Optimization of the performance of air-cathode MFC by changing Solid Retention Time

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Abstract

BACKGROUND: This work is focused on the optimization of the performances of air-cathode MFC by changing the solid retention time (SRT) of the suspended biomass culture.

RESULTS: Five MFCs inoculated with activated sludge obtained from a municipal wastewater treatment plant were fed with a highly-concentrated acetate solution (10000 ppm COD) and operated over two-month tests, in order to determine how SRT may influence the performances of the bio-electrogenic cells. The MFC operated at SRT of 2.5 days was found to overcome the performance of the other cells, operated at SRT of 1.4, 5.0, 7.4 and 10.0 days. In order to evaluate the possibility of using SRT as a manipulate parameter for the regulation of the operation of MFC, the SRT of the other MFCs was changed from their initial value up or down to 2.5 days.

CONCLUSIONS: As a result of the change in the SRT, production of electricity of the cells increased significantly in all cases, pointing out the relevance of the SRT control in
the optimization of the performances of MFCs. By operating at SRT 2.5 days, the current density was 4.2 A m$^{-2}$, the COD consumption rate was 1.53 g COD d$^{-1}$ L$^{-1}$ and the COD transformed by electrogenic microorganisms was estimated in 1.5 %.

Keywords

Microbial fuel cells; sludge age; solid retention time (SRT); acetate air-cathode

Highlights

- SRT has a great influence on the performance of a MFC
- MFC operated at SRT of 2.5 days overcomes efficiency of MFCs operated at 1.4, 5.0, 7.4 and 10.0 days
- Lactate occurrence during optimum performance of MFC
- Optimization of performances of MFCs by modification of SRT

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1. Introduction

Microbial fuel cells are energy transformation devices based on the action of electrogenic microorganisms that harvest energy contained in organic substrates producing directly electricity\(^1\). Although first manuscripts regarding this bio-electrochemical technology were published several decades ago, the search of novel technologies which can minimize the dependence on fossil fuels and be integrated to environmental friendly processes (such as remediation of wastewater technologies) has pushed up the research interest on this topic\(^3\)\(^-\)\(^5\). Therefore, hundreds of papers are being published every year, regarding the use of different substrates, electrode materials or cell designs. As a result, efficiencies in the use of organic matter have increased significantly and nowadays there are papers in which electric yields close to the maximum achievable according to a typical metabolism are attained\(^6\). However, there is still a very weak point in this technology and it is related to the scale up, both for the increase in the power obtained up to values that can supply real applications or the decrease in the size (miniaturization) in order to be used in compact applications\(^7\)\(^-\)\(^9\).

Regarding technological approaches, there are many types of MFC, ranging from the simpler one-compartment cells with flat electrodes to the double-compartment cells with 3-D electrodes and ion-exchange membranes, passing through a great deal of alternatives\(^10\)\(^-\)\(^12\). Likewise, many kinds of materials have been tested as anodes and cathodes, although carbonaceous materials are known to be the most reliable because of their microbial compatibility, low price and suitable chemical, electrochemical and physical resistance\(^13\)\(^-\)\(^16\).
One of the most interesting approaches is air-breathing MFC\textsuperscript{17-19}, which consists of microbial fuel cells equipped with air-cathodes. In this case, there are not two compartments in the microbial fuel cell but only one, being the cathode in direct contact with air and with the membrane that separates it from the anode chamber. Advantages of this configuration are important and previous results obtained with this approach are promising\textsuperscript{20-22}. In order to enhance the efficiency of these MFCs, the use of platinum catalyst onto the surface of the cathode is advisable. Additional advantages of this type of cell include the highest rate of oxygen transfer, the easiness in the management and the higher robustness of the cathodic reaction\textsuperscript{17, 23, 24}.

