Improving domestic wastewater treatment efficiency with constructed wetland microbial fuel cells: influence of anode material and external resistance

Clara Corbella[1] and Jaume Puigagut[1]*

[1]Group of environmental engineering and microbiology (GEMMA). Universitat Politècnica de Catalunya - BarcelonaTech

*corresponding author: jaume.puigagut@upc.edu

Postal address:
C/Jordi Girona 1-3, Building D1-105
08034 Barcelona (Spain)
Tel. +34 934015952

Abstract

For the past few years, there has been an increasing interest in the operation of constructed wetlands as microbial fuel cells (CW-MFCs) for both the improvement of wastewater treatment efficiency and the production of energy. However, there is still scarce information on design and operation aspects to maximize CW-MFCs, especially for the treatment of real domestic wastewater. The aim of this study was to quantify the extent of treatment efficiency improvement carried out by membrane-less MFCs simulating a core of a shallow un-planted horizontal subsurface flow constructed wetland. The influence of the external resistance (50, 220, 402, 604 and 1000 ohms) and the anode material (graphite and gravel) on treatment efficiency improvement were addressed. To this purpose, 6 lab-scale membrane-less MFCs were set-up and loaded in batch mode with domestic wastewater for 13 weeks. Results showed that 220 ohms was the best operation condition for maximising MFCs treatment efficiency, regardless the anode material employed. Gravel-based anode MFCs operated at closed circuit

showed ca. 21%, 18%, 15%, 31% and 25% lower effluent concentration than unconnected MFCs to the BOD_5, COD, TOC, PO_4^{-3} and NH_4^+-N, respectively. Main conclusion of the present work is that of constructed wetlands operated as MFCs is a promising strategy to improve domestic wastewater treatment efficiency. However, further studies at pilot scale under more realistic conditions (such as planted systems operated under continuous mode) shall be performed to confirm the findings here reported.

Keywords**:** constructed wetlands, microbial fuel cells, domestic wastewater, treatment efficiency

1. Introduction

Microbial Fuel Cells (MFCs) are bioelectrochemical devices that generate electricity from organic matter oxidation by means of exoelectrogenic bacteria as catalysts (Logan, 2008). In a MFC, organic matter is oxidised in the anodic compartment and electrons resulting from the oxidation are transferred to the electrode (anode) from where they flow through a conductive material and a resistor to reduce a higher electron acceptor, such as oxygen, at the cathode (Rabaey and Verstraete 2005). Therefore, to produce energy with a MFC enough redox gradient must exist between the anode and the cathode. To provide enough redox gradient MFCs are generally designed with a membrane permeable to protons (PEM) between electrodes (Logan et al., 2006). However, there are other MFCs configurations that exploit the naturally generated redox gradient in aquatic environments (such as rice paddy fields and marine sediments) that do not require the presence of a PEM (Bond et al., 2002; Kaku et al., 2008). This type of membrane-less MFCs are usually known as sediment MFCs. Recently, there has been an increasing interest on the application of membrane-less MFCs in constructed wetlands, especially in horizontal subsurface-flow configurations (HF CWs) (Doherty et

52 al., 2015) either for the improvement of wastewater treatment efficiency or the energy
53 production.

54 HF CWs are natural wastewater treatment systems in which pollutants are removed by
55 means of physical, chemical and biological processes (García et al., 2010). They are
56 capable of treating wastewater from a wide range of origins such as urban, industrial or
57 agricultural (Kadlec and Wallace, 2009). Due to their low energy requirements and their
58 easy operation and maintenance, HF CWs have become an alternative to conventional
59 intensified systems for the sanitation of small communities (Puigagut et al., 2007). They
60 consist of permanently flooded planted gravel beds that are considered to be mainly
61 anaerobic (Baptista et al., 2003). Therefore, the oxidation of the organic matter within
62 these systems is mainly carried out by means of anaerobic degradation pathways, which
63 are slower and less efficient than aerobic ones. Due to its anaerobic nature, HF CWs
64 have relatively larger surface requirements when compared to traditional intensive
65 technologies such as activated sludge systems. During the last years, research in HF
66 CWs has focused on the improvement of treatment performances and its consequent
67 reduction of surface requirement. Forced (or active) aeration of CWs has been recently
68 suggested as a suitable strategy to improve the removal of organic matter and nitrogen
69 species (Nivala et al., 2013; Wu et al., 2014). However, in spite of the advantages of
70 aeration strategies, its higher energy requirements results in higher costs of operation.
71 Therefore, there is a current need for innovative wetlands configurations that provide an
72 increase in treatment efficiency while keeping the energy consumption as low as
73 possible.

