Long-term assessment of best cathode position to maximise microbial fuel cell performance in horizontal subsurface flow constructed wetlands

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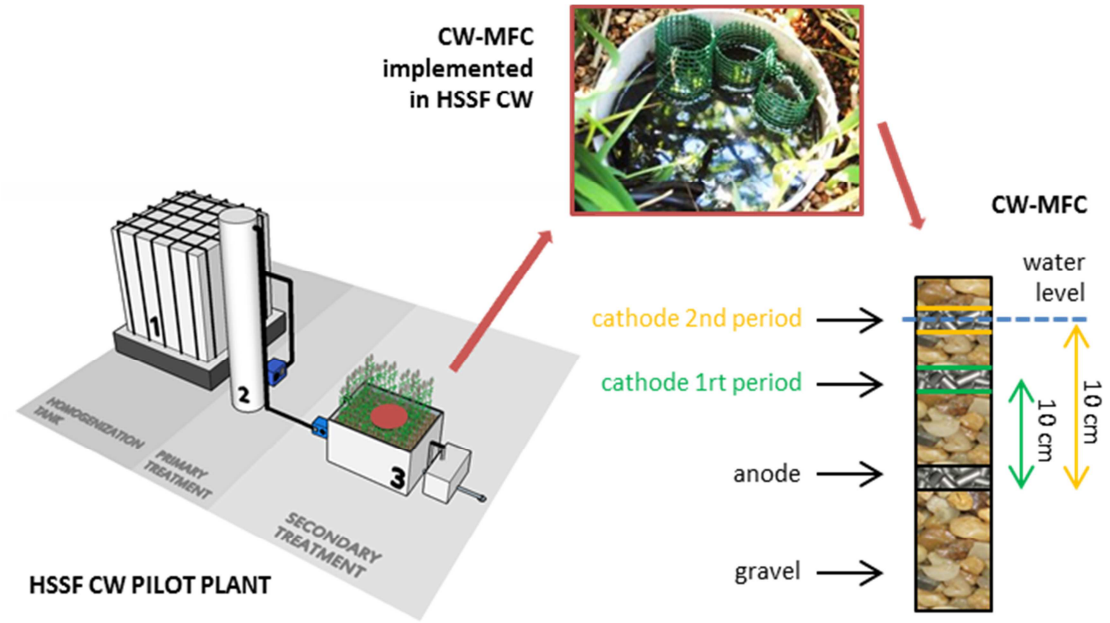


Corbella, C., Garfí, M., Puigagut, J. (2016) Long-term assessment of best cathode position to maximise microbial fuel cell performance in horizontal subsurface flow constructed wetlands. Science of the Total Environment, 563–564 (448–455)

Abstract

The cathode of microbial fuel cells (MFC) implemented in constructed wetlands (CW) is generally set in close contact with water surface to provide a rich oxygen environment. However, water level variations caused by plants evapotranspiration in CWs might decrease MFC performance by limiting oxygen transfer to the cathode . Main objective of this work was to quantify the effect of water level variation on MFC performance implemented in HSSF CW. For the purpose of this work two MFCs were implemented within a HSSF CW pilot plant fed with primary treated domestic wastewater. Cell voltage (E_{cell}) and the relative distance between the cathode and the water level were recorded for one year. Results showed that E_{cell} was greatly influenced by the relative distance between the cathode and the water level, giving an optimal cathode position of about 1 to 2 cm above water level. Both water level variation and E_{cell} were daily and seasonal dependent, showing a pronounced day/night variation during warm periods and showing almost no daily variation during cold periods. Energy production under pronounced daily water level variation was 40% lower (80±56 $mWh/m^2 \cdot day$) than under low water level variation (131±61 $mWh/m^2 \cdot day$). Main conclusion of the present work is that of the performance of MFC implemented in HSSF CW is highly dependent on plants evapotranspiration. Therefore, MFC that are to be implemented in constructed wetlands shall be designed to be able to cope with pronounced water level variations.

Keywords: microbial fuel cells, constructed wetlands; evapotranspiration; cathode limitation; energy production

1. Introduction

Microbial Fuel Cells (MFCs) are bioelectrochemical systems that generate electricity from organic matter oxidation using bacteria as catalysts (Logan, 2008). Electrons produced during the oxidation are transferred to the electrode (anode) from where they flow through a conductive material and a resistor to reduce an electron acceptor at the cathode (Rabaey and Verstraete, 2005; Logan et al., 2006). The current generation in MFCs depends on the redox gradient between the anode and cathode. For MFC to produce an electric current two areas are required, one under reduced redox conditions where organic matter is oxidized and the other one under higher redox potential where terminal electron acceptors are reduced. Horizontal subsurface flow constructed

wetlands (HSSF CWs) are engineered treatment basins filled up with granular media and planted with macrophytes that are used mainly for the treatment of domestic wastewater. In HSSF CWs the presence of both organic matter and naturally generated redox gradients can be exploited to produce energy via MFCs (Corbella et al., 2014; 2015). The implementation of MFCs in CWs (and in particular on HSSF CW) is in its first stage and current scientific information available on the topic is limited. So far, some studies have been developed in pilot-scale systems (Doherty et al., 2015; Villaseñor et al., 2013), though most of them were based on laboratory-scale experimental designs (Yadav et al., 2012; Fang et al., 2013; Zhao et al., 2013) and used synthetic wastewater instead of real domestic wastewater. In HSSF CWs, redox potential decreases with depth generating a vertical redox gradient between the upper layer, which is in higher redox conditions, and the deeper layers where anaerobic environment predominates (García et al., 2003; Pedescoll et al., 2013; Dusek et al., 2008). Main configuration for MFC implementation in HSSF CWs relies on setting a cathode at the surface of the system while the anode remains buried in the deeper zone of the treatment bed. Evapotranspiration caused by plants induces marked daily variations on water level within the treatment bed and therefore, has a notable influence on wetland's redox conditions (Mann and Wetzel, 1999; Pedescoll et al., 2013). Notable fluctuations of water table caused by plants evapotranspiration may vary MFC performance on daily and seasonal terms by changing the availability of oxygen at the cathode. Consistent with this, cathode has been considered to be one of the major sources of limitation in CW-MFCs due to the slow kinetics of oxygen reduction and the scarcity of oxygen in CW environment (Doherty et al. 2015; Corbella et al. 2015). However, although most of the reported CW-MFCs include plants in the experimental designs, none of them consider the effect of evapotranspiration on cathode performance (Fang et al., 2013; Villaseñor et al. 2013). Up to date daily fluctuations of CW-MFC performance have been attributed to the photosynthetic activity of plants (Villaseñor et al., 2013; Liu et al. 2014) with no specific mention to water level variation caused by plants evapotranspiration.Therefore, the purpose of this study was to determine the influence of water level fluctuations on the performance of MFCs implemented in HSSF CW that, as far as authors know, is currently unaddressed. To this purpose the relative distance between cathode and water level was continuously monitored for one year in two MFCs implemented in HSSF CW pilot plant fed with real domestic

