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Title: THE SALINITY EFFECTS ON THE PERFORMANCE OF A CONSTRUCTED WETLAND-MICROBIAL FUEL CELL

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Abstract: The objective of the present work is to study the influence of the wastewater salinity concentration on the performance of a Constructed Wetland-Microbial Fuel Cell (CW-MFC) for simultaneous water pollution control and electricity generation. The work has been carried out under the hypothesis that increasing the salinity may improve the electricity production because of a lower internal ohmic resistance, although it could damage the microbiological processes or the plants. A pilot-scale horizontal subsurface flow CW, modified to function as an MFC, was operated under a continuous operation mode over five consecutive experimental periods of approximately 2 months each. The wastewater salinity was increased in each new period by steeply increasing the NaCl concentration in the synthetic wastewater from 0.51 to 9.51 g L-1. The CW-MFC performance was monitored during every stationary period. The increasing salinity first improved the cell voltage, and the resultant maximum voltage (130 mV) under continuous operation corresponded to a salinity concentration between 4 and 5 g L-1. However, subsequently higher salinity levels caused the opposite effect. The maximum voltage was obtained in an unstable condition, as microbiological inhibition in the anode zone appeared early, at approximate salinity levels of only 3 g L-1. Batch experiments confirmed the results, and higher cell voltage values up to 600 mV were obtained if longer retention times were allowed. The wetland plants (Phragmites australis) were only damaged at a salinity concentration of 9.51 g L-1.

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Ecological Engineering
Editor

24-6-2017

Dear Editor:
Attached you will find the REVISED manuscript ECOLENG-D-16-01038 "*The salinity effects on the performance of a constructed wetland-microbial fuel cell*", by José Villaseñor Camacho, Luis Rodríguez Romero, Carmen María Fernández Marchante, Francisco Jesús Fernández Morales and Manuel Andrés Rodrigo Rodrigo (corresponding author: jose.villasenor@uclm.es) in order to be reviewed for a possible publication as original research paper in Ecological Engineering (subject classification: Treatment wetlands).

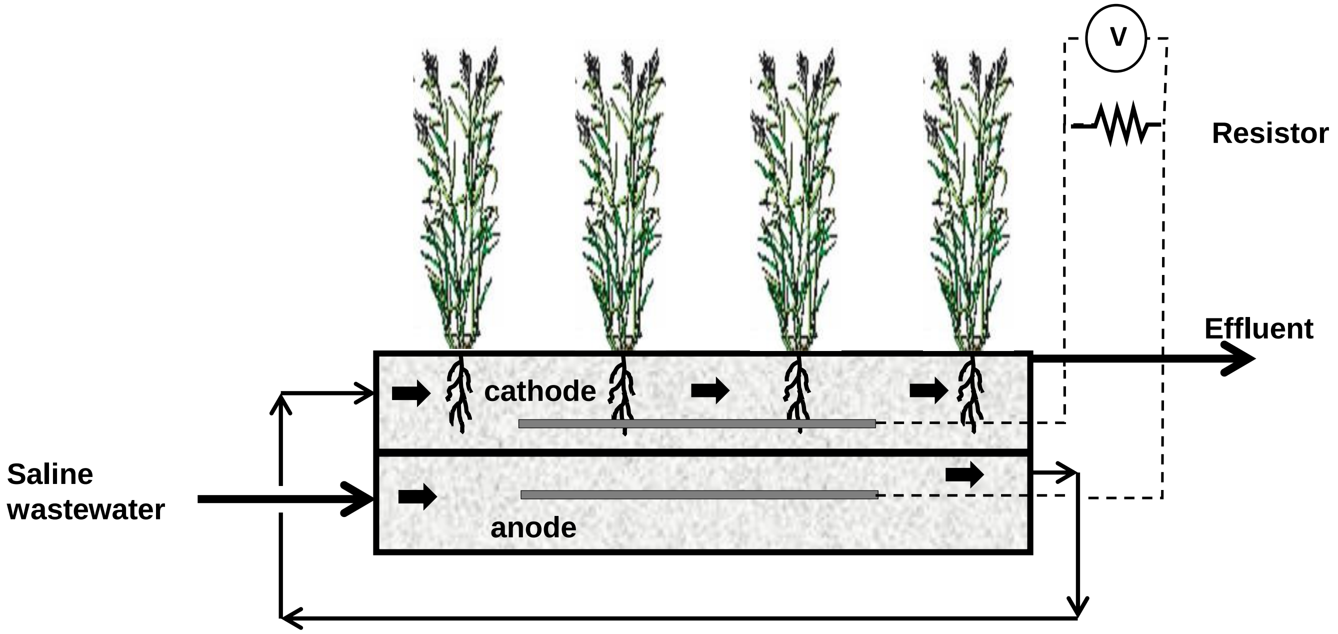
The following items are included in the new submission:

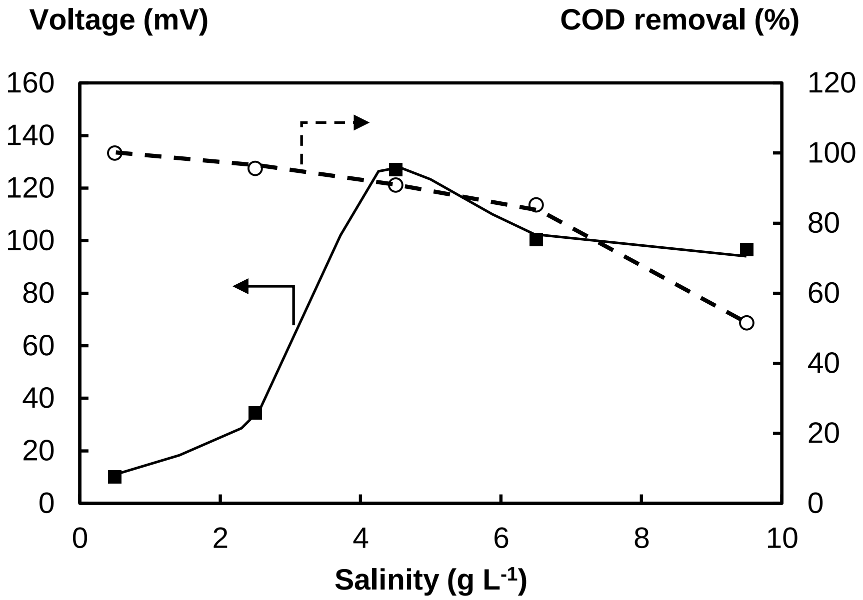
- The "**Revised Manuscript**" (using MS Word).
- The "**Highlighted Revised manuscript**", that is the same revision manuscript MS Word file, using the track changes mode, where you can easily find the modifications made to the text.
- The "**Responses to reviewers**": One MS Word document containing the detailed answers to each concrete reviewer's comments. Each answer indicates the position of the modifications in the highlighted revised manuscript (MS Word file).
- Revised figure **3**.

Yours sincerely

Dr. José Villaseñor Camacho

Graphical Abstract (for review)





Research Highlights

Highlights

CW-MFCs are efficient devices used to treat wastewater and produce electricity

Salinity affects the performance of CW-MFCs

Microbiological inhibition effects can be observed at a salinity of 3 g L^{-1}

Increasing salinity improved the cell voltage up to concentrations of 4 and 5 g L^{-1}

Wetland plants (*Phragmites australis*) are only damaged at salinities over 9.5 g L^{-1}

Revision Notes: Response to Reviewers

This document shows detailed responses to the reviewer`s comments. The responses indicate also the changes made in the revised manuscript. The changes are easily identifiable in the highlighted revised manuscript (revised manuscript changes marked document). The location of changes (page or line details in the responses) always refer to the highlighted revised manuscript MS Word file. Note that it is possible that the PDF generated by EES move lines.

Reviewers' comments:

Reviewer #2:

The manuscript describes the performance of a pilot-scale constructed wetland-microbial fuel cell under salinities ranging from 0.5 to 9.5 mg/L. The manuscript is comprehensive and skilfully written. In my opinion, it can be published after considering the following points:

L117: Is the composition of such high salinity industrial wastewaters suitable for biological treatment? Is there sufficient organic matter present? Can the authors be more specific which industrial wastewater would be suitable for such treatment?

It is well known that high salinity can be inhibitory for biological processes. Indeed, as indicated in the abstract and introduction (line 89, original manuscript) we worked under the hypothesis of a biological damage because of increasing salinity levels, and one of the objectives of the work is to know the salinity limit that could be supported by the CW-MFC (also indicated in the original manuscript). This limit was experimentally confirmed and indicated in the discussion section and conclusions.

Regarding the organic matter concentration in wastewater, we chose an intermediate COD level (300 mgL-1) as we considered that it is sufficient concentration to perform our experimental study. COD was maintained constant in the whole experimental period. A brief sentence about this comment has been included in the revised manuscript (lines 160 - 162)

There are recent research studies about industrial wastewater treatment (including high salinity effluents) using both CW (Wu et al., 2015) and MFC (Gude, 2016). For instance, leachate from domestic landfills, or washing effluents from agro-food industries could be suitable for such combined CW-MFC technology. This information has been included in the introduction (lines 117-119)

Wu, S., Wallace, S., Brix, S., Kuschk, P., Kirui, W.K., Masi, F., Dong, R., 2015. Treatment of industrial effluents in constructed wetlands: Challenges, operational strategies and overall performance. *Environ. Poll*ut., 201, 107-120.
Gude, V.G., 2016. Wastewater treatment in microbial fuell cells-an overview, *J. Cleaner Production*, 122, 287-307.

L172: 35 L d-1 instead of 35 l d-1

The change has been made (line 174 revised manuscript).
L269: "only accounts for" instead of "is only account for"

The change has been made (line 274 revised manuscript)

Figure 3: Is it possible to add error bars?

Figure 3a has been changed. Error bars for voltage measurements have been included in every period. Caption has been also changed.

L291: The cathode had a rather low DO (2 ppm). Would it be beneficial for the electricity generation to apply aeration in the cathode? Aeration is nowadays quite common in CWs.

Under the author's opinion, 2 ppm is high enough to ensure the aerobic environment in the cathode zone, and to maintain the necessary redox potential between anode and cathode (as indicated in the original manuscript line 292).

However, in the eventual case of a high organic loading, it would be possible that the organic matter could not be completely oxidized in the anode zone and it would cause depletion of DO in the cathode zone, producing anaerobic conditions which finally would cause MFC to stop working (see Villaseñor et al., 2013).

Thus, cathode aeration would be beneficial in such situation to maintain MFC working, but it must be considered also the aeration power consumption and costs in the global balance. This explanation has been included in the discussion section, lines 308-310 (revised manuscript).

L343 onwards: Could this explanation be simplified? Is the meaning that at higher salinity (4.5 mg/L) the system can obtain a higher maximum voltage but it takes longer to generate it than at lower salinity (2.5 mg/L)?

It is true, the final result is that under 4.5 mg/L it takes longer to generate voltage and (according to Figure 5b), the rate is lower. However, under the author's opinion, the reason of such behaviour must be discussed. Because of it, we stated in the original manuscript that using 4.5 mg/L is an unstable situation because of salt inhibition effects. The final sentence has been slightly modified in the revised manuscript (line 368).

L349: Could the authors summarize what the impact of these results is from practical point of view? Should one aim to obtain the highest maximum voltage or the highest average voltage generation rate? What should be the focus of further research?

According to the results, the authors considered that high salinity in industrial effluents could be a positive influence in the development of the CW-MFC technology as it improved voltage differences. However the challenge would be if halo-tolerant microorganisms and plants could be adapted to these conditions, which may be the focus of further research.

This summary has been included at the end of discussion, line 388.

THE SALINITY EFFECTS ON THE PERFORMANCE OF A CONSTRUCTED WETLAND-MICROBIAL FUEL CELL

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Abstract

The objective of the present work is to study the influence of the wastewater salinity concentration on the performance of a Constructed Wetland-Microbial Fuel Cell (CW-MFC) for simultaneous water pollution control and electricity generation. The work has been carried out under the hypothesis that increasing the salinity may improve the electricity production because of a lower internal ohmic resistance, although it could damage the microbiological processes or the plants. A pilot-scale horizontal subsurface flow CW, modified to function as an MFC, was operated under a continuous operation mode over five consecutive experimental periods of approximately 2 months each. The wastewater salinity was increased in each new period by steeply increasing the NaCl concentration in the synthetic wastewater from 0.51 to 9.51 g L^{-1}. The CW-MFC performance was monitored during every stationary period. The increasing salinity first improved the cell voltage, and the resultant maximum voltage (130 mV) under continuous operation corresponded to a salinity concentration between 4 and 5 g L^{-1}. However, subsequently higher salinity levels caused the opposite effect. The maximum voltage was obtained in an unstable condition, as microbiological inhibition in the anode zone appeared early, at approximate salinity levels of only 3 g L^{-1}. Batch experiments confirmed the results, and higher cell voltage values up to 600 mV were obtained if longer retention times were allowed. The wetland plants (*Phragmites australis*) were only damaged at a salinity concentration of 9.51 g L^{-1}.