Excluding the cell design, the composition of the biological culture seed in the MFC is, perhaps, the most important input, because not all microorganisms exhibit bio-electrogenic behavior and microorganisms contained in the microbial fuel cell are going to compete among them for the substrate\textsuperscript{25}. In the literature different populations has been described as electrogenic\textsuperscript{26}. However the use of mixed cultures is justified because they are easily operated and exert similar power densities to those obtained with pure cultures\textsuperscript{27}. The composition of microorganisms depends on many parameters such as the inoculum seed, temperature and fuel fed to the MFC\textsuperscript{28-30}. Among them, a parameter with a great influence on the performance of any type of biological process is the sludge age or solid retention time (SRT)\textsuperscript{31}. This parameter is used in most suspended-culture biological processes to select the population of microorganisms that allows to obtain the desired performance of the process, because it can wash up microorganisms with low growing rates. Thus, the SRT shows a great influence on the choice of a proper culture of microorganisms in many biological processes, such as the well-known biological nutrient removal in which its value can explain the difference between the uptake of phosphorus or the accumulation of glycogen as energy storage by the biological
It also affects the total amount of microorganisms (expressed typically in mass of total suspended solids) contained in a bioreactor. To regulate the sludge age in suspended culture processes, the sludge purge flowrate is manipulated. In biofilms-based processes, the meaning of sludge age is slightly different, because there is not a direct relationship of the sludge purged with the control of this parameter. In turn, biofilms are detached from solid support surface and this process cannot be controlled but just monitored. The cells in the mother layer grow, but once all attachment sites are saturated, any new daughter cells are shed into the fluid.

Initially, microbial fuel cells are complex processes which combine the outcome of both, suspended and fixed cultures. Processes on the anode surface can be directly produced by the microorganisms fixed onto it, but foreseeable, there is also an influence of the suspended solid culture on the transformation of the substrate and on the production of redox mediators. In fact, even now, it is still not clear which is the limiting process in MFC and electricity produced over the tests can be explained in terms of the outcome of both types of mechanisms. In literature, it has been stated that the miniaturized MFC yields better electrochemical performance, which could be related to a predominant role of the biofilm.

In the search of methodologies capable to raise the efficiency of MFC, manipulation of the SRT seems to be a good possibility because of the easiness of this regulation and the very sensitive response of the bio-electrochemical devices. Research portfolio of our Lab includes the characterization of air-cathode MFCs, and one of the most important parameters evaluated is the SRT because it was found to influence very importantly the performances of this type of bio-electrochemical devices. In particular, we have previously shown by preliminary experiments that the SRT can drastically affect the start-up of air-cathode MFCs. With this background, the goal of this paper is to evaluate
how changes in the sludge age of the suspended biomass culture can affect the performances of MFCs for long time operations and how they can be used to optimize the performances of this interesting energy conversion device.

2. Materials and Methods

2.1. Experimental setup

Five different air-breathing MFCs were simultaneously operated under different SRTs in order to evaluate the effect of this parameter on the performances of the bio-electrochemical reactor. The cells, made of methacrylate, consisted of two chambers of 0.346 cm³ each, separated by a Sterion® proton exchange membrane (Figure 1). Anodic chamber was connected to a 115 mL external reservoir by a peristaltic pump. Cathodic chamber was directly open to air (air-cathode MFC). Toray carbon papers were used as electrodes with an active area of 0.86 cm² in both chambers: the anode contained 10% Teflon (looking for improved mechanical properties) and the cathode contained 20% of Teflon and a microporous layer in order to favor a homogenous deposition of a catalytic layer of 0.5 mg Pt cm⁻² loading. This layer increases the reduction rate of the oxygen from the air. Both electrodes were connected by an external circuit with a resistance of 120 Ω. The electrodes and the proton exchange membrane underwent a very careful assembly to reduce the internal resistance. To do that, they were introduced between two stainless steel blocks equipped with heating surfaces brought under progressive and controlled heating until 120º C and a load of 1 ton for 4 minutes was applied at this temperature. More information about the procedure can be found elsewhere.
Regarding to the inoculum seed in each MFC, all the MFCs were inoculated with activated sludge from a conventional WWTP. This type of sludge has been previously characterized for its application in MFC and described elsewhere. In order to remove the organic pollutants contained in the activated sludge used to inoculate, it was overnight aerated. At the beginning of the test, 1 mL of activated sludge and 114 mL of synthetic wastewater were mixed in the external reservoir of the fed-batch anodic compartment.
2.2. Operation procedure