wastewater. Results shown in this study provide useful information to optimise the architecture of microbial fuel cells that are to be implemented in HSSF CWs.

2. Materials and methods

Pilot plant description

The pilot plant was located in Barcelona and it was set up in March 2011. It consisted of one wetland of 0.4 m^2 of surface with a gravel matrix having an initial porosity of about 40% and a depth of 35 cm (Figure 1). Water level inside the wetland was kept at about 30 cm depth (5 cm below the gravel surface). The wetland was planted with common reed (*Phragmites Australis*) and was very mature by the time this work was carried out (year 2013). The wetland had a sampling area of 20 cm diameter located in the centre of its surface. This area was not filled with gravel and was used to take samples and to place the probes and microbial fuel cells.

The wetland was fed with urban wastewater which was pumped directly from the municipal sewer. Initially wastewater was coarsely screened and after that it was stored in a 1.2 m^3 tank of five hours of hydraulic retention time (HRT) before being conveyed to the primary treatment. In the tank, wastewater was continuously stirred to avoid solids sedimentation. Primary treatment consisted of a hydrolytic up-flow sludge blanket reactor (HUSB reactor) of 115 L of total volume that was operated at four hours of HRT. Secondary treatment consisted of one horizontal sub-surface flow constructed wetland fed under a continuous flow of 0.875 l/h (design HRT was that of 2.6 days).

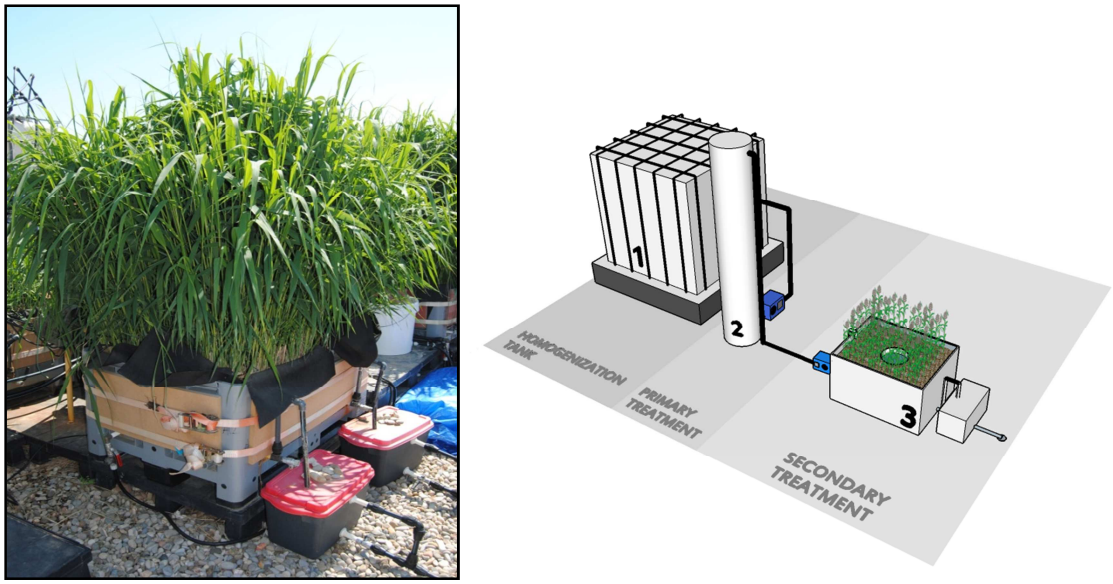


Figure 1. Constructed wetlands pilot plant. *Left picture*: the wetland during summer 2013. *Right figure*: a scheme of the pilot plant process line.

Wastewater physical and chemical analysis

Water quality parameters (total and soluble chemical oxygen demand (COD) and ammonia) were measured at the influent, middle part, and effluent of the wetland. They were analysed once every week or two weeks according to Standard Methods (APHA-AWWA-WEF, 2005). The influent and effluent flow was daily monitored which allowed us to calculated evapotranspiration rates and removal efficiencies on a mass balance basis. Air temperature was obtained from a close meteorological station (Department of Astronomy and Meteorology, University of Barcelona).

MFC's configuration and monitoring

Three MFC were implemented within the HSSF CW pilot plant. Two MFC were operated at closed circuit and one was left at open circuit. For the purpose of this work only the two MFC working under closed circuit will be considered. MFCs implemented consisted of a plastic mesh of 40 cm length and 5 cm of diameter filled with 35 cm of gravel in order to simulate wetlands' characteristics (Figure 2). The anode and cathode were made of twenty cylindrical graphite rods (1 cm length and 0.6 cm diameter) each, covered by a mesh of stainless steel (marine grade 316L) (Figure 2). They were cubic

shaped with a projected surface of 7.5 cm^2 and 1 cm high. The external circuit was closed by connecting both electrodes with one external resistance of 1000 ohms by means of cooper wires. Epoxy materials were used to preserve metal connections from wastewater corrosion. MFC were placed in the central part of the treatment bed which was empty of gravel (Figure 2).