74 In HF CWs, redox potential decreases with depth generating a vertical redox gradient
75 (García et al., 2003; Pedescoll et al., 2013) between the upper zone of the treatment
76 bed, which is in higher redox conditions due to the oxygen diffusion from the atmosphere
77 and the oxygen from plant supply, and the deeper zones where the anaerobic
78 environment predominates. Therefore, HF CWs can be exploited to produce energy via

79 MFCs (Corbella et al., 2016b, 2014). In MFCs implemented in HF CWs the anode is
80 placed in the anaerobic area while the cathode is placed in contact with the atmospheric
81 oxygen (Corbella et al., 2016b). Therefore, the anode constitutes an inert and non-
82 consumable electron acceptor with a high redox potential located in a very anaerobic
83 environment. Under this configuration bacteria would gain more energy when using the
84 anode as the electron acceptor in comparison to the commonly used electron acceptors
85 present in HF CWs like nitrates or sulfates, and overall treatment efficiency increases
86 (Srivastava et al., 2015).

87 In spite of the envisaged improvement in treatment efficiency derived from the operation
88 of a constructed wetland as a microbial fuel cell, there is still little information on design
89 and operation aspects that would lead to an enhancement of treatment efficiency,
90 especially under the treatment of real domestic wastewater.

91 The aim of this study was to quantify the extent of treatment efficiency improvement in
92 lab-scale membrane-less MFCs simulating a core of an unplanted shallow wetland. The
93 experimental MFCs were loaded with primary settled domestic wastewater and their
94 treatment efficiency was monitored as function of the external resistance implemented
95 (50, 220, 402, 604 and 1000 ohms) and the material of the anode employed (graphite
96 and gravel). Although the experimental systems here used cannot be fully considered as
97 constructed wetlands due to the lack of plants and the batch loading regime, results here
98 reported will be still of use towards an improvement on the design and operation of
99 constructed wetlands working as microbial fuel cells.