The MFCs were operated in a fed-batch mode until the pseudo steady state was achieved. When working in a fed-batch mode the process define cycles. During these cycles, significant changes may appear, however the steady state can be ratified if the cycles are repetitive. The composition of the synthetic wastewater fed to the MFC was 14.5 g L\(^{-1}\) of sodium acetate as carbon source (10000 ppm of COD) supplemented with the following trace minerals: 0.26 g L\(^{-1}\) of sodium carbonate, 0.18 g L\(^{-1}\) of ammonium sulfate, 0.11 g L\(^{-1}\) of potassium dihydrogen phosphate, 0.09 g L\(^{-1}\) of magnesium chloride, 0.07 g L\(^{-1}\) of calcium chloride and 0.02 g L\(^{-1}\) of ferric ammonium sulfate. Despite the concentration of organic matter is very high, and even far beyond the level of concentration that can be tolerated by the well-known bioelectrogenic Geobacter Sulfurreducens\(^{40}\), in the system studied is tolerable because instead of using a pure culture, the MFC was inoculated with an electrogenic mixed culture. Thus, in the literature it has been stated that mixed cultures are tolerant to these extreme conditions \(^{41, 42}\). The solution was fed to the MFC at a flow rate of 3 cm\(^3\)/min. In order to obtain MFCs working with different SRT, the anodic reservoir of each MFC was daily purged. After the purge, the discarded volume was replaced by fresh synthetic wastewater. The purge implemented in each MFC leaded to SRT of 10.0, 7.4, 5.0, 2.5 and 1.4 days. Five MFCs were operated simultaneously under the same conditions (including the volume of the cell and the flow-rate) with different SRT. We preferred to operate in this way and not to change the SRT in the same cell to ensure that the performances of the cells are not affected by the story of the MFC (e.g., by previously used SRTs).
2.3. Analytical techniques

Conductivity and pH were measured using a GLP22 Crison pH-meter. The COD was measured according to Standard Methods\textsuperscript{43} and samples were previously filtered through a fiber filter 45 µm pore size. Intermediates of reaction were monitored by HPLC (Agilent Technologies, Germany) with UV–DAD detector using a wavelength of 210/8 nm while the reference wavelength was 360/80 nm. The detector cell and the column operated under a temperature of 25° C. The mobile phase was a pH 2 buffer solution (20 Mm aqueous solution of NaH$_2$PO$_4$ and H$_3$PO$_4$) and the column used was a Zorbax SB-Aq 4.6 x 150 mm 5-microm (Agilent). Turbidity was measured by Hach DR/400U spectrophotometer at 860 nm. The evolution of the potential was continuously recorded in a computer connected to a digital multimeter (Keithley® 2000) and polarization curves were obtained by measuring the potential by varying the external resistance that closed the electrical circuit.

3. Results and Discussion

3.1. Performance of the MFC operated at SRT of 2.5 days

Figure 2 shows the changes in COD and current density for a two-month test, in which an air-cathode MFC (so-called MFC$_1$) is fed with a highly-concentrated acetate solution containing 10000 ppm of COD. SRT in MFC$_1$ was kept in 2.5 days by replacing each day 46 mL of mixed liquor with fresh wastewater. At this point, it is important to point out that in all tests carried out in this work, SRT matches with the hydraulic retention time (HRT) of the device, and this can be easily explained in terms of the experimental procedure applied, taking into account that auxiliary tanks of the MFC devices are stirred and, hence, concentration of solids in the auxiliary tank, hydraulic circuit and
anode compartment of the MFC can be considered uniform. It is also important to point out that the MFCs were operated for a month before the tests shown in this work, in order to allow microorganisms to form a biofilm in the anode surface. Over this first month, the same SRTs were kept in the biological systems. However, the resulting current produced was much lower and almost negligible as compared to the values shown in this work.