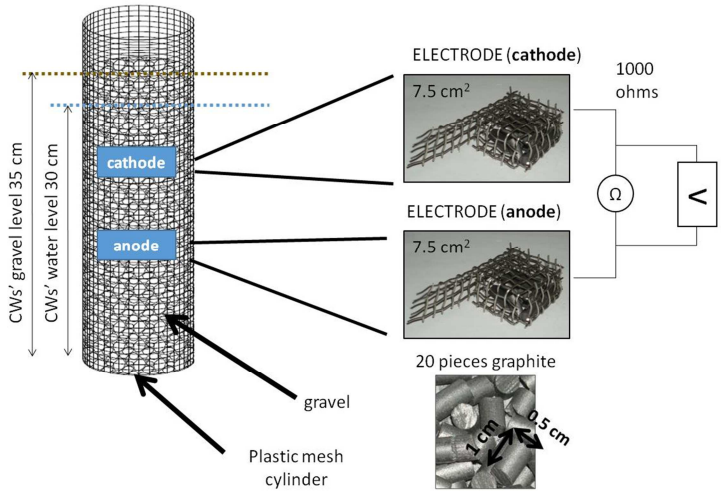


Figure 2. MFC experimental set up. On the upper picture we can see the MFC implemented within the wetland. On the bottom picture we can see the schematics of MFC and the picture of the electrodes implemented.

MFCs were monitored during two consecutive periods of six months each in which two different cathode positions were tested. Different cathode positions were applied in order to get a wider range of the relative distance between cathode and water level. During the first period (from February to July 2013) cathode was placed 5 cm below the design water level. During the second study period (from August 2013 to January 2014), cathode was placed at the same level than the design water level. In both cases, anodes were located 10 cm below cathodes (15 cm and 10 cm below the design water level, respectively).

MFCs were connected to a datalogger (Datataker DT50 series 3) which collected a value of voltage across the external resistance every 15 minutes. Current was calculated following ohms law and power calculated by means of $P=V^2/R$, where P represents power, V is voltage and R regards the external resistance. Current and power density

values were related to the projected anodic area, which was considered to be the base of the electrode (7.5 cm^2) in order to be able to express energy or power production per wetland surface.

Statistical analyses

Differences among experimental conditions for any of the considered parameters were determined by carrying out an ANOVA test, T-tests and Wilcoxon tests depending on the type of dataset considered. Data normality and homogeneity of variances were determined by performing the Kolmogorov-Smirnoff and Levenne tests, respectively. Differences among experimental conditions were considered significant at p values bellow 0.05. All statistical analyses were performed using the software package R 3.0.2.

3. Results and discussion

Plant treatment performance and organic matter available for MFC functioning

Soluble and total COD and ammonia were surveyed in the inlet, middle and outlet of the wetland along both periods. As it can be seen in Table 1, despite the high variability of results, the organic matter concentration in the inlet in terms of both soluble and total COD, was higher during the first period than during the second one (p value<0.05). However, no statistical differences were found between removal efficiencies in both periods. Accordingly, removal efficiencies for total COD were that of 61±19% and 60±10% to the first and second period, respectively. COD removal efficiencies were slightly lower than those found in the literature, where reported values range from 65 to 80 % (Puigagut et al., 2007). However, it is worth to mention that organic loading in our pilot plant (ca. 15 g COD. $m^{-2}.day^{-1}$) was slightly higher than that generally recommended for HSSF CW (Kadlec and Wallace, 2009). The amount of fuel for MFC functioning is of capital importance for MFC performance (Liu et al., 2004). In our study organic matter in the vicinity of the MFC was of similar extent in both study periods (ca. 140±50 mg/L and ca. 90±30 mg/L for the total and soluble COD, respectively for the first study period and ca. 170±70 mg/L and ca. 120±30 mg/L for the total and soluble COD, respectively for the second study period). Finally, it is worth mentioning that the effect that MFC might have exerted on wastewater treatment efficiency will not be considered in the present work since MFC active electrodes represented a very small proportion (≈0.02%) of the total treatment bed volume.

Table 1. Total and soluble chemical oxygen demand (COD) and ammonia concentrations at inlet, middle and outlet of the wetland during the first and second period. Note: average values are based on n=16 per experimental period.

			FIRST PERIOD			SECOND PERIOD		
			in	middle	out	in	middle	out
COD	Total	*mg O_2/L*	323±33	137±53	126±61	254±94	175±70	99±26
	Soluble	*mg O_2/L*	178±51	88±29	96±43	132±58	122±33	80±24
Ammonia		*mg NH_4-N/L*	41±7	-	19±19	29±7	-	24±18