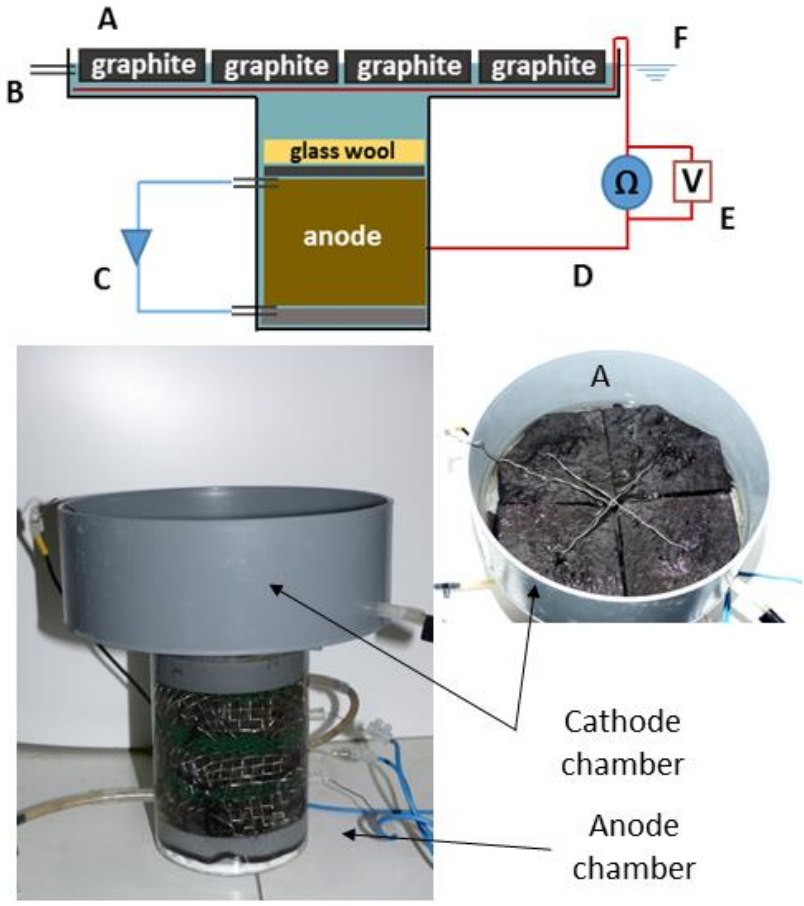
100 ## 2. Materials and methods

101 ### 2.1 MFC configuration

102 For the purpose of this work 6 lab-scale membrane-less microbial fuel cells (MFCs) were
103 operated for 13 weeks (Figure 1). At the moment of the experimental campaign all MFCs
104 had been previously loaded with domestic wastewater for 1 year producing a stable

105 electric signal and thus guaranteeing the development of a mature biofilm. According to
106 the objective of the study, the anode chamber of some of the experimental MFCs used
107 was designed to simulate a core of a shallow wetland. Therefore, anodes of 4 of the 6
108 systems employed were filled with granitic gravel of 0.8 cm in diameter (D60=7.3;
109 Cu=0.8; porosity=40%). Two of the gravel-based MFCs were operated at closed circuit
110 conditions (MFC^{+}) and two of them were operated at open circuit conditions (MFC^{-}). In
111 order to test the influence of the anode material used the other 2 experimental MFCs,
112 which were also operated at closed circuit conditions, had a graphite based anode (made
113 with graphite rods of 1 cm long 0.5 cm diameter) (Alfa Aesar, 99.9995%, metal basis,
114 ACKSP grade, Ultra “F” Purity).

115 Each MFC consisted of two chambers (the anodic and cathodic chambers) (Figure 1).
116 The anodic chamber was made of a PVC cylinder of 9 cm diameter and 15 cm of height
117 filled with graphite or gravel. The anode chamber had a total working volume of 0.5L.
118 Both materials were wrapped in a stainless steel mesh (marine grade 316L) that worked
119 as an electron collector In order to ensure adequate mixing conditions, water inside the
120 anode chamber was continuously recirculated by means of a peristaltic pump (Damova
121 MP-3035-6M; Toshiba VF-nC3). The cathode chamber consisted of a PVC cylinder
122 placed just above the anode chamber filled up with 4 pieces of graphite felt giving a total
123 surface area of 61 cm^2. Each piece of graphite felt was provided by Alfa (1.12 cm thick,
124 99.9 % carbon purity; metal basis). Graphite pieces of the cathode were inter-connected
125 using stainless steel wires (marine grade 316L). A layer of glass wool was placed
126 between the anode and the cathode chamber so as to avoid any oxygen leaking from
127 the cathode as recommended elsewhere (Venkata Mohan et al., 2008). The anode and
128 the cathode were externally connected by means of copper wires through an external
129 resistance that ranged from 50 to 1000 ohms during the first part of the experiment (first
130 6 weeks) and then was finally set at 220 ohms for the rest of the study period.



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133 **Figure 1.** Schematics (up) and pictures (down) of experimental MFCs used. A) Graphite
134 felt cathode; B) Outflow to guarantee a constant water level within the system; C)
135 Recirculation pump; D) External electric circuit; E) Data logger to measure and register
136 the voltage across the external resistance; F) Water level within the system.

