedure influences strongly the steady-state production of electricity

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1. Introduction

Microbial fuel cells (MFCs) are bio-electrochemical systems (BES) that exploit the capability of exoelectrogenic bacteria to use the surface of an electrode (the anode) as final electron acceptor for their metabolism, establishing an appreciable electrical current [1] through direct contact or indirectly through electron shuttles. One of the most attractive features of this technology is the possibility to use a large variety of wastewater as fuel source [2], opening the way to development of energetically independent wastewater treatment plants (WWTP) [3]. However MFC technology seems to embed some intrinsic disadvantages such as high activation overpotentials and concentration polarization, high cathodic potentials and ohmic losses [4]. In order to limit the losses associated with the cathodic process, an uncountable number of different catalyst have already been tested [5], including biocathodes [6]. More difficult seems the way to achieve an advance into the anodic process, owing to the biological complexity of microbes [7,8].

In order to search for highly active or resistant exoelectrogenic bacterial community, a large number of inoculation strategies were tested over time by MFC researchers. Since most of the bacteria involved into exoelectrogenic activity are also capable of a dissimilative extracellular reduction [9,10], Wang et al. have developed a specific culture medium based on a simple phosphate buffered basal medium using acetate as electron donor and Fe(III)-oxide as electron acceptor [11]. After series of dilution of a biofilm sample taken from a pre-existing MFC, they operated another MFC obtaining better performance than the first one, which was simply inoculated with the sludge of an activated sludge unit. Ieropoulos et al. have compared two different acclimation procedures with four different inoculum sources: anaerobic sludge, aerobic sludge, river water and pure culture of *Geobacter sulfurreducens* [12]. The aerobic sludge and the river

water were incubated aerobically while the *G. sulfurreducens* and the anaerobic sludge, anaerobically. Although no practical differences were found in the fully developed MFCs bacterial communities during power densities test at 1.5 mL h⁻¹ flow rate, the aerobically incubated communities had a less pronounced decrease in power production when operated at 125 mL h⁻¹, meaning that the most of the electrical activity was due to the attached biofilm and not to the suspended planktonic culture. Wang et al. tried to develop the most suitable procedure to simultaneously produce power and degrade pentachlorophenol (PCP) [13]. Two enrichment procedures were tested feeding the MFCs with PCP and substrate either simultaneously or sequentially. All MFCs reached the same power production after 4 cycles, but the simultaneous addition of glucose and PCP brought to a faster acclimation of just 10 days due to the growth of a more stable exoelectrogenic culture. Tanikkul and Pisutpaisal demonstrated that an heat treatment at 100 °C for 1 h can efficiently be used in order to reduce the amount of methanogens bacteria present into an inoculum extracted from an anaerobic digester [14]. When compared with the MFC operated with the untreated inoculum, a higher current production was always recorded at a similar carbon depletion rate, meaning that a more efficient community was established. Methanogens depletion was also the target of another work in which authors compare heat shock protocol with other kinds of environmental shock, such as acid pH or ultrasonication [15]. In that case, an acidic pre-treatment at pH 5 brought to a MFC 7-fold more active in terms of power density produced and columbic efficiency with respect to the untreated control. Thermal treatment was less effective and led to a 3-fold increase of aforementioned electric figure of merits with respect to the control cell. It must be reported that an acidophilic pre-treatment was already employed by Mohan's research group, with an inoculum from a previously operating H₂ lab-scale bioreactor [16]. In that case, the MFC was capable to

simultaneously produce energy and hydrogen gas with power production more than doubled with respect to the same reactor operated in other works with different conditions. Inspired from what was already done for bio-hydrogen production [17], the same research group tested a 24 hour incubation of a sludge collected from the local WWTP in 50 mM 2-iodopropane solution. The MFC inoculated with the resulting community had a power production of 180.6 mW m^{-2} against the 128.3 mW m^{-2} of an identical MFC operated with a heat-treated inoculum and 92.1 mW m^{-2} of the control MFC.

In addition, some electrochemical methods were proposed to get a rich exoelectrogenic inoculum. Indeed, it is possible to use a potentiostat in order to induce bacteria to close their respiratory electron chain on electrode surface by imposing a fixed anode potential [18]. With this technique Wang et al. demonstrated that it was possible to reduce the start-up time of 24 days with respect to a control cell [19], while Srikanth showed that the electrode potential has also an effect on substrate degradation and dissolved solids removal [20].