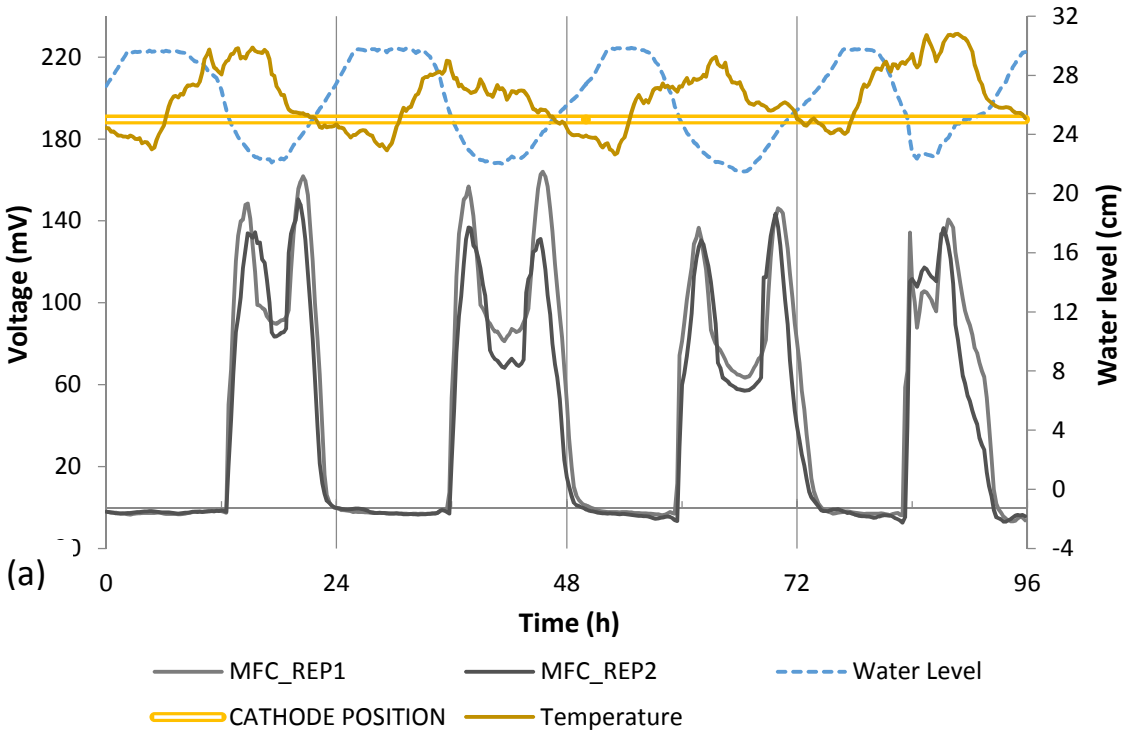
In terms of ammonia, again, inlet ammonia concentration was higher during the first period than during the second one (p value<0.05). Both periods were of six months each and, therefore, the performance of the pilot plant was affected by seasonal variations. It is reported that nitrogen removal is influenced by temperature (Vymazal et al., 2007) leading to larger removal efficiencies achieved on warm than colder periods. This behaviour was specially marked during the first study period. Thus, the average removal efficiency during all the period was that of 60±40%, but the mean value obtained from February to mid-May was that of 29±24% and from mid-May to end of July that of 98±2%.

Temperature is described to affect HSSF CW microbial processes and its treatment performance (García et al. 2010). CW-MFCs function in a microbial basis and therefore, as in conventional MFCs, temperature may also affect their performance (Jadhav and Ghangrekar, 2009). Accordingly, meteorological data from both periods was analysed to ensure they were comparable in terms of temperature. Results showed very similar average temperatures at the first and the second periods (16±6 and 17±6 ºC, respectively) thus being comparable despite the fact that both experimental conditions were tested during different periods (February-July and August-January).

Daily cell voltage pattern

Figure 3a and 3b depict a representative cell voltage pattern recorded under intense evapotranspiration during the first and second experimental periods, respectively. Figure 4 depicts a representative voltage pattern recorded under low evapotranspiration

conditions during the second experimental period. It is worth mentioning that during the first experimental period, where the cathode was set at 5 cm below the design water level, cell voltage was close to zero for the most part of the day during cold periods (low evapotranspiration conditions due to the absence of plants). From Figure 3a and 3b it is possible to see that cell voltage was dependent on the water level variation along the day. During the first study period (were cathode was located 5 cm below water level) higher MFC performances were generally recorded from noon to about 6 pm (Figure 3a) when cathode was exposed to the atmosphere due to a lower water level caused by evapotranspiration. During the second experimental period (were cathode was located at water level) higher MFC performances were recorded during hours were evapotranspiration was not intense (Figure 3b). Accordingly, cell voltages remained high during most part of the night and dropped during daylight when the cathode was well above the water level and reached a drying state that hampered any electrons flow (short-circuit conditions). Our results on cell voltage pattern are in accordance to that previously stated in current literature were marked daily oscillations of MFC voltage were recorded (Villaseñor et al., 2013). However, daily cell voltage oscillation of MFC implemented in wetlands has been attributed so far to the photosynthetic activity of plants (Villaseñor et al., 2013; Doherty et al., 2015). Our results suggest that, even though carbon exudates are a good carbon source for powering a MFC (De Schamphelaire et al., 2008), main factor governing the cell voltage in our system was the availability of oxygen at the cathode which was related to water level variation caused by plants evapotranspiration.



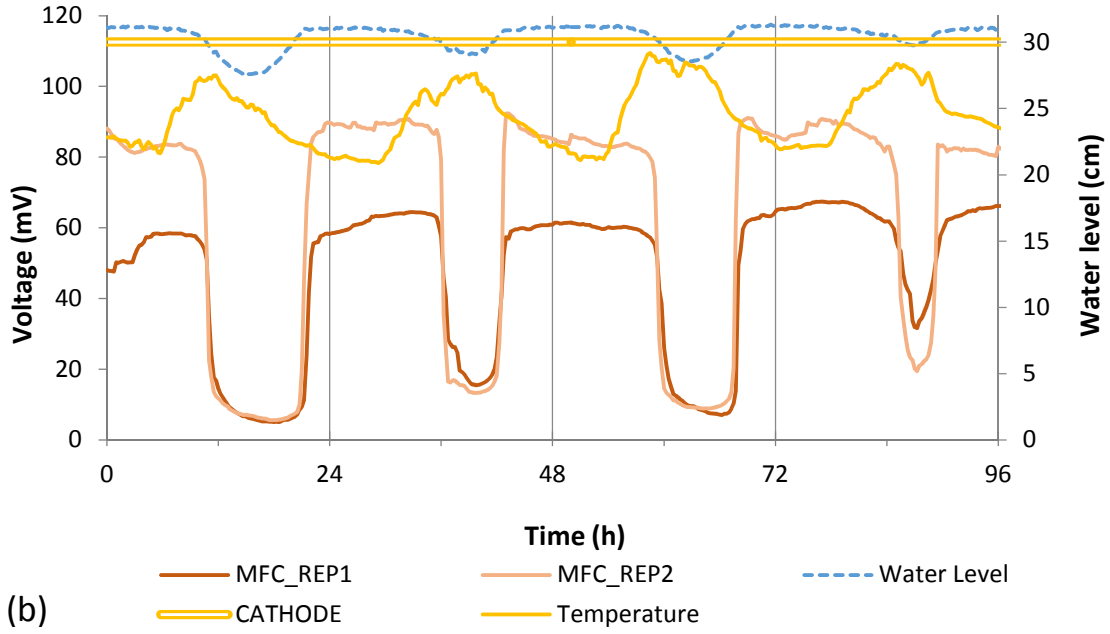


Figure. 3 Representative cell voltage patterns (two replicates, REP1 and REP2) recorded during high evapotranspiration conditions for the first experimental period (a)

238 and second experimental period (b). *Note*: negative cell voltage values are the result of
239 small inaccuracies of the measurement equipment.