137

138 **2.2 Experimental design**

139 External resistance is one of the most influencing parameters on MFCs performance
140 (Aelterman et al., 2008). Therefore, an experimental campaign was initially carried out in
141 order to determine the optimal external resistance that maximized MFCs treatment
142 efficiency. To this aim, 4 gravel-based anode MFCs (2 connected and 2 unconnected)
143 were operated during 6 weeks. Systems were loaded in batch mode every 24 hours with
144 primary settled domestic wastewater that was collected and frozen at the beginning of

the experiment and was defrosted every day before the feeding. Five different external resistances were tested: 50, 220, 402, 604 and 1000 ohms. Each one of the experimental MFC was operated for one week under a selected external resistance and influent and effluent water quality was monitored three times along the week. After one week the external resistance was changed to a new value and the system was left for about 24-48 hours until the electrical signal was stable under the new implemented external resistance. This procedure was repeated for all the tested external resistances considered. Soluble and total COD from inlet and effluent samples were analyzed according to APHA (2005). The optimal working conditions were selected by choosing the external resistance that maximized the organic matter difference between the effluent of the connected (MFC^{+}) and non-connected (MFC^{-}) systems.

Once the optimal resistance was determined, the experiment to assess the actual capacity of MFCs to improve CWs' treatment efficiency on a wider range of contaminants was carried out. To this purpose, 6 lab-scale MFCs were operated during 7 weeks. These MFCs consisted of 4 gravel-based MFC used in the first experiment plus 2 graphite-based MFCs that had been equally fed during the first experiment but not monitored regarding treatment efficiency. During this second experiment, MFCs were weekly fed with primary settled domestic wastewater and samples were taken at the inlet and at the outlet of the anode chamber of each cell after 7 days of contact time. To determine the effect of MFCs on the improvement of CWs' treatment efficiencies results from connected (MFC^{+}) and non-connected (MFC^{-}) gravel-based MFCs were compared. On the other hand, to assess the effect of the anodic material on removal rates, results from connected gravel and graphite-based MFCs were compared. Each experimental condition was tested in duplicate.

2.3 Physical-chemical analysis and electrical monitoring

Water quality parameters analyzed during the second experiment were that of total and soluble organic carbon (TOC_{tot} and TOC_{sol}), biochemical oxygen demand (BOD_5), total and soluble chemical oxygen demand (COD_{tot} and COD_{sol}), ammonium, nitrates and nitrites (NH_4^+-N, NO_3^--N, NO_2^--N) , sulphates (SO_4^{2-}S) and ortophosphates (PO_4^--P). Water quality parameters were analyzed following standard methods (APHA, 2005). Table 1 summarizes water quality parameters during the experiment.

Voltages generated by the 4 connected MFCs (2 of them with graphite-based anodes and the other 2 with gravel-based anodes) were recorded across the external resistance and stored every minute by means of a datalogger (Campbell Scientific CR1000). Current densities, power densities and coulombic efficiencies were calculated according to Logan et al. (2006).

Table 1. Physico-chemical parameters analyzed (TOCtot, TOCsol, CODtot, CODsol, BODtot, SO42--S, NH4+-N, NO3-N, NO2--N, PO4-P) at the influent and effluent of the graphite and gravel based connected MFCs and the gravel based non-connected MFC. Also removal rates are presented.