Hence, it is possible to conclude that the inoculation step can drastically affect the performances of MFCs. In particular, it would be necessary to select some simple and cheap inoculation procedures that can allow to improve the power production of MFCs and the organic removal. In this context, the aim of this work is to explore the potentiality of simple and inexpensive initial operations to achieve an efficient bacterial selection, favouring the exoelectrogenic ones over the others. For this purpose, anaerobic and aerobic sludge were fed to some MFCs without any external carbon source into a tight sealed environment during the first three days of start-up. For comparison, other two reactors were operated mixing initial sludge and an amount of sodium acetate as carbon source. Subsequently, microbial samples from the best-performing cell were used to

inoculate another reactor to evaluate how much the performance of the new MFC can be enhanced.

2. Materials and methods

Experimental set-up. The MFCs studied were air-breathing cathode cells in order to avoid limitations by the availability of oxygen among other reasons reported in literature [21]. The MFCs consists of two chambers made in poly(methylmethacrylate) and separated by a Nafion proton exchange membrane (DuPontTM Nafion PFSA Membrane). The electrodes and the membrane were as close as possible to decrease the internal resistance. The anodic electrode was made of carbon felt, due to its good benefits [22] and properties (specific area of $35.3 \text{ m}^2 \text{ g}^{-1}$ and $17700 \text{ m}^2 \text{ m}^{-2}$, areal weight of 500 g m^{-2} , porosity of 95% and roughness of $30 \text{ }\mu\text{m}$), with an active area of 0.866 cm^2 and was located in an anodic chamber of 0.346 cm^3 . An auxiliary tank of 115 ml was connected to the anodic chamber and contains the suspended bacteria and the wastewater. The inlet stream was pumped to the anodic chamber with a flow rate of 3 ml min^{-1} by a multichannel Heidolph (PD-5206 Peristaltic Dosing Dispensing Pump Drive) and the outlet stream was recirculated to the tank. The active area of the cathodic electrode was also 0.866 cm^2 and was made of carbon paper (C2 Freudenberg) on which surface was deposited a load of Pt 0.5 mg cm^{-2} to enhance the cathodic reduction reaction [23] following the procedure reported in literature [24]. To close the external electrical circuit anode and cathode were connected by an external resistance of $120 \text{ }\Omega$.

Enrichment Procedure. In order to study the effect of the nature of initial sludge (aerobic or anaerobic sludge) and the enrichment procedure, four different MFCs were started-up simultaneously with the same materials and the same environmental conditions. The initial sludge was taken from the aerobic and anaerobic reactors, respectively, of the

Wastewater Treatment Plant of Ciudad Real (Castilla La Mancha), ~~more information~~ whose description can be found elsewhere [25]. The sludge was decanted for a day. Subsequently, an initial sludge solution was obtained by mixing 50% of decanted sludge and 50% of the supernatant wastewater to ensure the same initial sludge concentration without depending on the environmental conditions that can water or thicken the sludge. The daily basis is detailed in Table 1. It is important to highlight that after the enrichment time, the bacteria was treated in order to work under a sludge age of 2.5 days due the greatest results obtained in this condition [26]. To get 2.5 days of sludge age, 46 mL of the anodic liquid was removed and replaced by fresh wastewater every day of operation.

Table 1: Overview on the adopted acclimation protocols.