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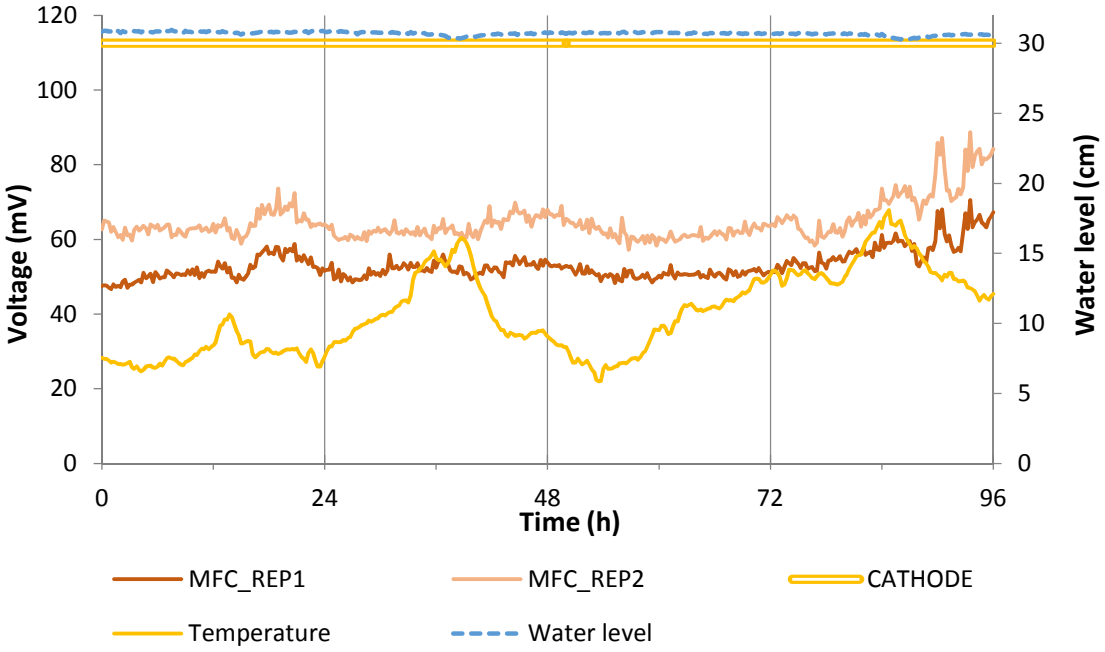

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242 Figure. 4 Representative cell voltage patterns (two replicates, REP1 and REP2)
243 recorded under low evapotranspiration conditions for the second experimental period.

244 <u>Best cathode position to maximise MFC performance</u>

245 Figure 5 depicts the cell voltage recorded along the one-year study period against the
246 relative position between cathode and the water level. Results show that both during the
247 first and the second period, only when cathode was slightly above water level (position
248 around 1 to 2 cm above water level) cell voltage was maximised. These results
249 suggested that, even though it is reported that the upper water layer of HSSF CWs is
250 under higher redox conditions (García et al., 2003; Pedescoll et al., 2013), the real
251 oxidized layer from which we can benefit by implementing MFC is of very little extend.
252 Consequently, when cathode was even slightly submerged there was not enough redox
253 gradient between electrodes (anode-cathode) for MFC to produce any significant
254 current. Performance of cathodes is therefore, considered for the authors of this work as
255 the main source for MFC performance limitation. Our results are in accordance to that
256 previously reported in conventional MFCs, where cathodes have been described as the
257 main limiting factor of microbial fuel cell performance (Anh et al., 2014; Fan et al.,

2008). Actually, it has been demonstrated that power generation is dependent on oxygen availability at the cathode (Oh et al., 2004; Ahn et al. 2014; Ferreira-Aparicio and Chaparro, 2014, Zhang et al., 2013) and also that air cathodes, compared to aqueous ones, lead to higher cell output not only due to higher oxygen concentrations but also to higher mass transfer rates (Fan et al., 2008). Furthermore, in conventional MFCs, cathodes' flooding (Ferreira-Aparicio and Chaparro, 2014; Zhang et al., 2013), air humidity and water pressure (Ahn et al. 2014) have been described to be factors affecting oxygen accessibility. For all that, the authors believe that in order to avoid cathode limitation in MFC implemented in CW, cell architecture shall address the possibility to cope with intense water level variations. To this regard, authors believe that a cathode based on a thick layer of graphite placed at the upper part of the treatment bed will allow the CW-MFC to have always an active cathode zone for oxygen reduction, regardless the water level within the wetland. According to our results, water level can vary up 10 cm from the design water level (Figure 3a; Figure 5). Therefore, the thickness of the graphite layer shall be, at least, that of 10 cm.