Figure 2. Time course of current density (◆), influent COD (×) and effluent COD (○) in the MFC operated at a SRT of 2.5 days.

Regarding the test at SRT of 2.5 d, COD in the effluent increases during a first stage, up to a pseudo steady state value of nearly 6400 ppm that is maintained, showing only the fluctuations (> ± 20%) which are typically associated to biological oxidation processes. At this point, for further understanding of the performances of this type of MFC, it is worth to take in mind that from the hydrodynamic point of view, the MFC device behaves as a semi-continuous reactor in long-term scenarios with a short-term
discontinuous response caused by daily feeding, which produces a daily profile in the concentration of COD in the mixed liquor. Hence, this parameter becomes maximum during the daily replacement of the volume of mixed liquor with fresh wastewater and then, it decays during the day (these decays are not shown in the Figure). COD concentrations shown in the figure correspond to the organic matter contained in the reactor at the end of the discontinuous daily cycle, before feeding with raw solutions and this value tends to a pseudo steady state within long-period tests, just as it could be expected for a continuous stirred tank reactor (CSTR), confirming the suitability of the hydrodynamic model proposed. This fact means that changes within long periods, such as that applied in the tests carried out in this work, have to be evaluated as if the MFC behaves as a CSTR (eq. 1, where $S_0$ and $S_1$ represent the influent and effluent COD, respectively, $V$ the solution volume, $r$ the mineralization rate, $q$ the ratio between the amount of solution fed every day and 86400 sec and $t$ the time). In turn, to evaluate the performances over short periods of time (time scale of hours), such as the daily changes of the COD, it is better to fit the performances of the cell to a discontinuous reactor model (eq. 2)

$$qS_0 - qS_1 + rV = V \frac{dS_1}{dt} \quad (1)$$

$$rV = V \frac{dS_1}{dt} \quad (2)$$

Taking into account this hydrodynamic description, the mass balance for the long-term response should fit to equation 1, which gives a pseudo steady state value of the COD consumption rate of 1.53 g COD d$^{-1}$ L$^{-1}$. This value is similar to those reported in the literature for analogous devices$^{39,44,45}$. 
Changes in the electric current produced by the MFC are related to changes in the COD, although they clearly show a time-delay. In this case, the production of electricity during the first ten-day period is negligible and then, it increases at a constant rate up to day 32th for which, after a small decrease, it meets a pseudo steady-state value of about 4.2 A m\(^{-2}\). Changes in the cell potential during the day (not shown) follow the same pattern than changes in the COD (previously described), and values plotted in Figure 2 correspond to the current density measured at the end of the feeding cycles, before replacing a volume of the mixed liquor with fresh wastewater.

Both values of the COD consumption rate and of the current density can allow estimating indirectly the value of the population of electrogenic microorganisms, by determining the fraction of the COD used by this microbial community. Thus, taking into account that Equation 3 shows the relationship between current and COD consumption rate for electrogenic microorganisms\(^{31,39}\), the ratio of COD transformed by electrogenic microorganisms can be easily calculated by Equation (4). For the MFC\(_1\) is 1.5 %, which is a typical value, although far below the maximum values obtained in previous studies about MFCs\(^{31}\).

\[ r_{COD-electrogenic} = \frac{jA}{4F} \]  
(3)

\[ ratio \; electrogenic = \frac{r_{COD-electrogenic}}{r_{COD}} \]  
(4)

Where \(r_{COD-electrogenic}\) corresponds to the COD consumption rate by electrogenic microorganisms and \(r_{COD}\) corresponds to the total COD consumption.