	Acclimatization procedure			
	A= MFC_100A.S	B= MFC_10A.S	C= MFC_50A.S	D = MFC_100N.A.S
Day/ Procedure	Start with 100% of aerobic sludge	Start with 10% of aerobic sludge	Start with 50% of aerobic sludge (intermediate case)	Start with 100% of anaerobic sludge
1 st	100 mL Total reservoir volume. 100 mL fresh <u>aerobic</u> sludge (50 mL decanted + 50 mL surnatant).	100 mL Total reservoir volume. 10 mL fresh <u>aerobic</u> sludge (Decanted) + 90 mL synthetic wastewater*.	100 mL Total reservoir volume. 50 mL fresh <u>aerobic</u> sludge (25 mL decanted + 25 mL surnatant) + 50 mL synthetic wastewater*.	100 mL Total reservoir volume. 100 mL fresh <u>anaerobic</u> sludge (50 mL decanted + 50 mL surnatant).
2 nd	50 mL withdrawn and replenish with fresh <u>aerobic</u> sludge (25 mL decanted + 25 mL surnatant).	50 mL withdrawn and replenish with synthetic wastewater*.	50 mL withdrawn. Replenishment with 25 mL of fresh <u>aerobic</u> sludge (12.5 mL decanted + 12.5 mL surnatant) + 25 mL of synthetic wastewater*.	50 mL withdrawn and replenish with fresh <u>anaerobic</u> sludge (25 mL decanted + 25 mL surnatant).
3 rd	As the 2 nd .	As the 2 nd .	As the 2 nd .	As the 2 nd .
4 th	Every cell starts standard feeding with the synthetic wastewater* and an Hydraulic Retention Time of 3 days (33 mL changed everyday).			

Materials. All chemicals were of analytical grade from PANREAC and Sigma-Aldrich and synthetic wastewater was prepared with MilliQ water. The feedstock consist of 12.08 g L⁻¹ ($\approx$ 7500 ppm of COD) of sodium acetate as carbon source. It was completed with many nutrients: 2.77 g L⁻¹ of sodium carbonate, 1.85 g L⁻¹ of ammonium sulfate, 1.11 g L⁻¹ of potassium dihydrogen phosphate, 0.92 g L⁻¹ of hexahydrate magnesium chloride, 1.25 g L⁻¹ of calcium chloride and 0.07 g L⁻¹ of ferric ammonium sulfate.

Analytical and electrochemical measurements. The daily output current density was calculated from the cell voltage measured by a Keithley 2000 voltmeter datalogger. Electrochemical characterization was carried out periodically by an AUTOLAB PGSTAT30. Polarization curves were started after leaving the MFCs in open circuit for two hours and the potential was varied at a scan rate of 0.001 V s⁻¹ from the OCV to 0.001 V. At the end of the experiment the cells were opened to further investigate the behavior of the attached biofilm through cyclic voltammetries. The anodic carbon felt was extracted and connected to a platinum wire into a common electrochemical cell, temperature conditioned at 25°C, housing a saturated calomel reference electrode (SCE) and a platinum counter electrode. A volume of 100 mL of fresh synthetic wastewater was used as electrolyte in every experiment, purged with N₂ gas for at least 15 min before operation. They were studied in a range of -0.6-0.3 V at a scan rate of 0.005 V s⁻¹. Coulombic efficiencies were calculated according to Logan et Al [27]. The COD removal was measured according to Standard Methods. Conductivity and pH were measured using a GLP22 Crison pH-meter. Suspended solids (SS) was monitored constantly by the difference of weight before and after vacuum filtration of 10 ml of a sample of the anodic reservoir using glass microfiber filters of 0.45 μ m previously dried at 105° C for 24 hours.

3. Results and discussions

3.1. Electricity production capacity

Reactors were operated as described in section 2 for one month. During first three days of operation, i.e. the acclimation period, reactors did not give a meaningful current density. This is an expected result since most of the bacteria present in a raw wastewater are not exoelectrogenic and their natural selection is a process that takes several replication cycles in which, in an oxygen deprived environment, they can prevail over the others because of their capability to use an electrode as final electron acceptor directly or by mediators [28]. Anyway, on the fourth day, as soon as the synthetic wastewater was fed to the reactor, the MFC_100A.S (involving 100 % of aerobic sludge) started producing an increasing current output (Figure 1). After 10 days the current density reached an almost stable value, meaning that the selection process was concluded.

Figure 1. A) Time course of the current density of MFCs: (◆) MFC_100AS; (▲) MFC_10AS; (□) MFC_50AS; (○) MFC_100NAS. Error bars represents the maximum and minimum values of current density achieved daily. B) Time required by the MFCs to start to generate electricity (■) and to operate under steady state (□). Average temperature: 23° C.