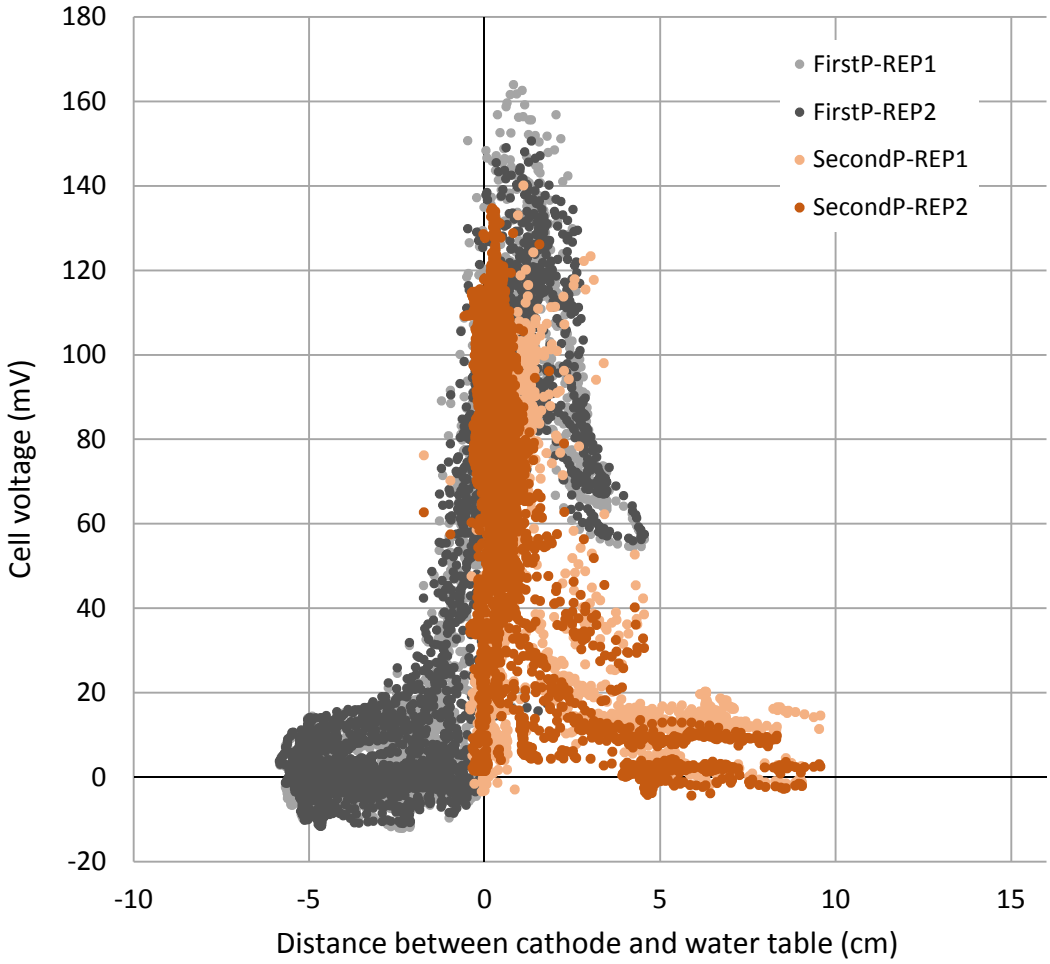


Figure 5. Voltage plotted against the relative distance between cathode and water table from both replicates (REP1 and REP2). *Note*[1]: Negative values of relative distance between cathode and water level means that the cathode is below water level. *Note*[2]: negative cell voltage values are the result of small inaccuracies of the measurement equipment.

Effect of water level variation on cell voltage and energy production

Cell voltage and energy production was assessed by comparing MFC performance under conditions of low water level variation (LWL) and high water level variation (HWL), regardless the study period considered. We want to point out that, as mentioned before, cell voltage along the day during cold months of the first study period the MFC was close to zero. Therefore, these data will not be considered for the purposes of this section. Voltage and energy produced under LWS conditions was calculated from the cold months of the second experimental period. Results showed that maximum cell voltage values were recorded for MFC working under HWL conditions. Accordingly,

under very intense evapotranspiration conditions most of the cell voltages recorded achieved values higher than 120 mV, while during the LWL these figures were rarely achieved. However, when daily maximums were statistically compared, no significant differences could be detected.

Contrary to voltages generated, energy produced (mWh/m^2·day) was significantly lower ($p<0.05$) under HWL conditions when compared to LWL conditions. More precisely, energy produced under HWL conditions was, in average terms, 40% lower (80±56 mWh/m^2·day) than that under LWL conditions (131±61 mWh/m^2·day) (Figure 6). This result was due to the fact that MFC operated under higher water level variations resulted in periods of high cell voltage and periods were cell voltage remained essentially constant and close to zero. On the contrary, MFC operated under low water level variations resulted in a nearly constant cell voltage signal along the day.

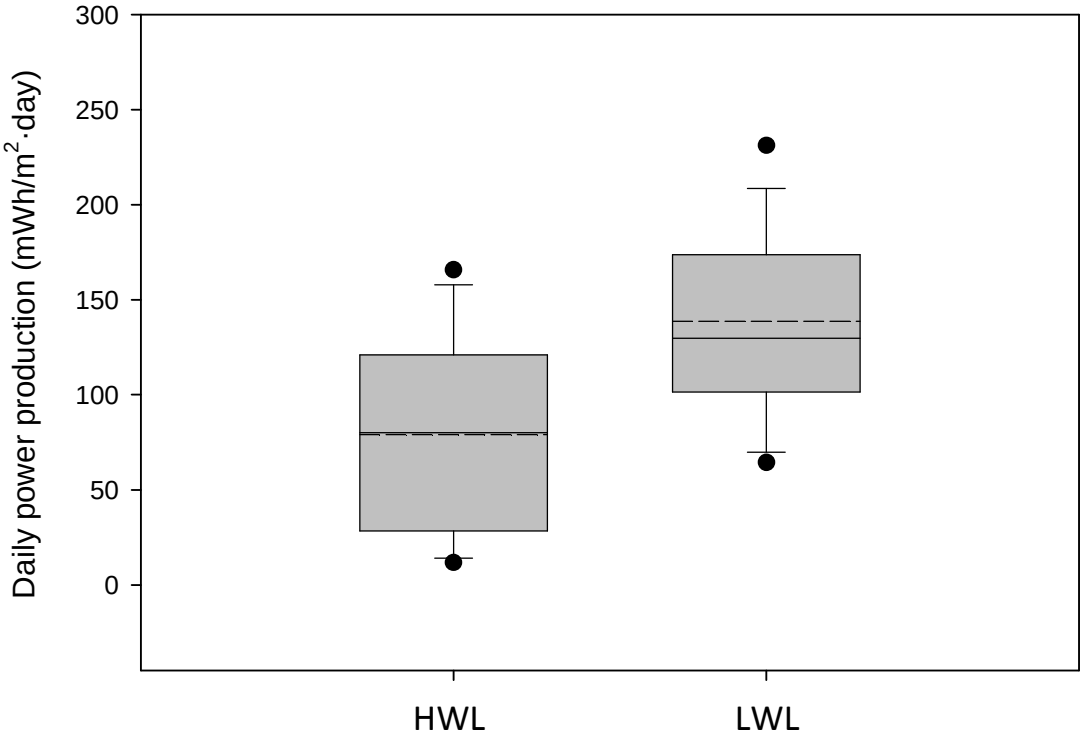


Figure 6. Daily energy production during high water level variation conditions (HWL) and low water level variation conditions (LWL).