			INFLUENT	EFFLUENT		
			-	GRAPHITE	GRAVEL	
			-	CONNECTED	CONNECTED	NOT CONNECTED
			mg/L	*mg/L*	*mg/L*	*mg/L*
TOC	**TOC tot**	**MEAN**	239.8	16.0	18.4	22.2
		SD	43.6	3.5	4.0	6.7
	TOC sol	**MEAN**	62.3	17.5	16.5	20.1
		SD	21.5	6.7	4.7	4.9
COD	**COD tot**	**MEAN**	625.1	107.8	112.1	134.4
		SD	169.8	28.5	25.6	24.2
	COD sol	**MEAN**	198.0	73.3	64.4	90.1
		SD	31.3	23.1	21.9	20.2
BOD_5		**MEAN**	239.3	134.9	132.7	163.4
		SD	96.6	29.2	33.0	41.7
SULPHATE-S		**MEAN**	23.4	12.1	10.6	7.9
		SD	2.5	4.1	3.9	2.3
AMMONIUM-N		**MEAN**	50.4	16.2	15.9	21.2
		SD	6.5	8.0	7.4	8.7
NITRITE-N		**MEAN**	0.0	0.2	0.2	0.5
		SD	0.0	0.3	0.3	1.0
NITRATE-N		**MEAN**	0.2	1.7	1.6	1.2
		SD	0.3	0.6	0.5	0.7
ORTOPHOSPHATE-P		**MEAN**	4.8	3.8	3.6	5.2
		SD	0.6	0.6	0.7	0.7

205 **2.4 X-ray diffraction analysis**

206 At the end of the study period, the cathode was sampled and subjected to X-ray difraction
207 analysis to determine which compounds had been precipitating during MFC operation.
208 To this purpose, 1g graphite felt pieces of the cathode of connected and un-connected
209 systems were taken and processed as follows.

210 Sample processing. Samples were grounded in an Agate mortar. The resulting powder
211 materials were placed back loaded in cylindrical cavities, of 16 millimetres of diameter
212 and 2.5 millimetres of thickness, of standard sample holders (PW1811/16).

213 Instrument and analytical conditions.

214 • PANalytical X'Pert PRO MPD Alpha1 powder diffractometer (radius = 240
215 millimetres)
216 • Cu Kα1 radiation (ʎ = 1.5406 Å).
217 • Work power: 45 kV – 40 mA.
218 • Focalizing Ge (111) primary monochromator
219 • Sample spinning at 2 revolutions per second
220 • Variable automatic divergence slit to get an illuminated length in the beam
221 direction of 10 millimetres.
222 • Mask defining a length of the beam over the sample in the axial direction of 12
223 millimetres
224 • Diffracted beam 0.04 radians Soller slits
225 • X'Celerator Detector: Active length = 2.122 º.
226 • ɵ/2ɵ scans from 4 to 80º 2ɵ with step size of 0.017º and measuring time of 50
227 seconds per step.

228 Determination. The determination method was that of the qualitative crystalline phase
229 analysis by means of the PDF (Powder Diffraction File) data base of the ICDD-JCPDS
230 (International Centre for Difraction Data – Joint Cometee of Powder Diffraction
231 Standards, 2015).

2.5 Statistical analysis

Differences among experimental conditions for all the considered parameters (physical-chemical and electrical parameters) were determined by carrying out an ANOVA test of variance. Homogeneity of variances and normality of data were assessed by performing a Levene test and a Kolmogorov–Smirnov test, respectively. Differences among experimental conditions were considered significant at p values bellow 0.1; p values below 0.05 were also indicated. All statistical analyses were performed using the software package R 3.0.2.

3. Results and discussion

3.1 Determination of the optimal external resistance

The relationship between the current and the voltage generated is determined by the external resistance (Logan et al., 2006). Different configurations (materials, dimensions, substrates, etc..) are linked to different internal resistances and hence, different optimal external resistances (Logan, 2008). Furthermore, depending on the purpose of the experiment, the control of the external resistance could foster either the power produced or the wastewater treatment (Katuri et al., 2011). Overall, it is generally accepted that the lower the external resistance the higher the current generated and the higher the organic matter removal rate (Aelterman et al., 2008; Gil et al., 2003; Katuri et al., 2011).