Considering the stationary phase, MFC_100A.S recorded an averaged value of 4.02 $A\ m^{-2}$ three log-units higher than what was achieved by MFC_10A.S (0.003 $A\ m^{-2}$), MFC_50A.S (0.001 $A\ m^{-2}$) or MFC_100N.A.S (0.004 $A\ m^{-2}$). The combination of low concentration of sludge and presence of carbon source leads to a fast removal of

electrogenic microorganisms from the bulk and to a no limitation for bacterial metabolism (i.e. a substrate surplus). This fact is reflected in the higher time required to exhibit electricity generation by MFC_10A.S and MFC_50A.S. In addition, observable electrogenic activity in the reactor inoculated with the anaerobic sludge takes longer, probably due to the higher competition between exoelectrogenic and methanogenic bacteria [29].

In order to check reproducibility of this behaviour, in particular for the comparison of the two extreme processes, two additional cells were started up and operated for 1 month operating at the same conditions than MFC_100A.S and MFC_10A.S. Results obtained in terms of daily exerted current density are shown in Figure 2, and they demonstrate that regardless the MFC are highly multiparametric systems, they can be considered also as robust systems showing always almost the same performance. Thus, cell operated under the same condition than MFC_100A.S stabilizes in a current density of 6.0 A m^{-2} while the cell operated under the same conditions than MFC_10A.S produces only 0.02 A m^{-2} .

Figure 2. Evolution of the current density of MFCs studied previously. (○) MFC started with low concentration of aerobic sludge. (●) MFC started increasing the initial concentration of aerobic sludge. Average temperature: 23° C.

Hence, the current across the external resistance reaches stationary values at the end of the first fortnight of operation, moment from which the systems can be considered stabilized. In Figure 3, the polarization curves registered for each of the four MFCs in this period are shown. Cell were left in open circuit for at least two hours in order to get the open circuit voltage (OCV). The best performing cell during MFC operation, the one inoculated with aerobic sludge under starving condition (MFC_100A.S), recorded an OCV of just 509 mV and the worse operating cells recorded always higher values: 809 mV for MFC_10A.S, 859 mV for MFC_50A.S and 859 mV for MFC_100N.A.S. Fuel mass transfer problems in the deeper layers of microorganisms might be the responsible for the decrease in the OCV of MFC_100A.S while the other MFCs show the theoretical value of OCV when operating with carbon felt as anode [30,31] as no electrogenic microorganisms grew up.

Figure 3. Polarization and power curves. (◆) MFC_100AS; (▲) MFC_10AS; (□) MFC_50AS; (○) MFC_100NAS. Data is an average of 3 polarization tests carried out in 3 consecutive days with a standard deviation in a range of $1.29 \cdot 10^{-4}$ (MFC_100AS) to approximately $3,34 \cdot 10^{-9}$ (MFC_10AS; MFC_50AS; MFC_100NAS).

The internal resistance (R_{int}) of the cell was calculated from the slope of the straight line interpolating the relation between potential and current on the E/CD plane [27]. The MFC fed only with the aerobic sludge (MFC_100A.S) had a R_{int} of $457 \, \Omega$, many order of magnitude less than the others: $2 \times 10^6 \, \Omega$ for MFC_10A.S, $8 \times 10^6 \, \Omega$ for MFC_50A.S and $1 \times 10^6 \, \Omega$ for MFC_100N.A.S. These high internal resistances can be attributed to a development of non electrogenic culture in these MFCs, because it greatly complicates the process of electron donation. This circumstance is also consistent with the OCV values reported previously and with the maximum achieved power densities. At the end of the tests, the cells were opened to further investigate the behaviour of the attached

biofilm through cyclic voltammetry (CV) analysis showed in Figure 4.

Figure 4. Cyclic voltammograms of anodic carbon felts extracted from the MFCs and operated into a common electrochemical cell with N_2 purged synthetic wastewater, platinum as cathode and saturated calomel as reference (SCE). Of three consecutive cycles recorded from -0.6 to 0.3 V vs SCE, only the second is here reported. Data were acquired at a scan rate of 0.005 V s^{-1} . Water at 25°C was circulated around the vessel through the jacket. (—) MFC_100AS; (---) MFC_10AS; (---) MFC_50AS; (····) MFC_100NAS; (— · —) Blank carbon felt.