The composition of the mixed liquor was monitored daily by HPLC, obtaining very important fluctuations in terms of concentrations of intermediates, which may only be explained in terms of the complexity of the reactions occurring in the anodic chamber of
the MFC. Anyhow, from the results obtained it can be concluded that most of the soluble COD of the mixed liquor can be associated to the acetate which does not react during the experiment. Regarding composition of other intermediates monitored by HPLC, it is important to point out that during a first period, only negligible concentrations of other species were detected. However, increase in the production of electricity (marked in the Figure as stage II) corresponds to the occurrence of a non-negligible concentration of formate, with an average value of around 6.7 ppm. This response changed when the system stabilizes and then, the only intermediate found at measurable concentration was lactate, with an average concentration more than one fold higher than that reported for formate in stage II. Production of lactate in a bioreactor fed with acetate was not expected, because lactate is a metabolite in the degradation of sugars like glucose but there are no direct metabolic pathways that relate it to acetic acid. Hence, this lactate has to be a byproduct in the endogenous degradation of microorganisms. There are several organisms, called lactic acid bacteria, that during their fermentation obtained lactate as end products. A common pathway for the lactate formation is the reduction of pyruvate. The absence of the latter specie in the HPLC results could be associated to a very fast consumption process of pyruvate. Because in the cytoplasm of some microorganisms are present granulations (inclusions) cytoplasmic of different composition having the meaning of accumulations of reserve material such as glycogen, the pyruvate can be obtained by glycolysis of these reserve compounds. The presence of these is frequent in many bacteria. Lactic acid bacteria, with Photosynthetic bacteria, Yeasts, Actinomycetes and Fermenting fungi, could be one of the several bacteria present in the mix of effective microorganisms used in a wastewater treatment plant.

3.2. Performance of MFC operated at other SRT and effect of its change to 2.5 d
Experiments carried out at higher sludge age result in the production of very small electric currents. This can be observed in Figure 3, where the performance of MFC\textsubscript{2} operated exactly under the same operating conditions of MFC\textsubscript{1} (except for the SRT which in this case is 10.0 d) is shown.

![Figure 3. Time course of current density (♦), influent COD (×) and effluent (○) COD in the MFC operated at a SRT of 10 days.](image)

As expected because of the higher retention time, the removal of COD is higher and, in fact, from a practical point of view, COD concentration is depleted after each feeding cycle as it can be clearly checked in the region Stage I, marked in this Figure. In comparing the values of COD depletion rate obtained by MFC\textsubscript{1} and MFC\textsubscript{2}, it can be observed a lower value of the MFC operated at SRT of 10.0 d, with a rate of 0.990 g COD d\textsuperscript{-1} L\textsuperscript{-1}, value 35.2% lower as compared to the value obtained in MFC\textsubscript{1}, in agreement with the expected less active microbial culture obtained at higher SRT. Over this stage, the production of electricity is very low, with average values close to 0.2 A.
m². By using equations 3 and 4, it can be stated that the current produced corresponds to a metabolism of only 0.11% of the COD by electrogenic microorganisms.

To try to determine the responsible of the improved electrogenic behavior observed in MFC₁ as compared to MFC₂, on day 33th the auxiliary tank of MFC₂ was re-inoculated with sludge coming from MFC₁ (operated at SRT of 2.5 days). This period is marked in the Figure as Stage II and no improvements in the performance of the cell were observed. However, what it is interesting is that in the composition of the mixed liquor, in addition to the acetate fed, small concentrations of formate and lactate were observed. These concentrations can be initially related to the effect of the seeding. After 10 additional days without any significant change in the response, it was decided to decrease the SRT down to 2.5. As it can be observed in so-called stages III and IV, the same pattern observed in Figure 1 for MFC₁ was reproduced for MFC₂, with a rapid increase of the COD up to concentrations near 6000 ppm and a time-delayed increase in the current density, up to almost 3 A m⁻². Concentrations of organics detected (in addition to acetate) followed also the same patterns with:

1) a zone previous to the raise in the electric current intensity in which formate becomes the most significant intermediate;

2) a final stage in which lactate is the most important species, in addition to the acetate not consumed by microorganisms.