Outlet COD concentrations in our MFC^{+} were generally lower than that of MFC^{-} (Figure 2-A). However, the highest significant differences ($p<0.05$) between connected and not-connected systems were recorded at 220 ohms of external resistance either for total or soluble COD (Figure 2-A). Furthermore the difference between MFC^{+} and MFC^{-} outlet concentrations tended to decrease with the external load applied (Figure 2-B). These results are in accordance to Katuri et al. (2011) who reported an increase of organic

matter removal of about 30% under lower external resistance (100 ohms) when compared to higher external resistance (50,000 ohms). Our results are also in accordance of that described by Song et al.(2010). These authors reported higher organic matter removal efficiencies at a resistance of 100 ohms when a range between 10 and 1000 ohms was tested in freshwater sediment MFCs. Overall, and according to our results, 220 ohms was the external resistance chosen for the following experiments that aimed at determining to which extent MFCs improve treatment efficiency for a wider range of contaminants.

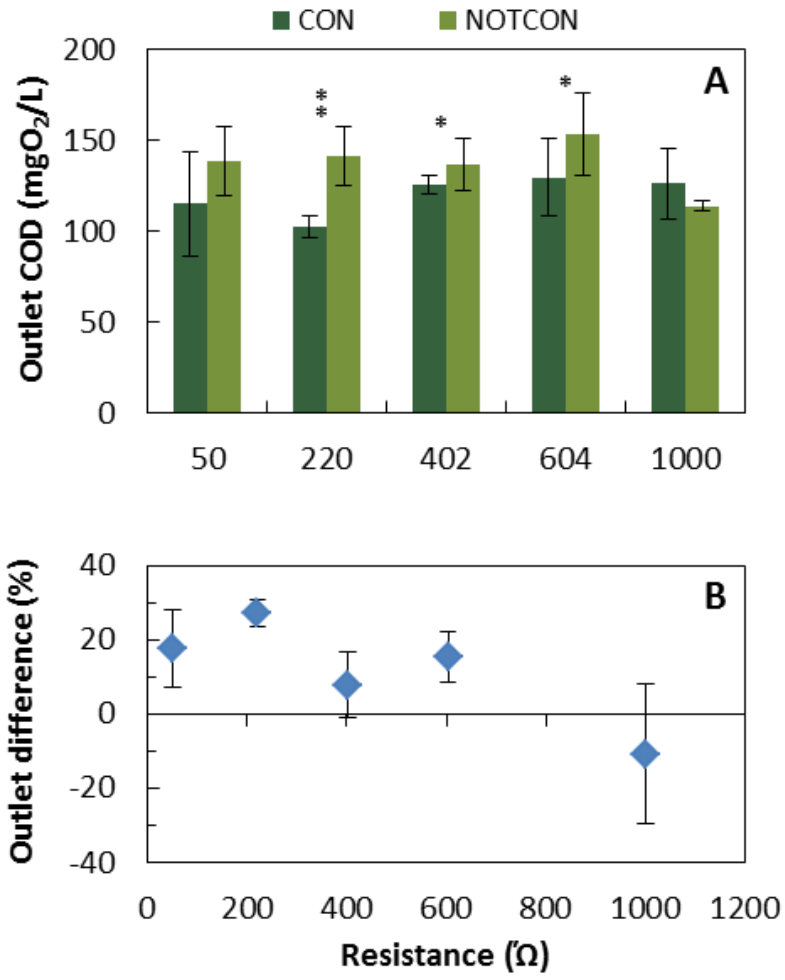

Figure 2. Effect of MFCs external resistance on MFC treatment efficiency improvement. A) Outlet soluble COD concentrations in connected (CON) and not-connected (NOTCON) MFCs as function of the external resistance. B) Difference between

connected and not-connected effluents (CODNOTCON – CODCON) in terms of total COD concentration. Statistical significances are indicated: ** : $p_{value}<0.05$; * : $p_{value}<0.1$. *Note*: for each bar/dot depicted in Figure 2 n=6.