CVs were obtained in turnover condition, i.e. in a carbon unlimited environment [32]. In the exactly same conditions as the ones described in Section 2.4, a blank carbon felt of the same kind and surface was subject to the same CV in order to get a blank reference to compare our results (Figure 4). The CVs of MFC_10A.S, MFC_50A.S and

MFC_100N.A.S gave current that are more comparable with this blank anode than the one given by MFC_100A.S. This fact is symptomatic of a reduced capacity to accomplish the exoelectrogenic pathway by the attached biofilm. By the way, MFC_100A.S anodic felt was capable to generate a high current of 3.7 mA, with a zero voltage of -560 mV vs SCE. About the shape of this CV, it is to be reported the absence of the typical sigmoidal shape with a plateau associated with membrane proteins direct electron transfer [33]. In this case the sigmoid is probably superimposed with an ever growing trend, typical of the mediated electron transfer operated by electron shuttles as flavins [34]. This might be explained by the presence into the fresh aerobic inoculum of facultative bacteria such as *Shewanella* sp. that are known to mainly exploit a mediated electron transfer through free flavins in both, oxidative and reductive direction [35,36]. When the cell is operated with initial anaerobic sludge, it can be observed that the shape described is quite different.

3.2. Wastewater treatment capacity

This section informs about the changes in the wastewater treatment capacity of the four MFCs studied in this work. To do this, Figures 5 and 6 focus on the two most relevant parameters: the organic matter consumption and the concentration of total suspended solids (TSSs). TSS is an indirect measurement of the concentrations of microorganisms contained in the bulk. It does not give a value of the microorganisms concentration and, of course, it does not inform about the biofilm, although when similar systems operated under the same conditions are compared, it may indicate which system has a higher or lower amount of microorganisms. As shown in Figure 6, the inoculation method clearly influences the initial concentration of microorganisms contained in the mixed liquor. However, after 5-10 days the concentration in the four MFCs tended to the same values,

in agreement with what could be expected in biological reactors operated with the same sludge age and fed with the same substrate.

Figure 5. Evolution of the suspended solids daily. (◆) MFC_100AS; (▲) MFC_10AS; (□) MFC_50AS; (○) MFC_100NAS. Error bars related to analytics. Onset with the average from daily SS during 8 days of steady state. Error bars related to standard deviation. Average temperature: 23° C.

Regarding the COD consumption rate (Figure 6), it can be clearly seen how, despite the initial consumption rate is rather different and strongly dependent on the amount of microorganisms contained in the bulk, after fifteen days of operation, almost the same value is achieved in the four MFC, as it happened with the TSS. Thus, from the point of view of the pollution removal capacity, the four cells have reached almost the same operation values, that could be easily expected taking into account that they underwent exactly the same sludge age.

Figure 6. Evolution of the COD consumed daily. ($\blacklozenge$) MFC_100AS; ($\blacktriangle$) MFC_10AS; ($\square$) MFC_50AS; ($\circ$) MFC_100NAS. Error bars related to analytics. Onset with COD consumed daily average during 8 days of steady state. Error bars related to standard deviation. Average temperature: 23° C.

In addition, the pH and the conductivity, were monitored. These parameters are less important from the viewpoint of the assessment of the treatment capacity, but they are of interest to understand the behaviour of the four MFCs. As shown in fig. 7, the different MFCs reached the same values after less than 10 days, regardless of the very different observed initial values.

Figure 7. Evolution of the pH (A) and of the conductivity (B) of the studied MFCs: (◆) MFC_100AS; (▲) MFC_10AS; (□) MFC_50AS; (○) MFC_100NAS. Error bars are related to analytical error.

3.3. Coulombic efficiency

Just after the acclimation period, every cell removed COD from the synthetic wastewater at very different rates. As an example, the 6th day MFC_100AS reduced the 96% of the initial COD, close to the 88% of MFC100NAS but far from the 56% and 45% of MFC_50AS and MFC10AS respectively. Once steady state operation was reached, the scenario was more balanced. Considering the average of the last five days of this first part of the experiment, MFC100_AS removed the $61 \pm 9\%$, MFC_50AS $60 \pm 3\%$, MFC_10AS

63±2%, MFC_100NAS 56±13%.

Figure 8. Coulombic efficiency at steady state. Data is an average of 8 days and error bars represent its standard deviation.