Keywords

Constructed Wetland; Microbial Fuel Cell; bioelectrogenic; salinity.

1. Introduction

Microbial Fuel Cells (MFCs) are electrochemical devices that can obtain electricity from organic matter by means of the activity of bioelectrogenic microorganisms. As conventional fuel cells, they also have two chambers, with electrodes working under a difference of electric potential. However, what makes MFCs unique is the fact that active microorganisms in the anode chamber are capable of oxidizing the organic matter using electric current instead of oxygen by various complementary mechanisms, becoming the real biological catalyst of the electrochemical device. MFCs have been extensively studied, and many scientific papers and books have been published in recent years. Because organic waste can be used as fuel for MFCs, this technology has been proposed for environmental remediation purposes, and the concept of bioelectrochemical wastewater treatment is currently receiving significant attention (Gude, 2016).

In this context, currently, a very promising variety of MFCs is being studied, consisting of integrating MFC technology into natural ecosystems or low-cost environmental remediation technologies. There are currently three of these technologies, including the Sediment Microbial Fuel Cells (SMFCs), also called Benthic MFCs; the Plant-type Microbial Fuel Cells (PMFCs); and MFCs coupled to Constructed Wetlands (CW-MFCs), and they all are based on the redox potential differences that naturally exist between the top water-air interface and the anaerobic bottom of these ecosystems. There is also a large amount of literature on the subject. All of these technologies have been described in a recent review (Fernández et al., 2015).

One of the most interesting options of these low-cost technologies for wastewater treatment are constructed wetlands (CWs). These systems are wetlands, isolated from the underground below them, that receive wastewater. Wastewater treatment in CWs is the result of a combination of natural physical, chemical, and biological phenomena (Zhi and Ji, 2012). There are different types of CWs; subsurface flow wetlands are one of the most implemented types (García et al., 2010). Basically, they consist of a wastewater subsurface flux flowing through a porous gravel bed, which includes macrophyte plants growing on the top surface and a mixed microbial population in the form of biofilms attached to the gravel and roots.

The study of the combination of CWs and MFCs is recent. The first work was published by Yadav (2010), and although the number of papers is still low, it is increasing exponentially in the last 5 years (Doherty et al., 2015a). The works studied the influence of the type or concentration of the organic pollutants (Corbella et al., 2015; Fang et al., 2015; Liu et al., 2014; Srivastava et al., 2015; Wu et al., 2015a), the role of plants and the effect of the position of the roots (Corbella et al., 2014; Fang et al., 2013; Liu et al., 2014), the water flow configuration (Corbella et al., 2014; Corbella et al., 2015; Doherty et al., 2015b), the type of cathode (Liu et al., 2014; Srivastava et al., 2015) and the distance between the electrodes (Doherty et al., 2015b; Doherty et al., 2015c). The works usually aimed to improve the cell efficiency through maintaining a high redox potential between the electrodes and reducing the internal resistance.

To the authors' knowledge, wastewater salinity may be an important aspect influencing the CW-MFC performance, and its effect has not yet been studied. Initially, wastewater salinity is expected to have a contradictory effect on the two main processes (biological and electrochemical) occurring in the device: negative in the biological process and positive in the electrochemical process. Regarding the possible biological effects, constructed wetlands have been extensively applied for industrial wastewater treatment (Gao et al., 2015; Wu et al., 2015b), and there are some works focused on high salinity wastewater treatment by CWs (Gao et al., 2015; Karajić et al., 2010). Klomjek and Nitisoravut (2005) reported that some plant species used in CWs were adversely affected by high salinity in wastewater. Gao et al. (2015) tested twelve different plants species and detected a salinity level at which the treatment efficiency begins to fall.

Regarding the microbial role in wetlands, Gao et al. (2012) reported that the increase in salinity strongly reduced the microorganism concentration, while Lin et al. (2008) reported inhibition of the microbial activity.

Regarding the electrochemical performance, Logan (2008) and Rozendal et al. (2008) described the expected positive effect of increasing the wastewater salinity in an MFC and explained it in terms of the increase in the electrical conductivity of the electrolyte, which reduces the internal ohmic resistance and results in an ohmic drop of the fuel cell. However, they also stated the expected negative effect in the microbial activity at high salinity levels. Likewise, Lefebvre et al. (2012) reported increased power and decreased internal resistances using increasing salinity levels but found a drastic power reduction at a NaCl level of 20 g/L because of the inhibition of microbial growth.

In this context, it could be considered that the wastewater salinity could be an important factor to be tested in the performance of the combined CW-MFC system, and this paper aims to describe and discuss the results of the study of a pilot-scale CW-MFC treating wastewater with a steeply increasing salinity. Until now, this subject has not been tested, and it is hypothesized that a moderate salinity could produce a positive effect on the fuel cell performance, although it could also negatively affect the role of plants or microorganisms in the subsurface system. Because of the complexity of the CW-MFC mechanisms, the global effect and the salt tolerance limit are still unknown. The results could be of great relevance because of their potential applicability to the treatment of high salinity industrial wastewater. For instance, leachate from domestic landfills, or washing effluents from agro-food industries could be suitable for such combined CW-MFC technology.

2. Materials and Methods

The materials, experimental procedures, and analytical methods have been described thoroughly elsewhere (Villaseñor et al., 2013; Villaseñor Camacho et al., 2014). The following subsections only describe the important details.

2.1. Constructed wetland - microbial fuel cell microcosm.

The experimental installation consisted of a pilot-scale horizontal subsurface flow CW for wastewater treatment, modified to function as an MFC (figure 1).

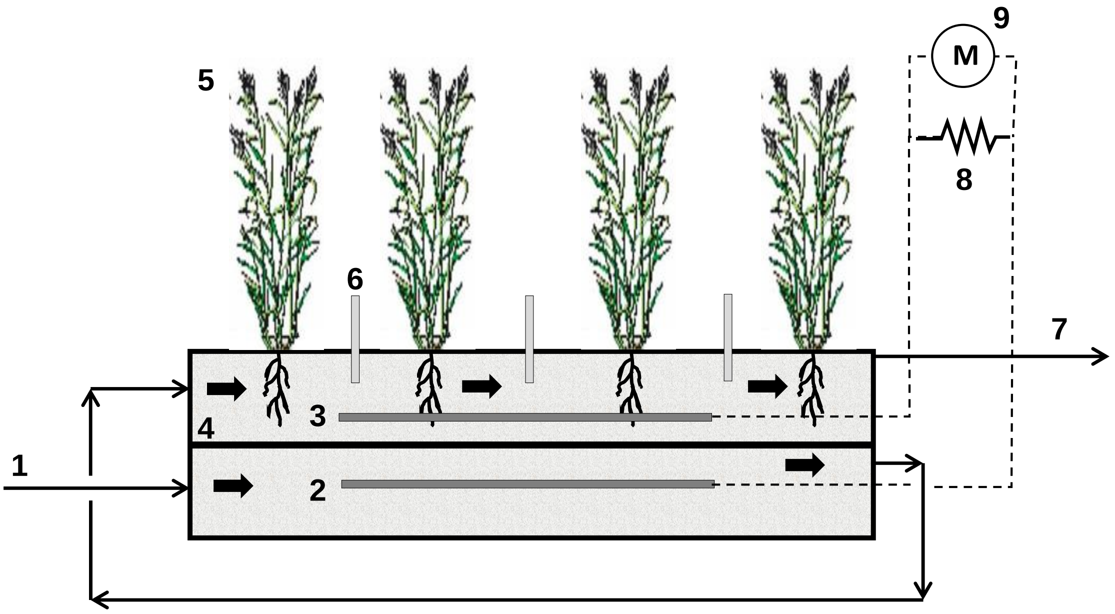

Figure 1

The installation was located in the greenhouse facility of the Institute for Chemical and Environmental Technology of the University of Castilla La Mancha, Ciudad Real (Spain). The wetland consisted of a 115 cm × 47 cm plastic channel with a bed depth of 50 cm, and it was filled with gravel with an average particulate diameter of 9 mm and bed porosity of 0.4. Sampling points were placed along the wetland, and they made it possible to introduce temperature or dissolved oxygen probes. *Phragmites australis*, which was purchased from a commercial greenhouse, was planted in the wetland in autumn 2011 (20 plants m^{-2}), although this work was performed in the period February-October 2014, and the plants were completely developed during the experimental work. Regarding the MFC elements, rectangular (each was 70 cm × 15 cm and 3 cm thick) graphite plate electrodes were located in the gravel bed, and the distance between them was 26 cm. The anode plate was located 12 cm above the bottom of the wetland, and an identical graphite cathode plate was also located 12 cm below the wetland surface. Both electrodes were located in the subsurface water flow. The anode and cathode were connected by a 120 Ω resistor.

A 2-cm-thick layer of calcium bentonite (*Bentonil A*, from *Süd-Chemie*) separated the anode and cathode compartments in order to limit the growth of roots to the upper area only, where the cathode was located. The raw wastewater flow passed through the anode compartment, and subsequently, the outlet flow was pumped to the cathodic compartment via horizontal subsurface flow and finally left the wetland.

2.2 Synthetic wastewater

The synthetic domestic wastewater composition included glucose (175 mg L^{1}), $CH_3COONa{\cdot}3H_2O$ (175 mg L^{-1}), $NaHCO_3$ (144 mg L^{-1}), KH_2PO_4 (58 mg L^{-1}), $MgCl_2{\cdot}6H_2O$ (48 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (39 mg L^{-1}), $(NH_4)_2SO_4$ (146 mg L^{-1}), and $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ (109 mg L^{-1}). The resulting main inlet wastewater parameters of this synthetic wastewater were as follows: total suspended solids (TSS): 0-5 mg L^{-1}, chemical oxygen demand (COD): 300 mg L^{-1}, inorganic salt concentration: 515 mg L^{-1}, and electrical conductivity: 0.9 mS cm^{-1}. Over the course of the experiment, the salinity concentrations were modified as described later in this paper. It was considered that the COD level was high enough, but not excessive, to perform the experimental study, and it was always maintained constant.

2.3. Experimental procedure.

A start-up period was not necessary because the wetland had been continuously working since autumn 2011, using synthetic wastewater (Villaseñor Camacho et al., 2014). The wetland worked under a continuous operation mode over five consecutive experimental periods of approximately 2 months each. The wastewater salinity was increased in each new period by increasing the NaCl concentration in the synthetic wastewater. The salinity was the only variable under study, so all the others parameters were kept constant. The CW-MFC performance was monitored during every stationary period. Also, a batch experiment was performed at the end of each period by stopping the water flow during 9 days. Table 1 shows the NaCl concentration, the total inorganic salt concentration and the electrical conductivity of the wastewater used in every period. A constant wastewater flow of 35 L d^{-1} was used to maintain a hydraulic residence time of 2.75 days (except at the end of each period in which the batch experiment was performed). The wastewater pH was always maintained at approximately 7.4 by means of the buffer capacity of the synthetic medium, and the air-conditioning system available in the greenhouse maintained the room temperature between 19 and 27ºC.

Table 1. Salinity concentration and wastewater conductivity during the consecutive experimental periods.

Period	NaCl added to wastewater (g L^{-1})	Total inorganic salt concentration (g L^{-1})	Average electrical conductivity (mS cm^{-1})
I	0	0.51	0.9
II	2	2.51	4.7
III	4	4.51	9.1
IV	6	6.51	11.7
V	9	9.51	15.1

2.4. Sampling, analysis, and electrochemical monitoring.

The whole system was monitored twice every week. Samples of the influent and effluents of the two electrode compartments were taken, and the soluble COD, TSS and volatile suspended solids (VSS) concentrations were analysed in the laboratory according to standard methods (A.P.H.A., 1998). The dissolved oxygen (DO) level in the anodic compartment was measured in the anodic effluent, and the DO was also measured in the cathodic compartment *in situ* using the sampling points. The DO was measured using a YSI-5000 dissolved oxygen probe. The potentials between the edges of the external resistance were continuously monitored by a multimeter in order to continuously monitor the value of the cell potential.