Figures 4 and 5 summarize the information obtained in three additional tests carried out with other three MFCs (named MFC₃, MFC₄ and MFC₅) for which a similar SRT change sequence was carried out, up or down to the 2.5 days fixed in the base experiment (MFC₁). Thus, the test with the three additional MFCs started from different
devices stabilized at SRT below (MFC$_3$ from 1.4 days) and above (MFC$_4$ from 5.0 and MFC$_5$ from 7.4 days) the value of MFC$_1$.

Figure 4. Average concentration of intermediates and pseudo steady state current density attained in the four MFCs that underwent the change in the SRT from different initial values up or down to 2.5 days. a) Values measured just before changing the initial SRT to 2.5 d. b) Values stabilized following 30 days of operation after the change in the SRT to 2.5 d.
A very important piece of information comes up from these figures: the change of SRT from any value up or down to 2.5 days results in an improvement in the electric current production and in the predominance of lactate as the key intermediate (primary species measured by HPLC in addition to the surplus acetate) as shown by figures 4 and 5, respectively. It is interesting to observe that maximum current produced improves with a high-value in the previous sludge age (Fig. 5). At this point, it is worth to take in mind that values represented do not correspond to pseudo steady state values but to values recorded after one month of operation of the MFCs, for which the 2.5 days of SRT was kept (value selected arbitrarily). This fact can be clearly observed in Figure 5, in which it is also of interest to see that the higher the initial SRT, the lower is the time required to activate the electrogenic response (measured as the time for which the current started to increase exponentially). Initially, this behavior may be explained in terms of the presence of more types of microorganisms in the biological culture of MFC operated at high SRT, which help in a faster adaptation to the conditions of the 2.5 days tests. Inset of Figure 5 shows the decreasing relationship of this time-lag with the SRT, pointing out the relevance of the kinetic of the activation process.
Changes in the current density produced by the change of the SRT of MFCs to 2.5 days.  MFC2 (10 d);  MFC3 (1.4 d); O MFC4 (5.0 d) and × MFC5 (7.4 d).

Onset: time lag till exponential increase of the electric current intensity produced vs. SRT.

A very important observation is relative to the transient response of the COD during the SRT-change tests, which is shown in Figure 6. As it can be observed, values of COD in the mixed liquor of the MFC changes rapidly from a practically total removal (in the cells with initial SRT ranging from 5.0 to 10.0 d) up to the same value obtained in the 2.5 days test, indicating that the systems evolves to the same pseudo steady state, regardless of the initial starting conditions. As expected, COD values obtained initially by the MFC3 (operated at 1.5 d) are slightly higher than that of MFC1 (2.5 d) but with the change in the SRT, there is an enhancement in the COD removal rate and COD in this test also stabilizes within the same range than the other tests. It is important to take into account that in order to evaluate the COD removal rate, not only the concentration...
but also the flowrate should be accounted and this flowrate increases when decreasing the SRT.

Figure 6. Changes in the COD of the mixed liquor produced by the change of the SRT of MFCs to 2.5 days. ◆ MFC₂ (10 d); ♦ MFC₃ (1.4 d); ○ MFC₄ (5.0 d) and × MFC₅ (7.4 d). △ MFC₁ (2.5 d).

Regarding pH and conductivity, changes are shown in Figure 7. Initially, not a very relevant information, although it can be clearly seen how the highest SRT microbial fuel cells exhibit a higher pseudo steady state value of the pH and how, with the change in the SRT value, the system is led to a narrower zone of variation closer to that obtained for the 2.5 days tests. Regarding conductivity, the only remarkable point is the sudden increase in the value obtained when the SRT is changed to 2.5 days, which is rapidly compensated and which should be explained in terms of the adaptation of the microbial cultures to the new conditions. Anyhow, the most relevant information is the reproducibility of final conditions, regardless of the initial SRT conditions, that it is again pointed out with these two globalizing parameters.
Figure 7. Changes in the pH and conductivity produced by the change of the SRT of MFCs to 2.5 days. ◆ MFC2 (10 d); ◇ MFC3 (1.4 d); ○ MFC4 (5.0 d) and × MFC5 (7.4 d).