Significance of energy production with MFC in the context of constructed wetlands

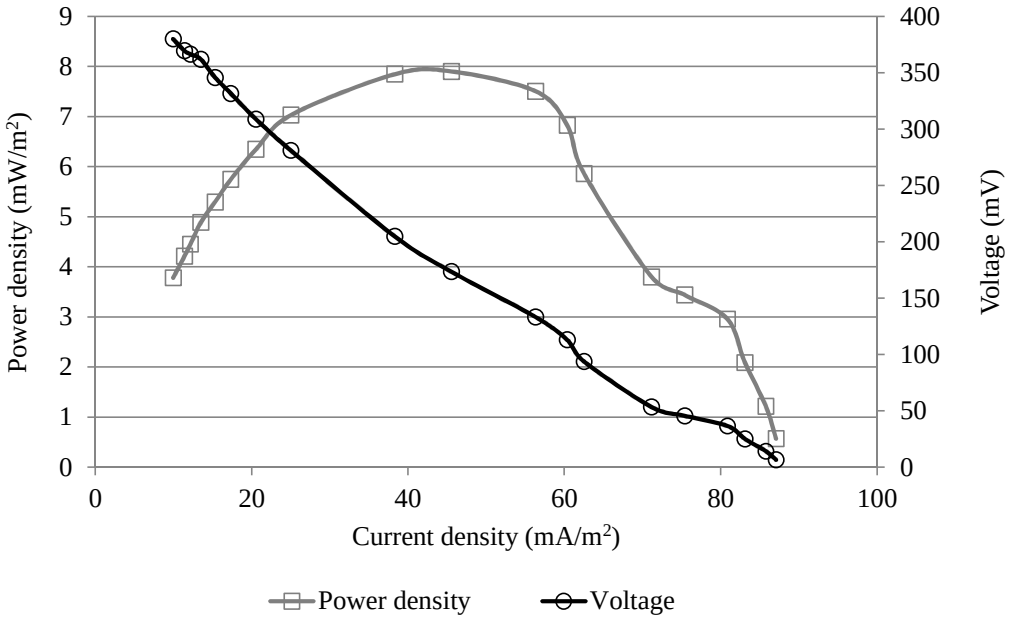
Constructed wetlands (CWs) is a technology that consumes little energy for the treatment of domestic wastewater (<0.1 KWh/m^3) (Kadlec and Wallace, 2009). However, due to its large surface requirements (about 5 m^2/PE) CWs implementation is generally restricted to the sanitation of small human settlements (<10,000 inhabitants). In order to not only overcome the large surface requirements of CWs but also to increase its treatment efficiency, active aeration has been considered a suitable strategy (Kadlec and Wallace, 2009; Austin and Nivala, 2009). However, CWs aeration increases the energy devoted to the treatment of wastewater when compared to passive, more traditional wetlands configuration. Current figures of energy consumption for completely aerated wetlands range from 0.16 to 0.49 kWh/m3 (Kadlec and Wallace, 2009; Austin and Nivala, 2009). Under this condition we can estimate that MFC implemented in CWs using the energy production figures here reported may cover less than 1% of the total energy demand (note that for the estimation we have assumed that one PE generates about 0.15 m^3/day of wastewater and that MFC energy production is that of 131 mWh/m^2.day and that only one third of the wetland is actually suitable for energy production via MFC since most of the COD is consumed within the first third of the wetland length). However, recent works carried out on the optimization of wetlands aeration have shown that the aeration of a small surface area of horizontal sub-surface flow constructed wetlands (8% of the total area) might be enough to increase treatment efficiency and reduce surface requirements (Labella et al., 2015). Under this situation the energy requirements are lower when compared to full surface aeration (0.029 kWh/$m^2 \cdot$ year). Therefore, under this lower energy requirements for aeration the energy provided to the wetlands by means of MFCs would be about the 50% of the total energy demand (note that for the estimation we have assumed the same conditions that in the previous calculation).

Influence of oxygen availability on the internal resistance: Impedance analysis

As it has been stated before, water level variation influenced the oxygen availability at the cathode, what in turn affected cells' performance. In this section this influence is assessed in terms of MFCs' overpotentials and ohmic losses. To this aim, two polarization curves were performed during the first period of MFC operation. One of the polarization curves was performed when cathode position was under sub-optimal

conditions (cathode submerged 2 cm below water level) (Figure 6a) whereas the other polarization curve was performed close to optimal conditions (cathode was in line with water level) (Figure 6b). Overpotentials are generally current dependent and can occur both at the anode and the cathode. Logan et al. (2006) divides them into activation losses, bacterial metabolism losses and mass transport or concentration losses. The latter arise due to insufficient mass transfer of chemical species to or from the electrode what limits the reaction rate (Logan, 2008). Results showed in Figure 3 confirm that the lack of oxygen availability at the cathode when it was submerged increased concentration losses to the extent of preventing electrons to flow. Accordingly, Rabaey et al., (2008) reports that oxygen reduction at non-catalysed materials cause large overpotentials. Moreover, open circuit voltage and internal resistance are also a manner to analyse MFCs' performance. The internal resistance, which is also current dependent, is defined as the sum of all internal losses (Logan et al., 2006) and it is reported that cathode is one of the main factors contributing to it (Fan et all, 2008). In fact, the cathode as the power limitation has also been reported in a plant-MFC (Strik et al., 2008).