3. Results and Discussion

Figure 2 shows the changes in the performance of the CW-MFC over the five periods of study, for which increasing salinity (with step disturbances) was fed to the bioelectrochemical device. The COD and cell voltage were monitored in order to determine the changes in performance of the CW-MFC. COD, the first parameter, describes the operation of the device as an environmental remediation technology, while the cell voltage describes its performance as an energy production technology.

The CW-MFC functioned for over approximately 2 months in the first experimental period, showing stationary and stable performance. As expected, the wastewater treatment results were successful. The soluble COD at the anode compartment outlet was approximately 25 ppm, while the soluble COD concentration at the cathode compartment outlet (the final effluent) was negligible. The VSS concentration in the effluent varied between 0 and 10 ppm, and this variation was caused by the probable detachment and drag of organic material from inside the wetland because the VSS concentration of the inlet wastewater was negligible.

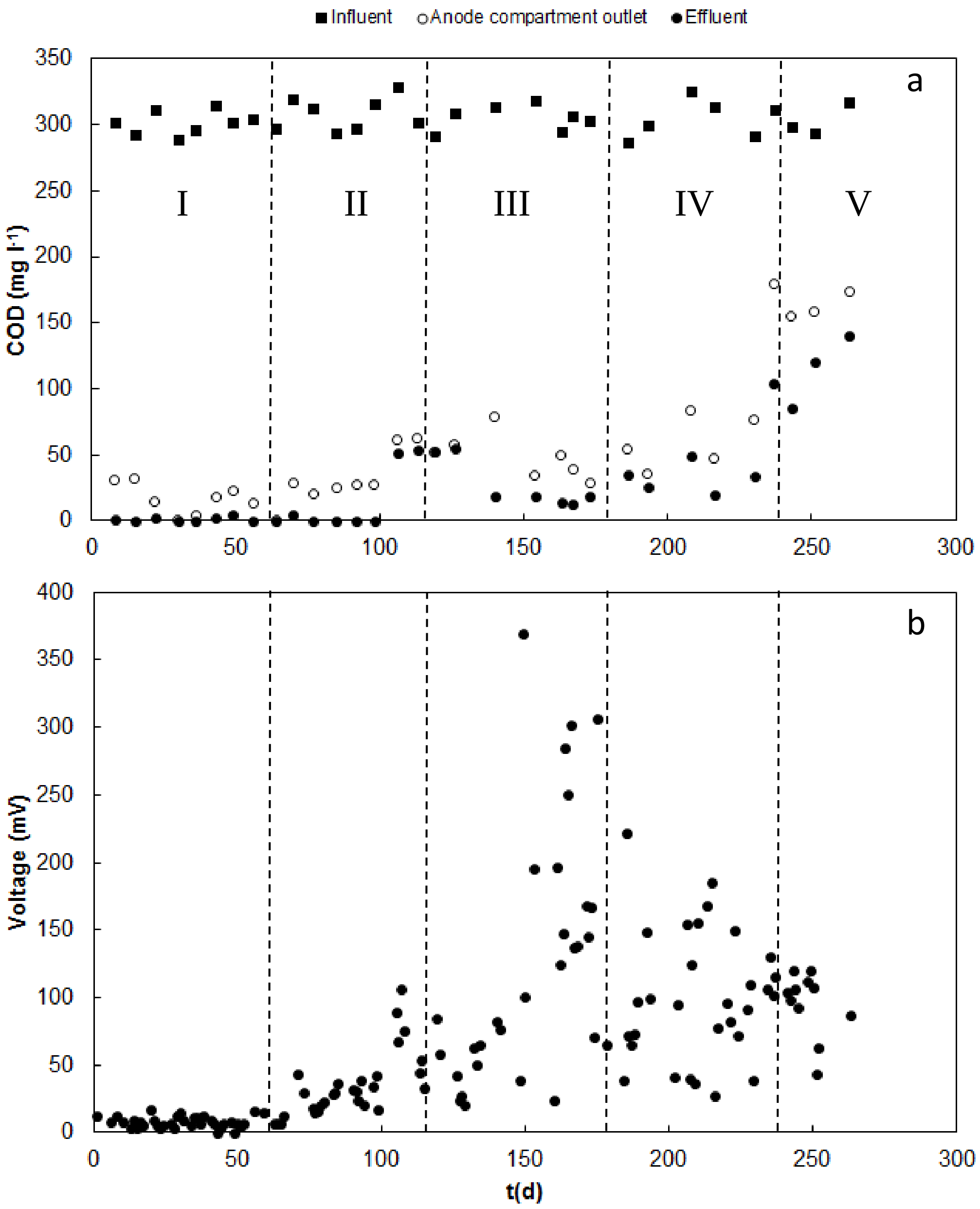

Figure 2.

From an electrochemical point of view, the CW also worked as an MFC over period I, and it generated electricity. However, the voltage values monitored were not as high as expected (average cell voltage was 10 mV with a load of 120 ohm), in particular when this result is compared with the results obtained in previous operation periods, for which the wetland worked under very similar conditions. At this point, it is important to note that the CW-MFC had been operating uninterrupted for more than three years. Thus, over the first stationary operation period (2012), the CW-MFC generated approximately 18 mV per unit of the influent wastewater organic loading rate (g_{COD} m^{-2} d^{-1}) (Villaseñor et al., 2013), and over the second operation period (2013), it generated approximately 13 mV per unit of the organic loading rate (Villaseñor Camacho et al., 2014). The results shown in the present work (obtained over the 3rd year of continuous operation) reached only 0.6 mV per unit of the organic loading rate. Thus, the MFC efficiency strongly decreased over three years, although a high wastewater treatment efficiency remained. Some previous research suggested that it is important to study the long-term performance of these types of MFCs based on natural ecosystems. The decrease in the efficiency has been previously related to the deterioration of the cathode (Zhang et al., 2012) or to the clogging phenomena in the cathode zone (Zhang et al., 2011). It must be noted that clogging is one of the classical problems associated with the long-term operation of subsurface constructed wetlands (García et al., 2010). The reasons for such a low efficiency are unknown, although the system showed stable operation, which is necessary for continued study in this work.

Figure 2a shows the COD concentrations in the inlet wastewater, the anode compartment outlet and the cathode compartment outlet (final effluent) over the consecutive step disturbances as a function of the value of the salinity. The increase in salinity between periods I and II (from 0.51 to 2.51 g L^{-1}) was observed to cause a slight decrease in the efficiency of the anodic oxidation of the organic matter, but the COD concentration in the final effluent remained negligible. Moreover, an increasing progressive deterioration can be observed in the anodic oxidation in periods III to V, but in this case, it adversely affected the final effluent COD concentrations as the values measured were increasingly higher. This observation points out the influence of the salinity on the metabolic activity of the microorganisms and that high values of the salinity negatively affect the application of CW as environmental remediation technology. Therefore, high salinity values should be prevented in order to assure a good performance with regards to wastewater treatment.

Figure 2b shows the cell voltages (120 ohm resistance) generated by the CW-MFC over the entire experimental period. The cell voltage increased as the wastewater salinity increased. Although there were some variability and fluctuations in the data, a clear increasing trend was observed until the end of period III (150 d). However, the fluctuations became very large from that point onwards. High cell voltages were observed, although they were unstable. Instability would be probably associated with the declining performance of the microbial metabolism caused by the higher salinity. Finally, a clear decreasing trend was observed in the last period (marked as V).

Figure 3a summarizes the average values of the cell voltage measured during each period of this experimental study and the “electrogenic ratio”, that is, the percentage of the COD consumed by bioelectrogenic microorganisms. The electrogenic ratio is calculated by eq. 1. $r_{COD\text{-}electrogenic}$ is the COD consumption rate by bioelectrogenic microorganisms and is calculated by eq. 2, where j is the current density, A is the anode area, n is the number of electrons donated per mol of COD consumed, and F is the Faraday constant. r_{COD} is the total COD consumption rate at the anode outlet, which can be calculated by the mass balance shown in eq. 3, which takes into account the flow-pattern of the CW-MFC, where q is the volumetric flow rate. Figure 3b shows the values calculated by equations 2 and 3.

$$electrogenic\ ratio\ (\%) = \left(r_{COD-electrogenic}/r_{COD}\right) \cdot 100 \qquad (1)$$

$$r_{COD-electrogenic} = (jA/4F) \qquad (2)$$

$$qCOD_{in} - qCOD_{out} + rCOD \cdot V = V(dCOD/dt) \qquad (3)$$

Figure 3b shows that the $r_{\text{COD-electrogen}}$ increased when the salinity increased from 0.5 to 4.5 g L^{-1}, and almost remained unchanged up to 9.5 g L^{-1}, while the r_{COD} decreased with increase in salinity, resulting in high electrogenic ratio at high salinity levels. It seems that the electrogens could be more tolerant to salinity increase than other COD degradation microorganisms. Moreover, the percentage $r_{\text{COD-electrogen}}$ only accounts for a small part of the COD removal, indicating that the MFC functioning would not be well established in the test CW-MFC system.

Taking all these points into account, figure 3a shows that the best electric current generation result (130 mV) was obtained when using a wastewater salinity concentration between 4 and 5 g L^{-1}. Regarding the efficiency in the use of the COD by electrogenic microorganisms, figure 3a also shows an optimal salinity between 4 and 5 g L^{-1}. Upward of these concentrations, the efficiency of the degradation of the COD in the anode decreases (figure 3b). This phenomenon caused a relatively high percentage for the electrogenic ratio (figure 3a, using 9.51 g L^{-1} salinity), but as will be discussed later, the authors consider that this is not a stable condition.