A last important parameter monitored in the mixed liquor of the MFCs is the turbidity, which is related to the microorganisms contained in the bioreactor not associated to flocs. Due to the high shear stress (high flowrate in comparison with the diameter of pipes and use of peristaltic pumps) it is expected that biomass is not associated in large flocs and hence turbidity can be used as an indirect measurement of the population or at least as a first approach. As it can be seen in the Figure 8, no changes are observed before the change and after it, turbidities monitored in all tests tend to approximately the
same value, pointing out once again the reproducibility of the biological systems tested, even after undergoing a very different change (because of the different SRTs applied).

Figure 8. Changes in the turbidity produced by the change of the SRT of MFCs to 2.5 days. ◆ MFC₂ (10 d); ◇ MFC₃ (1.4 d); ○ MFC₄ (5.0 d) and × MFC₅ (7.4 d).

The characterization of the MFC needs for electrochemical measurements, in addition to the monitoring of the main parameters of the mixed liquor. For this reason, periodically polarization curves were recorded. In Figure 9, it is compared one polarization curve for each MFC taken the same day of the change in the sludge age (where the changes are greater). Values clearly reflected the better performance of the cell operated with a sludge age of 2.5 days. Maximum power produced by MFC₁ is 0.65 W m⁻², while the maximum power recorded for the other cells are much lower and they range between 0.0005 and 0.015 W m⁻², more than one fold below, in agreement with the response observed in the current produced. OCP is low as compared to other MFCs and the more
important difference is the lower resistance observed in MFC\(_1\), with may suggest a
different metabolic pathway in this type of cells.

![Figure 9](image_url)

Figure 9. Potential vs current density for polarization curves recorded after 30 days of
operation. ☐ MFC\(_2\) (10 d); ☀ MFC\(_1\) (2.5 d); □ MFC\(_4\) (5.0 d) and ▲ MFC\(_3\) (7.4 d). ●
MFC\(_2\) (10 d).

Hence, SRT is a very important parameter that contribute to fix the mechanisms of
acetate oxidation in MFC, by controlling the amount and quality of microorganisms
populations\(^{46}\). Although its response is not easily foreseeable because of the complexity
of the microbial processes, this work demonstrate that it is very reproducible even using
different MFCs operated initially under extremely different SRTs. This is of a great
relevance and further work are being made in order to understand why the performance
of the MFC operated at SRT of 2.5 days is significantly better than at any other SRT. In
this context, better performance of MFC operated at lower SRT in terms of electricity
production can be explained in terms of the competition between microorganisms. The improvement in the current production with the decrease means that the electrogenic population increases at lower SRT and this can only be explained if their growing rate is higher as compared to the growing rate of competing microorganisms. Thus, a higher SRT contributes to wash up these competing populations. Regarding the performance of MFC operated at 1.4 days of SRT, the only way to explain the worse performance is by taking into account that the change from 2.5 to 1.4 contributes to the washing up of the population of electrogenic bacteria. Anyhow, more work has to be carried out in order to understand this behavior and particularly, the relevance of the intermediates found when electricity production is activated.

4. Conclusions

From this work the following conclusions can be drawn on the effect of the SRT on MFC for long-time operations:

- Air-cathode MFC fed with a synthetic fuel consisting of a solution of 10000 ppm of acetate and nutrients produce electricity when operated in the range of SRT 1.4-10.0 days.
- Most effective performance of the MFC is obtained operating at a sludge age of 2.5 days. Under those conditions, lactate is always formed as intermediate.
- Electric current produced by MFC can be improved by changing the SRT to 2.5 days. Seeding with the sludge is not enough to enhance energy production.
- Time required to activate the electrogenic behavior once change the SRT depends on the initial SRT at which the MFC were acclimated. The higher the SRT the lower is the required lag-time.
Acknowledgements

Financial support from Spanish Ministry of Economy and Competitiveness (MINECO) through project CTQ2013-49748-EXP (Explora Program) is gratefully acknowledged.

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