However, despite of a very similar carbon removal, the current produced by the last three reactors were very low, which led to a very inefficient process of energy generation as reflected by the values of CE calculated for the last five days (Figure 8), understating it as steady state: $6.7 \cdot 10^{-4} \pm 5.9 \cdot 10^{-5} \%$, $2.2 \cdot 10^{-4} \pm 4.4 \cdot 10^{-5} \%$ and $8.6 \cdot 10^{-4} \pm 4.1 \cdot 10^{-5} \%$ for MFC_50AS, MFC_10AS and MFC_100NAS, respectively. Data obtained reflect CVs results, were was clear that MFC_50AS, MFC10AS and MFC100NAS have not developed a proper exoelectrogenic community. In the very same way it was possible to observe how MFC_100AS was the only cell which used a defined amount of the energy consumed in order to produce an electron current of $1.5 \pm 0.5\%$.

3.4. Selection and re-inoculation tests

Since it was clear that MFC_100AS was the most successful of the four at the start-up, the test was prosecuted with this one for 76 days in order to verify if further improvement could be achieved. Comparing data reported in section 3.1 to those presented in Table 2, it is clear how the resistance of the cell decreased in time from 472 Ω to 232 Ω , leading to a higher operational current and maximum power densities: 6.78 A m⁻² at steady-state and 1.942 W m⁻², respectively. In the meanwhile also the Coulombic efficiency increased from about 1.6% to just above 8%.

According to the procedure proposed by Wang et al. [11], a second microbial fuel cell was inoculated (MFC_100RE) with the sludge of MFC_100AS, which was almost an electrogenic sludge according to the results obtained. The same starving procedure used for MFC_100AS was adopted also in this case. Table 2 shows the complete electrochemical characterization of MFC_100AS just before a sample of its anolyte was collected and used to inoculate MFC_100RE, whose analysis is also reported.

Table 2. Electrochemical characterization at steady state of MFC_100AS and MFC_100RE.

	OCV (V)	R _{int} (Ω)	J _{max} (A m ⁻²)	J _{steady-state} (A m ⁻²)	Power density (W m ⁻²)	CE (%)
MFC_100AS Day76	0.306	232.93	17.92	6.78	1.942	8.068
MFC_100RE	0.374	82.67	34.33	14.58	2.946	66.38

As shown in table 2, the resulting MFC was capable to greatly overcome the performance of its parent just 6 days after re-inoculation. This significant improvement of performance can be explained on the basis of further enrichment of exoelectrogenic bacteria operated on a community that was already well performing. The biofilm is colonized since the first

moment with electrogenic bacteria so the process of direct donation bacteria-electrode is favoured and the internal resistance is drastically reduces up to $82\ \Omega$. Both behaviours are observed in the smooth trend of the polarization curve at the steady state of the re-inoculated MFC. This steady state is achieved after prolonging the experiment up to 40 days and the MFC was capable to produce up to $34\ \text{A m}^{-2}$ and $3\ \text{W m}^{-2}$ (Figure 9).

Figure 9: Polarization (A) and Power density (B) curves of MFC_100AS and MFC_100RE. (—) MFC_100A.S start-up ; (—) MFC_100A.S after 28 days ; (---) MFC_100A.S after 76 days; (---) MFC_100RE start-up; (—) MFC_100RE.

Despite of the low percentage of COD consumed, about 6%, it can be confirmed that a very efficient and robust bacterial community has growth in this device as it is capable of convert 66% of the energy content of the synthetic wastewater into electricity. The huge amount of not oxidized organic matter can be attributed to the limited presence (or to the low concentration) of other not-electrogenic communities.

Conclusions

The performance of the air-breathing MFCs are hardly influenced by the enrichment process and the origin of the initial sludge. When operating AB-MFCs, the higher is the dose of sludge during the inoculation days, the more energy density is reached at the steady state by the AB-MFC. Exoelectrogenic bacteria can be developed from aerobic sludge as demonstrated by current densities of 4.0 A m^{-2} achieved during the start-up and the 6.8 A m^{-2} at the steady state. Anaerobic sludge results in a bad inoculum for these devices. The re-inoculation of another MFC whose sludge is involved in exoelectrogenic conditions leads to an exoelectrogenic colonization of the biofilm, increasing the performance of the MFC up to 14.6 A m^{-2} and 66 % of CE and decreasing the internal resistance up to $82 \text{ } \Omega$. This last fact shows how efficient can be a MFC when a predominantly exoelectrogenic biofilm is achieved, being the direct electron transfer the main donation mechanism. .

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