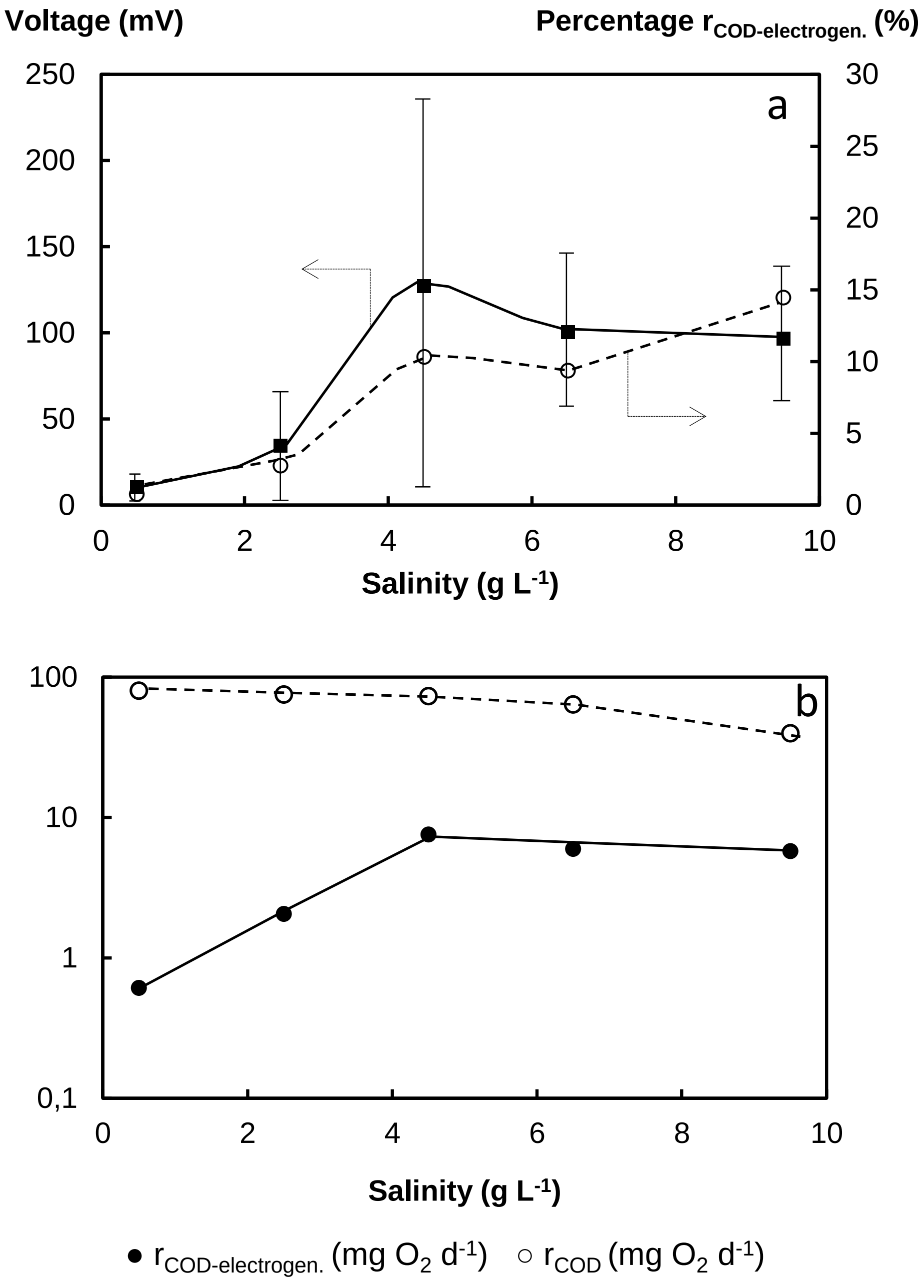

Figure 3

Figure 4 shows the time-course of the dissolved oxygen and VSS concentrations at the outlet of the anode and cathode compartments over the five stages. As expected, the anode compartment worked under nearly completely anaerobic conditions, while the cathode compartment showed concentrations very close to 2 ppm, which are similar to the DO concentrations previously reported in this system (Villaseñor et al., 2013). This condition ensures enough oxygen for the proper operation of the cathode reduction process. Subsequently, a continuous DO concentration decrease was observed as the salinity increased. There may be several reasons for this decline, such as the decrease of oxygen solubility in water or a negative impact in the plants' growth and, hence, a decrease in the roots' aeration potential. However, according to the COD profile observed in figure 2a, it may be hypothesized that the DO level decreased because of the salinity inhibitory effect on the anode microbiological performance, as the organic matter was not completely oxidized, and it produced an oxygen demand in the cathode compartment. Thus, the aeration potential of the plants would not be high enough to replace the oxygen consumption. Then, cathode aeration could be beneficial in such situation to maintain DO level and so maintain MFC working, but it must be considered also the aeration power consumption and costs in the global balance.

Figure 4b shows a progressive increase in the VSS concentration in the outlet of both compartments, especially in period II. The VSS could be related to the detachment phenomenon, which is usually associated with the deterioration of a biofilm due to the inhibition of microbial growth. It is assumed again that the inhibition occurred in the anode zone because both VSS profiles were very similar, and sometimes, even higher VSS concentrations were measured at the outlet of the anode compartment.

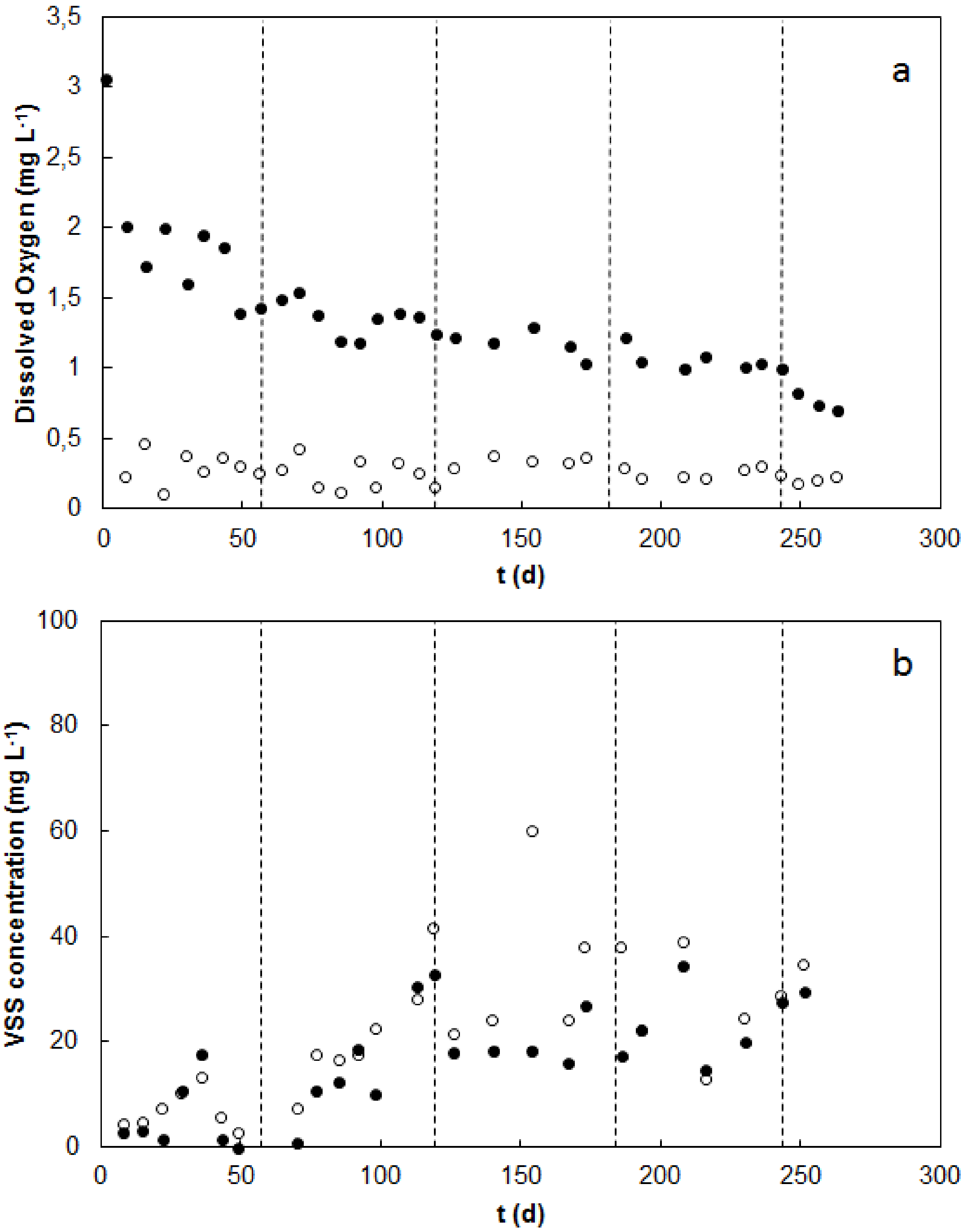

Figure 4

Some previous works have reported the influence of high salinity levels on the performance of CWs or MFCs. Regarding MFCs, Lefebvre et al. (2012) tested an MFC treating wastewater under high NaCl concentrations. They observed a decrease in the internal ohmic resistance and an increase in the electric power generation, but they also found problems in the anodic biological process. They concluded that the antagonistic effect of NaCl on the anolyte conductivity and biofilm growth made bioelectricity production advantageous at salinity concentrations of up to 20 g L^{-1} of NaCl, but the inhibition of anodophilic microorganisms began at a concentration of 10 g L^{-1} NaCl. Liu et al. (2005) tested an MFC using NaCl concentrations of up to 400 mM, and the electric power generation always increased. They estimated that the maximum concentration of NaCl at which the microbial growth inhibition would occur is approximately 3%. Regarding CWs, there are some works that reported salinity inhibition of the microbial processes inside the wetland (Gao et al., 2012; Lin et al., 2008). Regarding the possible negative impact of the high salinity on the plants, in the present work, only a clear deterioration during the last period has been observed, that is, under 9.51 g L^{-1} of salt. Different macrophyte plants in constructed wetlands treating high salinity wastewater have been tested in previous reported works. Gao et al. (2012) tested 12 different plants and observed that high salinity damaged their growth and decreased their nutrient uptake capacity and aeration potential in the roots zone. However, *Phragmites australis* was the most resistant species, and it functioned correctly up to a salt concentration of 20 g L^{-1}.

According to our results and previous research, it is assumed that higher salinity levels would reduce the internal ohmic resistance and, thus, increase the electric power generation, although there is a maximum admissible level for plants and microorganisms. The maximum level is between 4 and 5 g L^{-1} under the continuous operation mode. However, lower salt concentrations (approximately 3 g L^{-1}) begin to damage the anodic biological process. We assumed that the role of the plants remained constant, and plant damage was only observed from salinity concentrations of 9.51 g L^{-1}

and higher. The electric power generation under 9.51 g L^{-1} is high, although we consider that this condition is unstable.

Figure 5a shows the results of the batch experiments performed at the end of each continuous period. The wastewater inflow was stopped in the batch experiments in order to allow enough retention time to oxidize the organic matter in the anode compartment. A voltage increase up to a maximum value can be observed in every experiment, and subsequently, there is a decrease until the "fuel" is depleted. Figure 5b shows the maximum cell voltage measured and the average voltage generation rate (calculated from the beginning to the maximum value). The figure also shows that the positive effect of the salinity increases as higher voltage differences are measured until the salt concentration reaches 4.51 g L^{-1}. Above this concentration, the maximum voltage decreases. Additionally, it can be observed that the voltage values are much higher than the values obtained under continuous operation, and this result suggests that higher retention times would be advisable. Figure 5b shows that the higher voltage generation rate corresponds to a relatively low salinity concentration of 2.51 g L^{-1}, although the maximum cell voltage (600 mV) corresponds to 4.51 g L^{-1}. This result would confirm the results obtained under continuous operation; that is, under 4.51 g L^{-1}, it is possible to obtain transiently higher potential differences in the MFC, although it takes longer to generate electricity because it is an unstable condition, as the microbiological process would begin to deteriorate at a concentration of approximately 3 g L^{-1}.

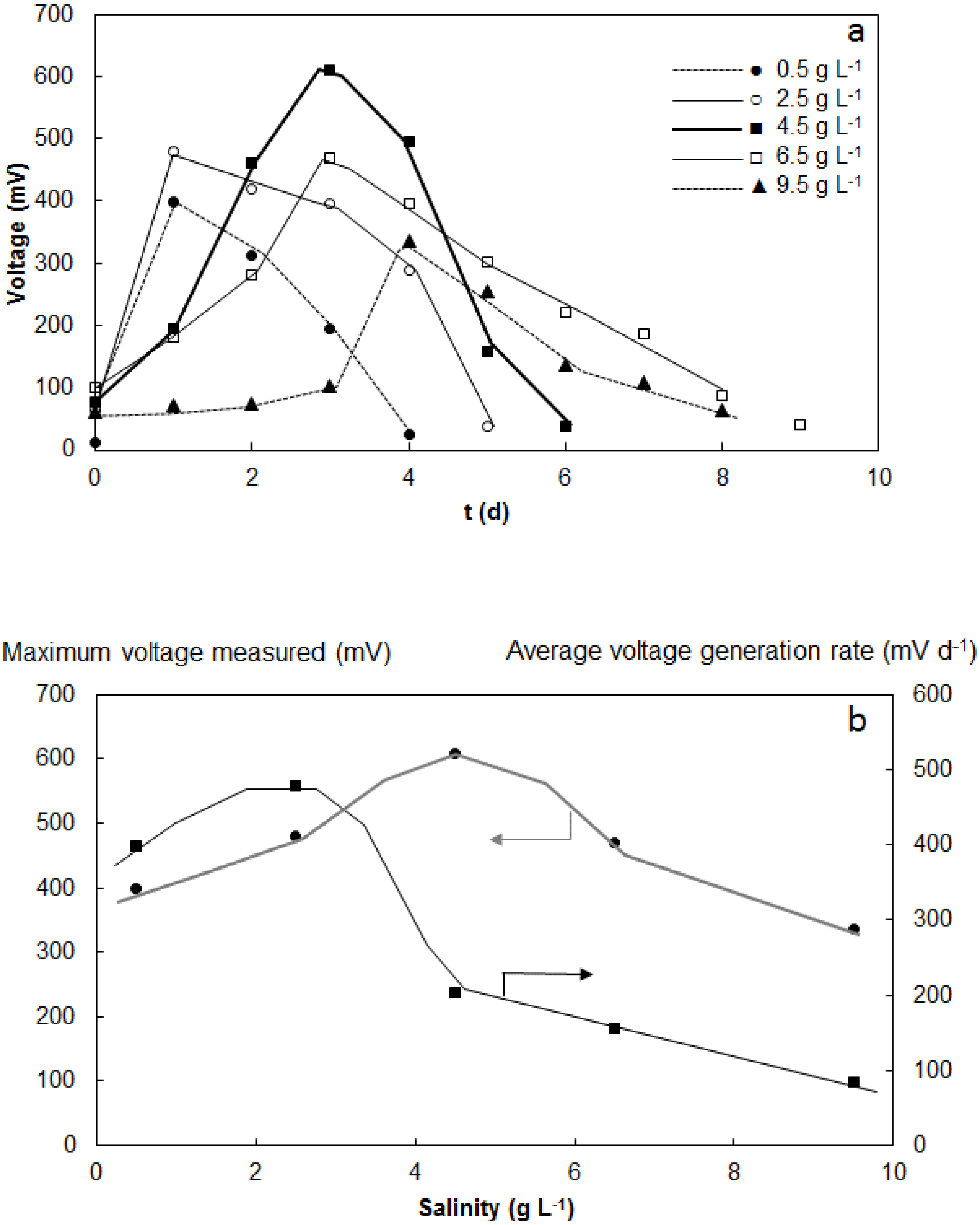
a
Voltage (mV)
t (d)
0.5 g L-1
2.5 g L-1
4.5 g L-1
6.5 g L-1
9.5 g L-1
0
100
200
300
400
500
600
700
0
2
4
6
8
10
b
Maximum voltage measured (mV)
Average voltage generation rate (mV d-1)
Salinity (g L-1)
0
100
200
300
400
500
600
700
0
100
200
300
400
500
600
0
2
4
6
8
10