3.2 Effect of anodic material on treatment efficiency and electrical output

The electrode material used in MFCs architecture is a key parameter regarding their performance. Good electrical conductivity is one of the most important properties to optimize MFCs functioning (Zhou et al., 2011). Therefore, non-conductive materials are rarely used as anodic materials in conventional MFCs due to its incapacity to collect and transfer electrons. In this study we compared the effect of using a very common conductive electrode material (graphite) with a non-conductive material typically described as filling media in constructed wetlands (gravel). Previous studies of our group demonstrated the feasibility of using gravel as anodic material when an electron collector is provided (stainless steel mesh) (Corbella et al., 2016a). In that case, the gravel matrix did not transfer the electrons itself but provided a physical surface for the electrical conductive biofilm to stablish. (Corbella et al., 2015).

Unexpectedly, graphite and gravel-based anodes of MFCs did not produce any significant difference on the removal efficiency for any of the analyzed water quality parameters (Figure 4 – Table 1). MFCs used in this experiment were previously operated during 1 year before the experiment was conducted. Domestic wastewater is known to have high content of suspended solids (Pedescoll et al., 2011) which progressively accumulates within the system. This solids accumulation have been demonstrated to decrease MFCs performance in gravel based systems (Corbella et al., 2016a). Accordingly, the authors of the present paper believe that the accumulation of solids

within the systems might have decreased the capacity of the graphite-base system to transfer electrons to the external circuit to the point that no differences can be detected between as the conductive media (graphite) and a non-conductive media (gravel). Therefore, it remains still unknown whether at early stages of MFC functioning graphite-based electrodes would outperform non-conductive media in terms of pollutants removal.