a)



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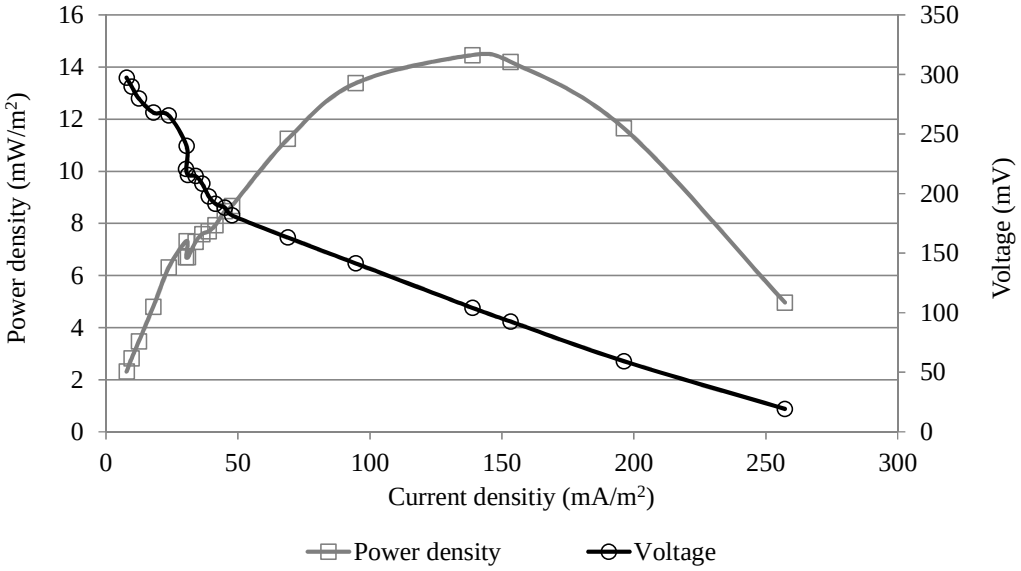

Figure 6. Polarization curves made on the same MFC (the same replicate) during normal operation: a) under optimal cathode position (in line with water level) and b) under suboptimal cathode position (2 cm below water level).

Polarization curves were made in order to electrochemically characterize MFCs working under two different cathode positions relative to water level. Maximum power and their corresponding current densities registered were 7.9 mW/m^2 and 45.5 mA/m^2 for the case of MFC under suboptimal cathode conditions (that is to say cathode submerged about 2 cm) and 14.5 mW/m2 and 138.8 mA/m^2 for the case of MFC close to optimal conditions (cathode in line with water level). Accordingly, the internal resistances estimated from the polarization curves were that of 5080Ω and 1000Ω, respectively. This result is in accordance to the fact that the maximum power is achieved when the internal resistance is equal to the external (Lefebvre et al., 2011). Internal resistance can be estimated as the slope of the linear section of the polarization curve (Logan et al., 2006). Results obtained from this estimations showed that under both conditions internal resistance estimated was in the range of external resistances at which maximum power was reached (6466Ω and 1064Ω for suboptimal and optimal cathode conditions respectively) and therefore that at maximum point $R_{ext}=R_{int}$. Overall, this analysis based on impedance shows that cathode position under optimal conditions reduces the internal resistance of the system and, therefore, maximises cell performance.

4. Conclusions

- Water level variation in constructed wetlands caused by evapotranspiration has a great effect of microbial fuel cell performance.
- Cathode shall remain between 1 to 2 cm above water level in order to optimize cell performance.
- Microbial fuel cell having a fixed cathode and operated under high water level variation produced about 40% less energy (80±56 mWh/m^2·day) than that under low water level variation (131±61 mWh/m^2·day). Therefore, cell architecture shall address the possibility to cope with intense water level variation to optimise cell performance.
- Water level variation influences cathode performance by increasing the overall internal resistance of the system.

5. Acknowledgments

This study was funded by the Spanish Ministry of Science and Innovation (MICINN) (project CTM2010-17750). Clara Corbella kindly acknowledges her PhD scholarship (2014 FI_AGAUR, Generalitat de Catalunya).

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Supplementary material

Figure S1. Average monthly temperatures.

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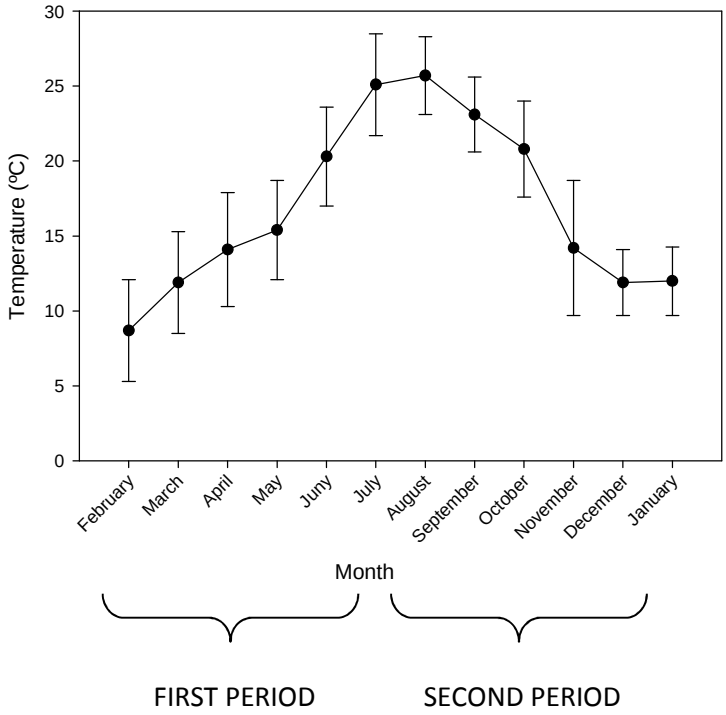
30
25
20
15
10
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Temperature (ºC)
February
March
April
May
Juny
July
August
September
October
November
December
January
Month
FIRST PERIOD
SECOND PERIOD

499