Figure 5

From the results in the present work, it could be argued that the CW-MFC would improve the electric power generation while treating wastewater with high salinity, although there is a maximum salt level that first inhibits the microbial process. Later, the salt level damages the plants' performance. Some researchers proved previously that it is possible to use halotolerant plant species (Webb et al., 2012) or to inoculate halotolerant microorganisms (Karajić et al., 2010) in CWs. Regarding MFC, Monzon et al. (2015) proposed using extreme halophilic microbes. The authors of the present work consider that the application of such halotolerant microbes and plants could allow for the application of CW-MFC to simultaneously treat high salinity industrial wastewater and produce electricity.

According to these results, the authors considered that high salinity in industrial effluents could be a positive influence in the development of the CW-MFC technology as it improved voltage differences. However the challenge would be if halo-tolerant microorganisms and plants could be adapted to these conditions, which may be the focus of further research.

4. Conclusions

The increasing salinity first improved the cell voltage under continuous operation, and the maximum voltage corresponded to a salinity concentration between 4 and 5 g L^{-1}, while subsequently higher salinity levels caused the opposite effect. However, according to the COD, DO and VSS measurements, microbiological inhibition in the anode zone appeared early at an approximate salinity of 3 g L^{-1}. The batch experiments confirmed this behaviour. The wetland plants remained without apparent damage up to salinity levels of 9.51 g L^{-1}.

Acknowledgements

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Figure 1. Experimental installation. (1) Wastewater feeding; (2) Anode; (3) Cathode; (4) Bentonite layer; (5) Reed plants; (6) Sampling points; (7) Treated effluent; (8) Resistance; and (9) Multimeter.

Figure 2. Influent and effluent COD concentrations (a) and voltage generation (b) during the entire experimental period.

Figure 3. (a) Average voltage generation values and error bars, and percentage of the COD consumed by bioelectrogenic microorganisms in the MFC depending on the salinity of the wastewater and (b) the total and electrogenic COD consumption rates.

Figure 4. Dissolved oxygen concentrations (a) and VSS concentrations (b) in the anode (○) and the cathode (●) effluents.

Figure 5. Cell voltage generated (a) and the maximum and average cell voltage change rates (b) in the batch experiments.

THE SALINITY EFFECTS ON THE PERFORMANCE OF A CONSTRUCTED WETLAND-MICROBIAL FUEL CELL

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Abstract

The objective of the present work is to study the influence of the wastewater salinity concentration on the performance of a Constructed Wetland-Microbial Fuel Cell (CW-MFC) for simultaneous water pollution control and electricity generation. The work has been carried out under the hypothesis that increasing the salinity may improve the electricity production because of a lower internal ohmic resistance, although it could damage the microbiological processes or the plants. A pilot-scale horizontal subsurface flow CW, modified to function as an MFC, was operated under a continuous operation mode over five consecutive experimental periods of approximately 2 months each. The wastewater salinity was increased in each new period by steeply increasing the NaCl concentration in the synthetic wastewater from 0.51 to 9.51 g L^{-1}. The CW-MFC performance was monitored during every stationary period. The increasing salinity first improved the cell voltage, and the resultant maximum voltage (130 mV) under continuous operation corresponded to a salinity concentration between 4 and 5 g L^{-1}. However, subsequently higher salinity levels caused the opposite effect. The maximum voltage was obtained in an unstable condition, as microbiological inhibition in the anode zone appeared early, at approximate salinity levels of only 3 g L^{-1}. Batch experiments confirmed the results, and higher cell voltage values up to 600 mV were obtained if longer retention times were allowed. The wetland plants (*Phragmites australis*) were only damaged at a salinity concentration of 9.51 g L^{-1}.

Keywords

Constructed Wetland; Microbial Fuel Cell; bioelectrogenic; salinity.

1. Introduction

Microbial Fuel Cells (MFCs) are electrochemical devices that can obtain electricity from organic matter by means of the activity of bioelectrogenic microorganisms. As conventional fuel cells, they also have two chambers, with electrodes working under a difference of electric potential. However, what makes MFCs unique is the fact that active microorganisms in the anode chamber are capable of oxidizing the organic matter using electric current instead of oxygen by various complementary mechanisms, becoming the real biological catalyst of the electrochemical device. MFCs have been extensively studied, and many scientific papers and books have been published in recent years. Because organic waste can be used as fuel for MFCs, this technology has been proposed for environmental remediation purposes, and the concept of bioelectrochemical wastewater treatment is currently receiving significant attention (Gude, 2016).

In this context, currently, a very promising variety of MFCs is being studied, consisting of integrating MFC technology into natural ecosystems or low-cost environmental remediation technologies. There are currently three of these technologies, including the Sediment Microbial Fuel Cells (SMFCs), also called Benthic MFCs; the Plant-type Microbial Fuel Cells (PMFCs); and MFCs coupled to Constructed Wetlands (CW-MFCs), and they all are based on the redox potential differences that naturally exist between the top water-air interface and the anaerobic bottom of these ecosystems. There is also a large amount of literature on the subject. All of these technologies have been described in a recent review (Fernández et al., 2015).

One of the most interesting options of these low-cost technologies for wastewater treatment are constructed wetlands (CWs). These systems are wetlands, isolated from the underground below them, that receive wastewater. Wastewater treatment in CWs is the result of a combination of natural physical, chemical, and biological phenomena (Zhi and Ji, 2012). There are different types of CWs; subsurface flow wetlands are one of the most implemented types (García et al., 2010). Basically, they consist of a wastewater subsurface flux flowing through a porous gravel bed, which includes macrophyte plants growing on the top surface and a mixed microbial population in the form of biofilms attached to the gravel and roots.

The study of the combination of CWs and MFCs is recent. The first work was published by Yadav (2010), and although the number of papers is still low, it is increasing exponentially in the last 5 years (Doherty et al., 2015a). The works studied the influence of the type or concentration of the organic pollutants (Corbella et al., 2015; Fang et al., 2015; Liu et al., 2014; Srivastava et al., 2015; Wu et al., 2015a), the role of plants and the effect of the position of the roots (Corbella et al., 2014; Fang et al., 2013; Liu et al., 2014), the water flow configuration (Corbella et al., 2014; Corbella et al., 2015; Doherty et al., 2015b), the type of cathode (Liu et al., 2014; Srivastava et al., 2015) and the distance between the electrodes (Doherty et al., 2015b; Doherty et al., 2015c). The works usually aimed to improve the cell efficiency through maintaining a high redox potential between the electrodes and reducing the internal resistance.

To the authors’ knowledge, wastewater salinity may be an important aspect influencing the CW-MFC performance, and its effect has not yet been studied. Initially, wastewater salinity is expected to have a contradictory effect on the two main processes (biological and electrochemical) occurring in the device: negative in the biological process and positive in the electrochemical process. Regarding the possible biological effects, constructed wetlands have been extensively applied for industrial wastewater treatment (Gao et al., 2015; Wu et al., 2015b), and there are some works focused on high salinity wastewater treatment by CWs (Gao et al., 2015; Karajić et al., 2010). Klomjek and Nitisoravut (2005) reported that some plant species used in CWs were adversely affected by high salinity in wastewater. Gao et al. (2015) tested twelve different plants species and detected a salinity level at which the treatment efficiency begins to fall.

Regarding the microbial role in wetlands, Gao et al. (2012) reported that the increase in salinity strongly reduced the microorganism concentration, while Lin et al. (2008) reported inhibition of the microbial activity.

Regarding the electrochemical performance, Logan (2008) and Rozendal et al. (2008) described the expected positive effect of increasing the wastewater salinity in an MFC and explained it in terms of the increase in the electrical conductivity of the electrolyte, which reduces the internal ohmic resistance and results in an ohmic drop of the fuel cell. However, they also stated the expected negative effect in the microbial activity at high salinity levels. Likewise, Lefebvre et al. (2012) reported increased power and decreased internal resistances using increasing salinity levels but found a drastic power reduction at a NaCl level of 20 g/L because of the inhibition of microbial growth.

In this context, it could be considered that the wastewater salinity could be an important factor to be tested in the performance of the combined CW-MFC system, and this paper aims to describe and discuss the results of the study of a pilot-scale CW-MFC treating wastewater with a steeply increasing salinity. Until now, this subject has not been tested, and it is hypothesized that a moderate salinity could produce a positive effect on the fuel cell performance, although it could also negatively affect the role of plants or microorganisms in the subsurface system. Because of the complexity of the CW-MFC mechanisms, the global effect and the salt tolerance limit are still unknown. The results could be of great relevance because of their potential applicability to the treatment of high salinity industrial wastewater. For instance, leachate from domestic landfills, or washing effluents from agro-food industries could be suitable for such combined CW-MFC technology.

2. Materials and Methods

The materials, experimental procedures, and analytical methods have been described thoroughly elsewhere (Villaseñor et al., 2013; Villaseñor Camacho et al., 2014). The following subsections only describe the important details.

2.1. Constructed wetland - microbial fuel cell microcosm.

The experimental installation consisted of a pilot-scale horizontal subsurface flow CW for wastewater treatment, modified to function as an MFC (figure 1).

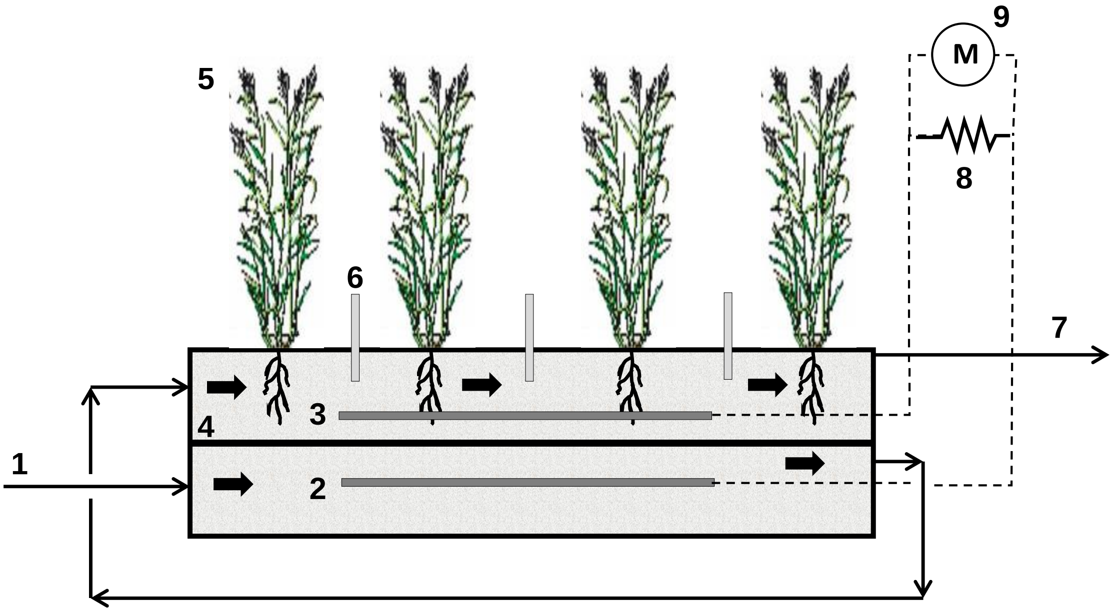


Figure 1

The installation was located in the greenhouse facility of the Institute for Chemical and Environmental Technology of the University of Castilla La Mancha, Ciudad Real (Spain). The wetland consisted of a 115 cm × 47 cm plastic channel with a bed depth of 50 cm, and it was filled with gravel with an average particulate diameter of 9 mm and bed porosity of 0.4. Sampling points were placed along the wetland, and they made it possible to introduce temperature or dissolved oxygen probes. *Phragmites australis*, which was purchased from a commercial greenhouse, was planted in the wetland in autumn 2011 (20 plants m^{-2}), although this work was performed in the period February-October 2014, and the plants were completely developed during the experimental work. Regarding the MFC elements, rectangular (each was 70 cm × 15 cm and 3 cm thick) graphite plate electrodes were located in the gravel bed, and the distance between them was 26 cm. The anode plate was located 12 cm above the bottom of the wetland, and an identical graphite cathode plate was also located 12 cm below the wetland surface. Both electrodes were located in the subsurface water flow. The anode and cathode were connected by a 120 Ω resistor.

A 2-cm-thick layer of calcium bentonite (*Bentonil A*, from *Süd-Chemie*) separated the anode and cathode compartments in order to limit the growth of roots to the upper area only, where the cathode was located. The raw wastewater flow passed through the anode compartment, and subsequently, the outlet flow was pumped to the cathodic compartment via horizontal subsurface flow and finally left the wetland.

2.2 Synthetic wastewater

The synthetic domestic wastewater composition included glucose (175 mg L^{1}), $CH_3COONa{\cdot}3H_2O$ (175 mg L^{-1}), $NaHCO_3$ (144 mg L^{-1}), KH_2PO_4 (58 mg L^{-1}), $MgCl_2{\cdot}6H_2O$ (48 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (39 mg L^{-1}), $(NH_4)_2SO_4$ (146 mg L^{-1}), and $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ (109 mg L^{-1}). The resulting main inlet wastewater parameters of this synthetic wastewater were as follows: total suspended solids (TSS): 0-5 mg L^{-1}, chemical oxygen demand (COD): 300 mg L^{-1}, inorganic salt concentration: 515 mg L^{-1}, and electrical conductivity: 0.9 mS cm^{-1}. Over the course of the experiment, the salinity concentrations were modified as described later in this paper. It was considered that the

COD level was high enough, but not excessive, to perform the experimental study, and it was always maintained constant.

2.3. Experimental procedure.

A start-up period was not necessary because the wetland had been continuously working since autumn 2011, using synthetic wastewater (Villaseñor Camacho et al., 2014). The wetland worked under a continuous operation mode over five consecutive experimental periods of approximately 2 months each. The wastewater salinity was increased in each new period by increasing the NaCl concentration in the synthetic wastewater. The salinity was the only variable under study, so all the others parameters were kept constant. The CW-MFC performance was monitored during every stationary period. Also, a batch experiment was performed at the end of each period by stopping the water flow during 9 days. Table 1 shows the NaCl concentration, the total inorganic salt concentration and the electrical conductivity of the wastewater used in every period. A constant wastewater flow of 35 L~~l~~ d^{-1} was used to maintain a hydraulic residence time of 2.75 days (except at the end of each period in which the batch experiment was performed). The wastewater pH was always maintained at approximately 7.4 by means of the buffer capacity of the synthetic medium, and the air-conditioning system available in the greenhouse maintained the room temperature between 19 and 27ºC.

Table 1. Salinity concentration and wastewater conductivity during the consecutive experimental periods.

Period	NaCl added to	Total inorganic salt	Average electrical

	wastewater (g L^{-1})	concentration (g L^{-1})	conductivity (mS cm^{-1})
I	0	0.51	0.9
II	2	2.51	4.7
III	4	4.51	9.1
IV	6	6.51	11.7
V	9	9.51	15.1

2.4. Sampling, analysis, and electrochemical monitoring.

The whole system was monitored twice every week. Samples of the influent and effluents of the two electrode compartments were taken, and the soluble COD, TSS and volatile suspended solids (VSS) concentrations were analysed in the laboratory according to standard methods (A.P.H.A., 1998). The dissolved oxygen (DO) level in the anodic compartment was measured in the anodic effluent, and the DO was also measured in the cathodic compartment *in situ* using the sampling points. The DO was measured using a YSI-5000 dissolved oxygen probe. The potentials between the edges of the external resistance were continuously monitored by a multimeter in order to continuously monitor the value of the cell potential.

3. Results and Discussion

Figure 2 shows the changes in the performance of the CW-MFC over the five periods of study, for which increasing salinity (with step disturbances) was fed to the bioelectrochemical device. The COD and cell voltage were monitored in order to determine the changes in performance of the CW-MFC. COD, the first parameter, describes the operation of the device as an environmental remediation technology, while the cell voltage describes its performance as an energy production technology.

The CW-MFC functioned for over approximately 2 months in the first experimental period, showing stationary and stable performance. As expected, the wastewater treatment results were successful. The soluble COD at the anode compartment outlet was approximately 25 ppm, while the soluble COD concentration at the cathode compartment outlet (the final effluent) was negligible. The VSS concentration in the effluent varied between 0 and 10 ppm, and this variation was caused by the probable detachment and drag of organic material from inside the wetland because the VSS concentration of the inlet wastewater was negligible.

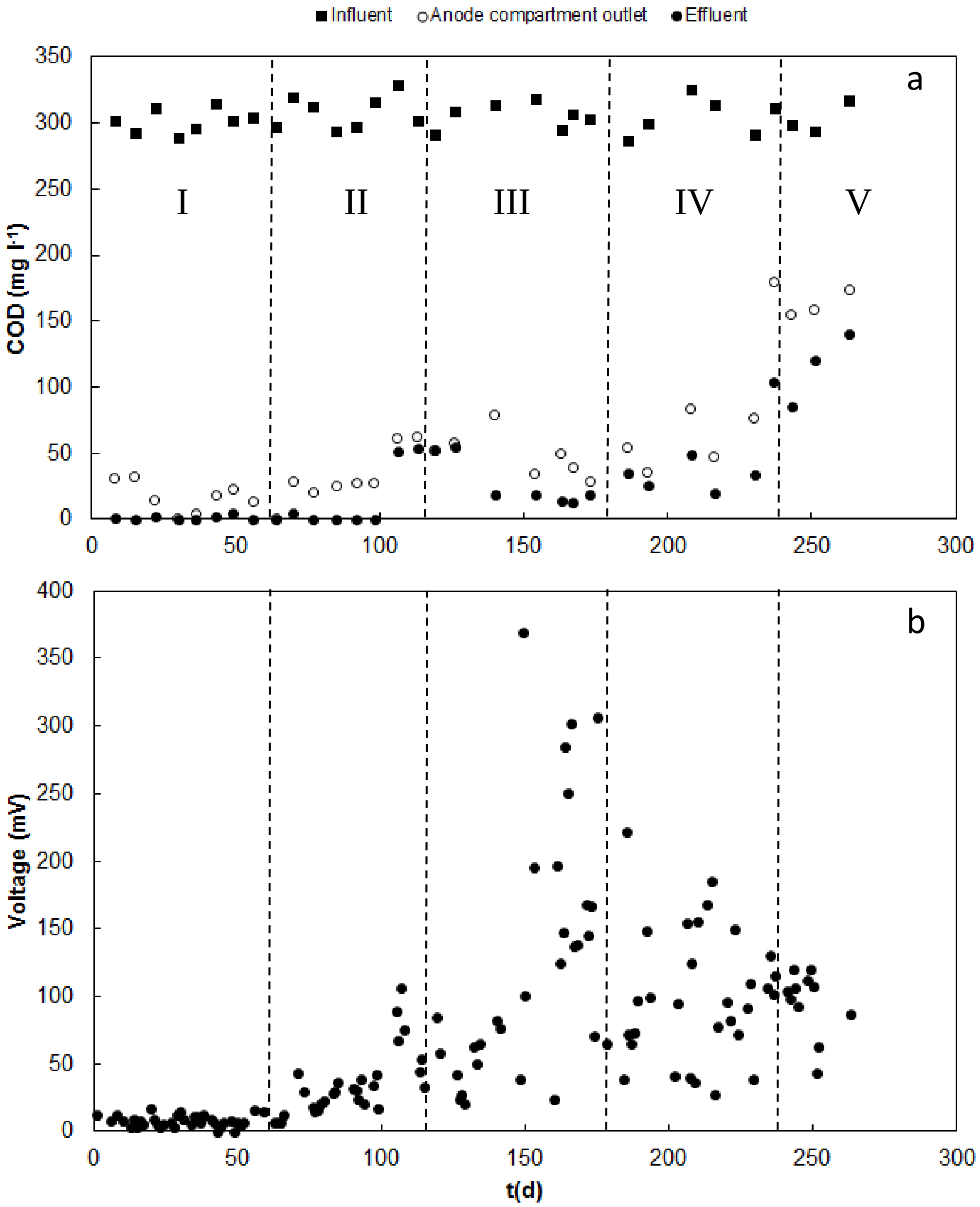


Figure 2.

From an electrochemical point of view, the CW also worked as an MFC over period I, and it generated electricity. However, the voltage values monitored were not as high as expected (average cell voltage was 10 mV with a load of 120 ohm), in particular when this result is compared with the results obtained in previous operation periods, for which the wetland worked under very similar conditions. At this point, it is important to note that the CW-MFC had been operating uninterrupted for more than three years. Thus, over the first stationary operation period (2012), the CW-MFC generated approximately 18 mV per unit of the influent wastewater organic loading rate (g_{COD} m^{-2} d^{-1}) (Villaseñor et al., 2013), and over the second operation period (2013), it generated approximately 13 mV per unit of the organic loading rate (Villaseñor Camacho et al., 2014). The results shown in the present work (obtained over the 3rd year of continuous operation) reached only 0.6 mV per unit of the organic loading rate. Thus, the MFC efficiency strongly decreased over three years, although a high wastewater treatment efficiency remained. Some previous research suggested that it is important to study the long-term performance of these types of MFCs based on natural ecosystems. The decrease in the efficiency has been previously related to the deterioration of the cathode (Zhang et al., 2012) or to the clogging phenomena in the cathode zone (Zhang et al., 2011). It must be noted that clogging is one of the classical problems associated with the long-term operation of subsurface constructed wetlands (García et al., 2010). The reasons for such a low efficiency are unknown, although the system showed stable operation, which is necessary for continued study in this work.

Figure 2a shows the COD concentrations in the inlet wastewater, the anode compartment outlet and the cathode compartment outlet (final effluent) over the consecutive step disturbances as a function of the value of the salinity. The increase in salinity between periods I and II (from 0.51 to 2.51 g L^{-1}) was observed to cause a slight decrease in the efficiency of the anodic oxidation of the organic matter, but the COD concentration in the final effluent remained negligible. Moreover, an increasing progressive deterioration can be observed in the anodic oxidation in periods III to V, but in this case, it adversely affected the final effluent COD concentrations as the values measured were increasingly higher. This observation points out the influence of the salinity on the metabolic activity of the microorganisms and that high values of the salinity negatively affect the application of CW as environmental remediation technology. Therefore, high salinity values should be prevented in order to assure a good performance with regards to wastewater treatment.

Figure 2b shows the cell voltages (120 ohm resistance) generated by the CW-MFC over the entire experimental period. The cell voltage increased as the wastewater salinity increased. Although there were some variability and fluctuations in the data, a clear increasing trend was observed until the end of period III (150 d). However, the fluctuations became very large from that point onwards. High cell voltages were observed, although they were unstable. Instability would be probably associated with the declining performance of the microbial metabolism caused by the higher salinity. Finally, a clear decreasing trend was observed in the last period (marked as V).

Figure 3a summarizes the average values of the cell voltage measured during each period of this experimental study and the “electrogenic ratio”, that is, the percentage of the COD consumed by bioelectrogenic microorganisms. The electrogenic ratio is calculated by eq. 1. $r_{\text{COD-electrogenic}}$ is the COD consumption rate by bioelectrogenic microorganisms and is calculated by eq. 2, where j is the current density, A is the anode area, n is the number of electrons donated per mol of COD consumed, and F is the Faraday constant. r_{COD} is the total COD consumption rate at the anode outlet, which can be calculated by the mass balance shown in eq. 3, which takes into account the flow-pattern of the CW-MFC, where q is the volumetric flow rate. Figure 3b shows the values calculated by equations 2 and 3.

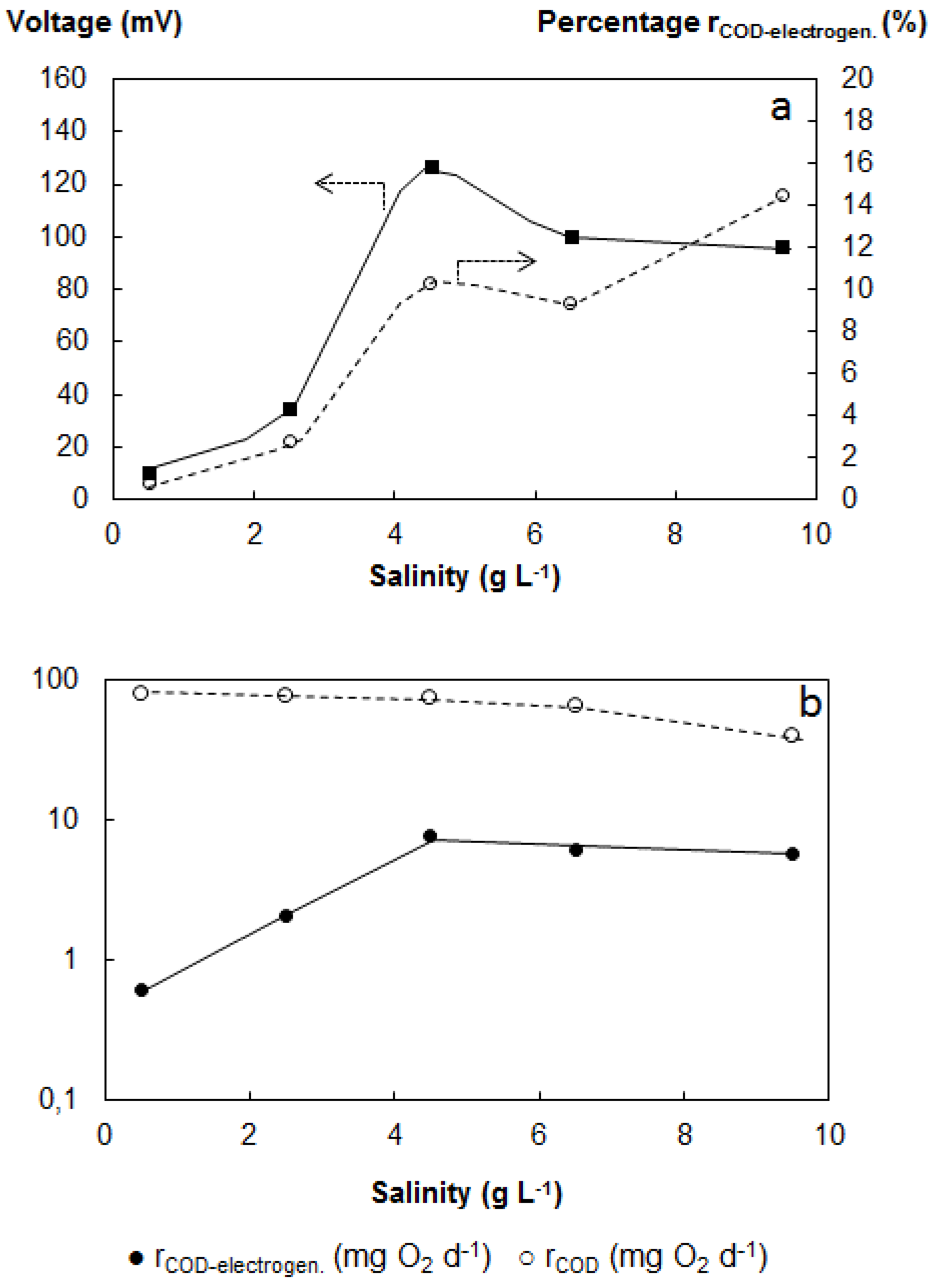
$$electrogenic\ ratio\ (\%) = \left(r_{COD-electrogenic}/r_{COD}\right) \cdot 100 \qquad (1)$$

$$r_{COD-electrogenic} = (jA/4F) \qquad (2)$$

$$qCOD_{in} - qCOD_{out} + rCOD \cdot V = V(dCOD/dt) \qquad (3)$$

Figure 3b shows that the $r_{COD\text{-}electrogen}$ increased when the salinity increased from 0.5 to 4.5 g L^{-1}, and almost remained unchanged up to 9.5 g L^{-1}, while the r_{COD} decreased with increase in salinity, resulting in high electrogenic ratio at high salinity levels. It seems that the electrogens could be more tolerant to salinity increase than other COD degradation microorganisms. Moreover, the percentage $r_{COD\text{-}electrogen}$ ~~is~~ only accounts for a small part of the COD removal, indicating that the MFC functioning would not be well established in the test CW-MFC system.

Taking all these points into account, figure 3a shows that the best electric current generation result (130 mV) was obtained when using a wastewater salinity concentration between 4 and 5 g L^{-1}. Regarding the efficiency in the use of the COD by electrogenic microorganisms, figure 3a also shows an optimal salinity between 4 and 5 g L^{-1}. Upward of these concentrations, the efficiency of the degradation of the COD in the anode decreases (figure 3b). This phenomenon caused a relatively high percentage for the electrogenic ratio (figure 3a, using 9.51 g L^{-1} salinity), but as will be discussed later, the authors consider that this is not a stable condition.


Voltage (mV)
Percentage r COD-electrogen. (%)
160
140
120
100
80
60
40
20
0
20
18
16
14
12
10
8
6
4
2
0
a
0
2
4
6
8
10
Salinity (g L-1)
100
10
1
0,1
b
0
2
4
6
8
10
Salinity (g L-1)
● r COD-electrogen. (mg O2 d-1)
○ r COD (mg O2 d-1)

287

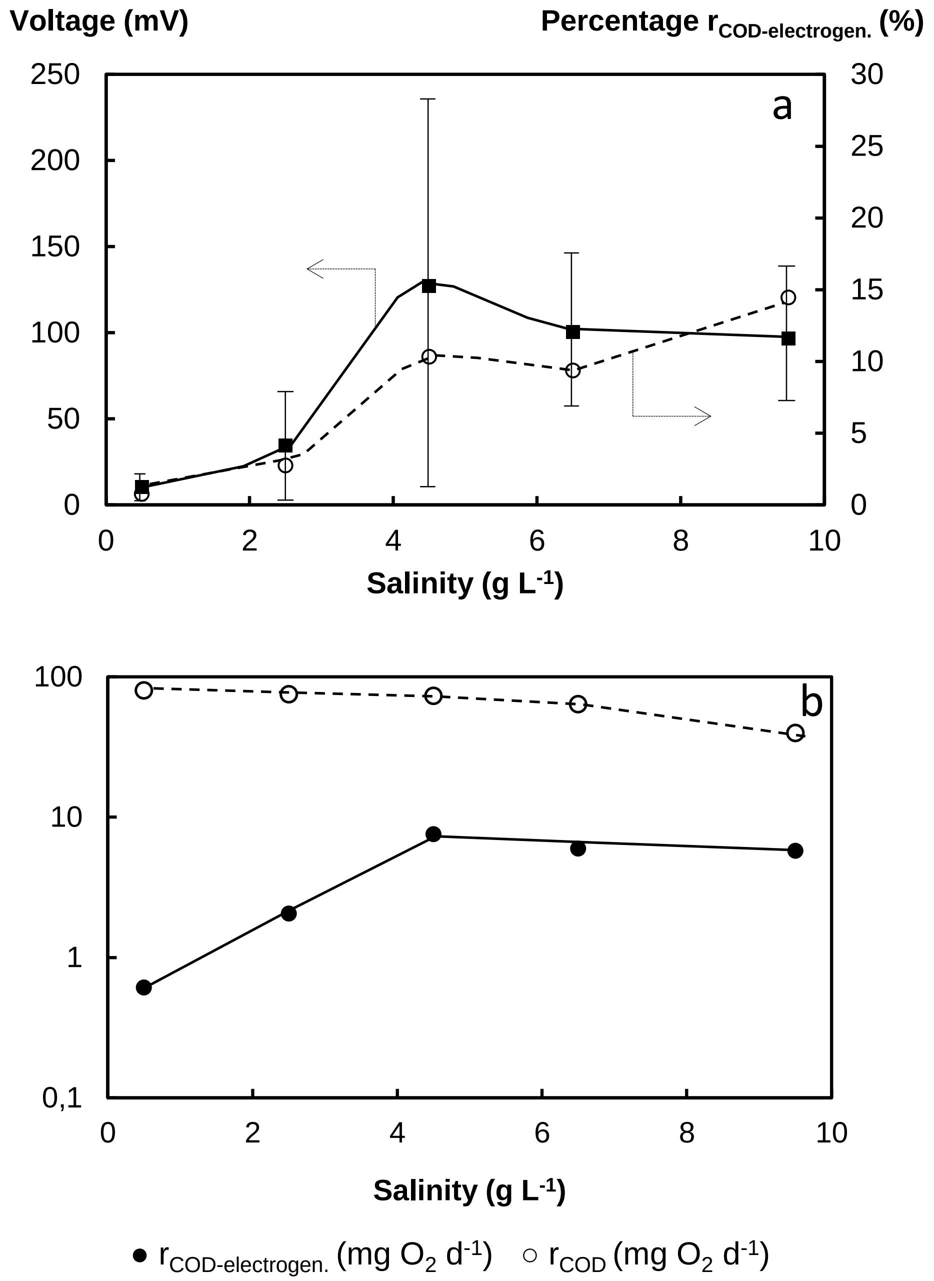


Figure 3

Figure 4 shows the time-course of the dissolved oxygen and VSS concentrations at the outlet of the anode and cathode compartments over the five stages. As expected, the anode compartment worked under nearly completely anaerobic conditions, while the cathode compartment showed concentrations very close to 2 ppm, which are similar to the DO concentrations previously reported in this system (Villaseñor et al., 2013). This condition ensures enough oxygen for the proper operation of the cathode reduction process. Subsequently, a continuous DO concentration decrease was observed as the salinity increased. There may be several reasons for this decline, such as the decrease of oxygen solubility in water or a negative impact in the plants' growth and, hence, a decrease in the roots' aeration potential. However, according to the COD profile observed in figure 2a, it may be hypothesized that the DO level decreased because of the salinity inhibitory effect on the anode microbiological performance, as the organic matter was not completely oxidized, and it produced an oxygen demand in the cathode compartment. Thus, the aeration potential of the plants would not be high enough to replace the oxygen consumption. Then, cathode aeration could be beneficial in such situation to maintain DO level and so maintain MFC working, but it must be considered also the aeration power consumption and costs in the global balance.

Figure 4b shows a progressive increase in the VSS concentration in the outlet of both compartments, especially in period II. The VSS could be related to the detachment phenomenon, which is usually associated with the deterioration of a biofilm due to the inhibition of microbial growth. It is assumed again that the inhibition occurred in the anode zone because both VSS profiles were very similar, and sometimes, even higher VSS concentrations were measured at the outlet of the anode compartment.

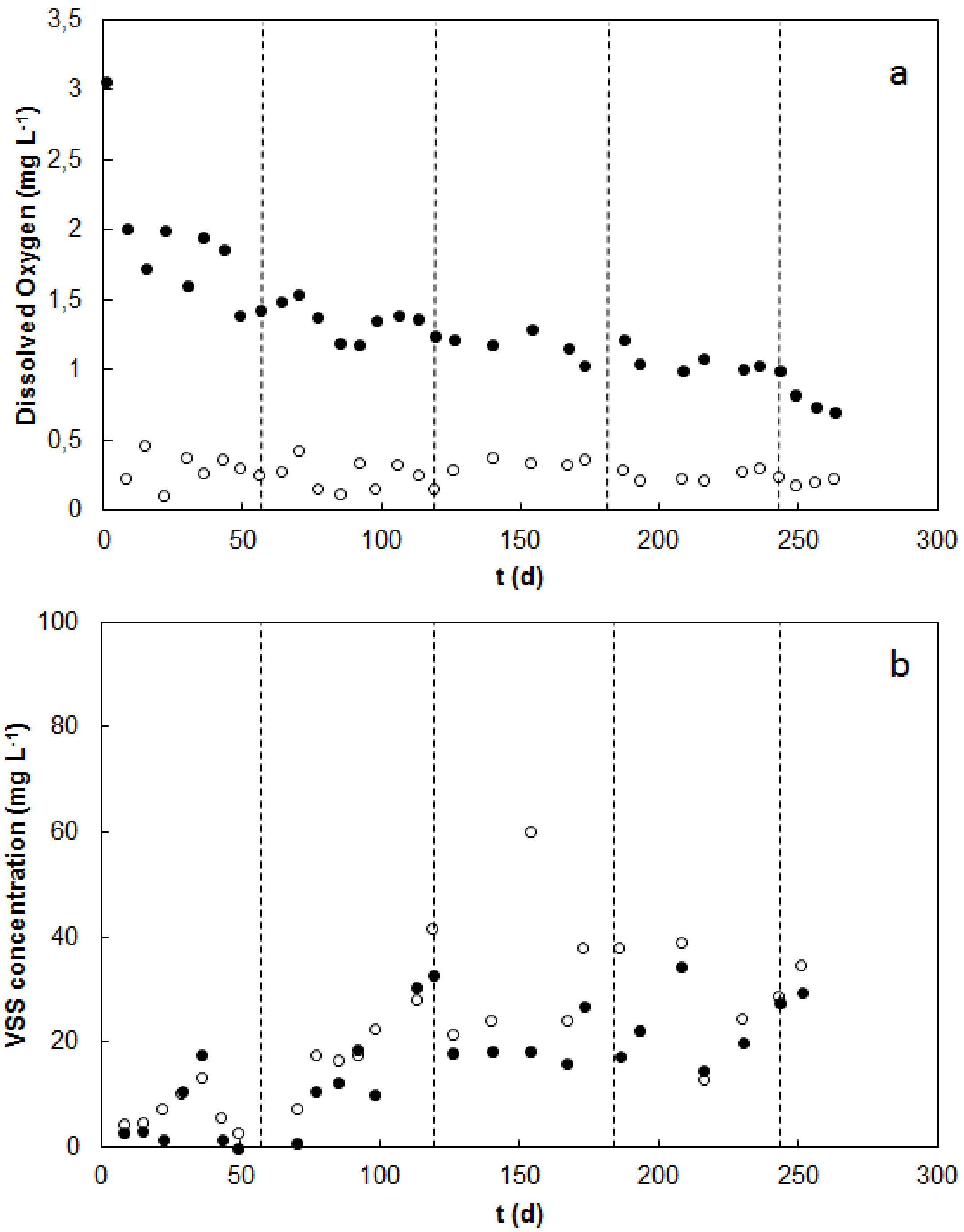


Figure 4

Some previous works have reported the influence of high salinity levels on the performance of CWs or MFCs. Regarding MFCs, Lefebvre et al. (2012) tested an MFC treating wastewater under high NaCl concentrations. They observed a decrease in the internal ohmic resistance and an increase in the electric power generation, but they also found problems in the anodic biological process. They concluded that the antagonistic effect of NaCl on the anolyte conductivity and biofilm growth made bioelectricity production advantageous at salinity concentrations of up to 20 g L^{-1} of NaCl, but the inhibition of anodophilic microorganisms began at a concentration of 10 g L^{-1} NaCl. Liu et al. (2005) tested an MFC using NaCl concentrations of up to 400 mM, and the electric power generation always increased. They estimated that the maximum concentration of NaCl at which the microbial growth inhibition would occur is approximately 3%. Regarding CWs, there are some works that reported salinity inhibition of the microbial processes inside the wetland (Gao et al., 2012; Lin et al., 2008). Regarding the possible negative impact of the high salinity on the plants, in the present work, only a clear deterioration during the last period has been observed, that is, under 9.51 g L^{-1} of salt. Different macrophyte plants in constructed wetlands treating high salinity wastewater have been tested in previous reported works. Gao et al. (2012) tested 12 different plants and observed that high salinity damaged their growth and decreased their nutrient uptake capacity and aeration potential in the roots zone. However, *Phragmites australis* was the most resistant species, and it functioned correctly up to a salt concentration of 20 g L^{-1}.

According to our results and previous research, it is assumed that higher salinity levels would reduce the internal ohmic resistance and, thus, increase the electric power generation, although there is a maximum admissible level for plants and microorganisms. The maximum level is between 4 and 5 g L^{-1} under the continuous operation mode. However, lower salt concentrations (approximately 3 g L^{-1}) begin to damage the anodic biological process. We assumed that the role of the plants remained constant, and plant damage was only observed from salinity concentrations of 9.51 g L^{-1}

and higher. The electric power generation under 9.51 g L^{-1} is high, although we consider that this condition is unstable.

Figure 5a shows the results of the batch experiments performed at the end of each continuous period. The wastewater inflow was stopped in the batch experiments in order to allow enough retention time to oxidize the organic matter in the anode compartment. A voltage increase up to a maximum value can be observed in every experiment, and subsequently, there is a decrease until the “fuel” is depleted. Figure 5b shows the maximum cell voltage measured and the average voltage generation rate (calculated from the beginning to the maximum value). The figure also shows that the positive effect of the salinity increases as higher voltage differences are measured until the salt concentration reaches 4.51 g L^{-1}. Above this concentration, the maximum voltage decreases. Additionally, it can be observed that the voltage values are much higher than the values obtained under continuous operation, and this result suggests that higher retention times would be advisable. Figure 5b shows that the higher voltage generation rate corresponds to a relatively low salinity concentration of 2.51 g L^{-1}, although the maximum cell voltage (600 mV) corresponds to 4.51 g L^{-1}. This result would confirm the results obtained under continuous operation; that is, under 4.51 g L^{-1}, it is possible to obtain transiently higher potential differences in the MFC, although it takes longer to generate electricity because it is an unstable condition, as the microbiological process would begin to deteriorate at a concentration of approximately 3 g L^{-1}.

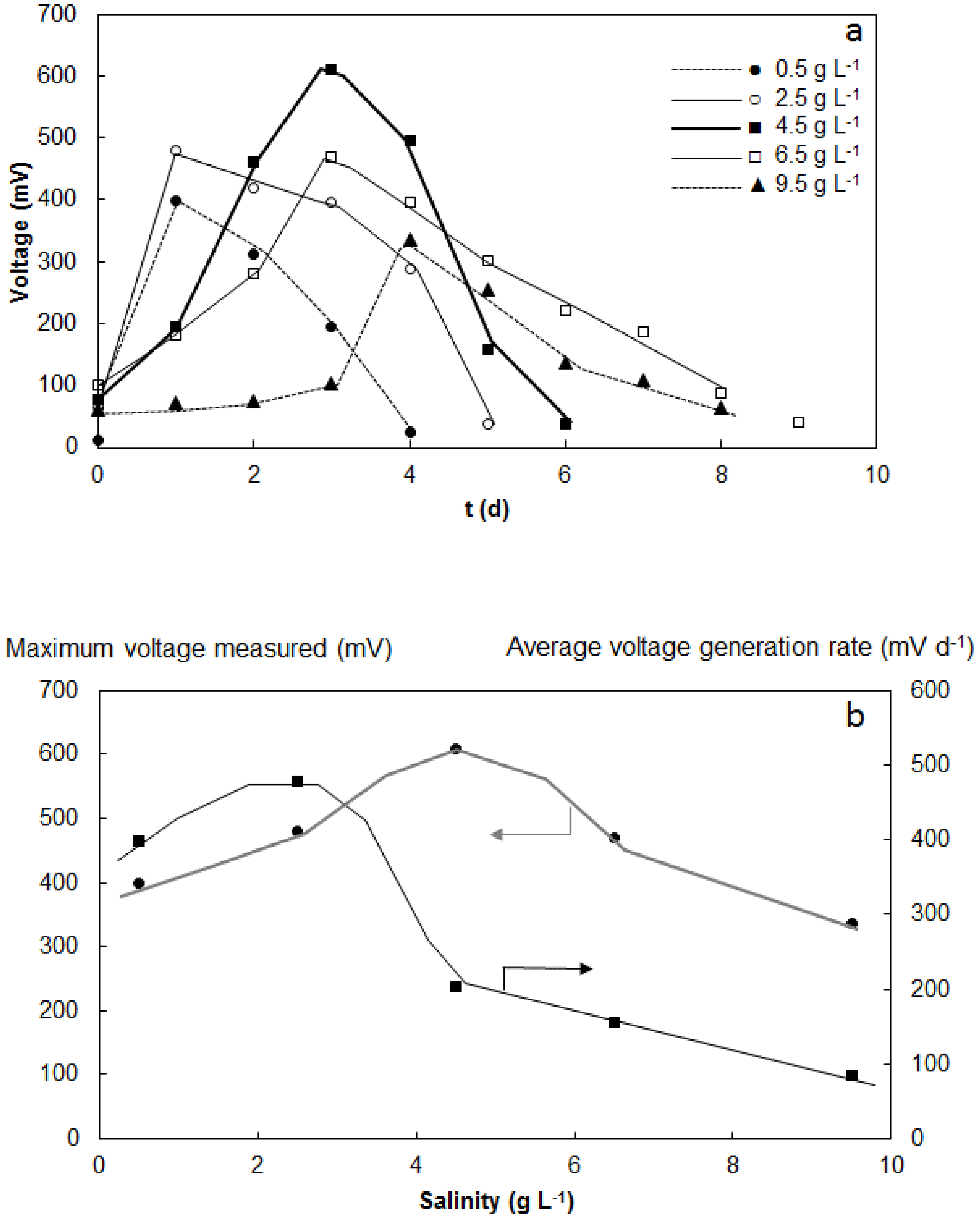

a
Voltage (mV)
t (d)
0.5 g L-1
2.5 g L-1
4.5 g L-1
6.5 g L-1
9.5 g L-1
0
100
200
300
400
500
600
700
0
2
4
6
8
10
b
Maximum voltage measured (mV)
Average voltage generation rate (mV d-1)
Salinity (g L-1)
0
100
200
300
400
500
600
700
0
100
200
300
400
500
600
0
2
4
6
8
10

Figure 5

From the results in the present work, it could be argued that the CW-MFC would improve the electric power generation while treating wastewater with high salinity, although there is a maximum salt level that first inhibits the microbial process. Later, the salt level damages the plants' performance. Some researchers proved previously that it is possible to use halotolerant plant species (Webb et al., 2012) or to inoculate halotolerant microorganisms (Karajić et al., 2010) in CWs. Regarding MFC, Monzon et al. (2015) proposed using extreme halophilic microbes. The authors of the present work consider that the application of such halotolerant microbes and plants could allow for the application of CW-MFC to simultaneously treat high salinity industrial wastewater and produce electricity.

According to these results, the authors considered that high salinity in industrial effluents could be a positive influence in the development of the CW-MFC technology as it improved voltage differences. However the challenge would be if halo-tolerant microorganisms and plants could be adapted to these conditions, which may be the focus of further research.

4. Conclusions

The increasing salinity first improved the cell voltage under continuous operation, and the maximum voltage corresponded to a salinity concentration between 4 and 5 g L^{-1}, while subsequently higher salinity levels caused the opposite effect. However, according to the COD, DO and VSS measurements, microbiological inhibition in the anode zone appeared early at an approximate salinity of 3 g L^{-1}. The batch experiments confirmed this behaviour. The wetland plants remained without apparent damage up to salinity levels of 9.51 g L^{-1}.

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Figure 1. Experimental installation. (1) Wastewater feeding; (2) Anode; (3) Cathode; (4) Bentonite layer; (5) Reed plants; (6) Sampling points; (7) Treated effluent; (8) Resistance; and (9) Multimeter.

Figure 2. Influent and effluent COD concentrations (a) and voltage generation (b) during the entire experimental period.

Figure 3. (a) Average voltage generation values and error bars, and percentage of the COD consumed by bioelectrogenic microorganisms in the MFC depending on the salinity of the wastewater and (b) the total and electrogenic COD consumption rates.

Figure 4. Dissolved oxygen concentrations (a) and VSS concentrations (b) in the anode (○) and the cathode (●) effluents.

Figure 5. Cell voltage generated (a) and the maximum and average cell voltage change rates (b) in the batch experiments.