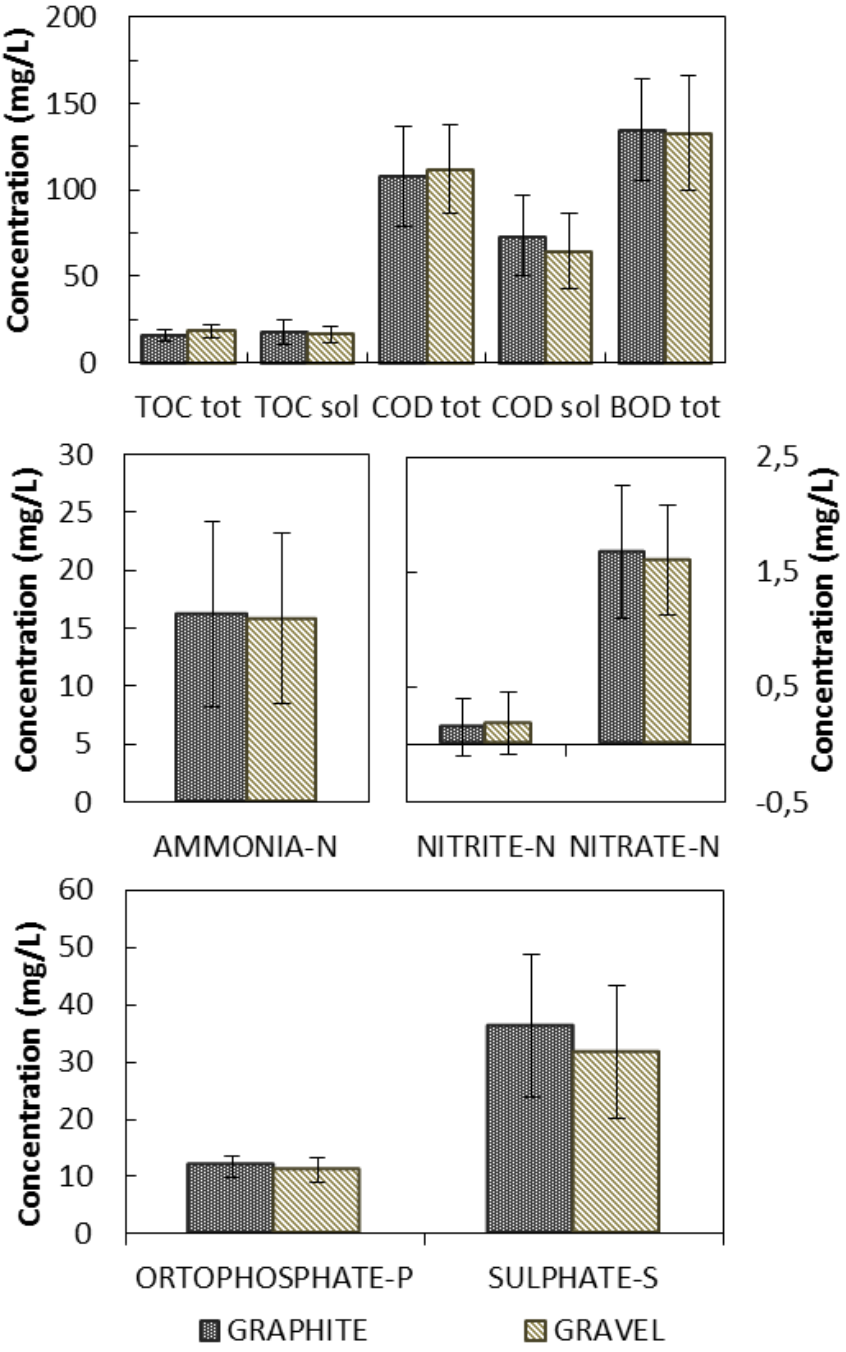

Figure 4. Effluent concentrations of physical-chemical parameters surveyed for graphite and gravel-based MFCs. *Note*: statistical analysis were performed and no differences were found between materials regardless the parameter considered.

Same conclusions as that stated above can be outlined regarding cell voltage recorded as function of the anode material. Mean voltages obtained all along the experiment were that of 123±49 mV and 102±60 for gravel and graphite-based systems, respectively. Furthermore, maximum voltages obtained were also very similar among tested anode materials (238 and 245 mV for gravel and graphite basted CW-MFCs, respectively). The same trend was observed in terms of weekly charge produced (305±134 C and 253±88 C, for gravel and graphite MFCs, respectively). Similar maximum power densities were recorded for gravel and graphite based MFCs (288 and 346 mW/m^3 CW, respectively). Also similar maximum current densities were found (1.17 A/m^3_{anode} and 1.28 A/m^3_{anode} for gravel and graphite based MFCs). Overall, these results are in the range of those reported for CW-MFCs set under different configurations (Doherty et al., 2015; Oon et al., 2015; Srivastava et al., 2015).

Due to the fact that no differences were found between the anode materials here considered, only results from gravel based MFCs are presented below. Therefore, results shown from this moment on compare treatment efficiencies between connected (MFC^+) and non-connected (MFC^-) gravel-based MFCs.

3.3 Organic matter removal

Average coulombic efficiencies (CE) were that of 10±6% and 42±14% for total and soluble COD, respectively. Such CEs are common in MFCs loaded with complex organic substrates such as the wastewater here employed (Larrosa-Guerrero et al., 2010). Reported CE values for MFCs implemented in wetlands are actually lower than those here reported (between 0.05% and 3.9%) (Doherty et al., 2015). Furthermore, our results also agree with that previously stated in literature regarding higher CE for soluble COD

than for total COD (Huang et al., 2011).This is due to the fact that exoelectrogenic bacteria are only able to oxidize simple carbohydrates. Therefore, particulate organics need to be hydrolyzed to volatile fatty acids before being utilized by exoelectrogens (Kiely et al., 2011). Moreover, physical removal of particulate organic matter by filtration in MFCs contributes to total OM removal but it is just a physical-based removal process, thus lowering the total OM CE.

Organic matter removal efficiency in this study was assessed by means of different parameters such as BOD_5, total and soluble COD and total and soluble organic carbon (OC). The concentration of organic matter at the effluent of MFC^- was always statistically higher than in MFC^+, suggesting a stimulation of the organic matter oxidation in connected systems (Table 1 – Figure 5). More precisely, effluent concentrations in connected systems were 21±2 %, 18±11% and 15±16% lower than not-connected systems, for BOD_5, COD and total OC, respectively. These results are in accordance to current literature where an improvement on treatment efficiency of MFCs operated at closed circuit conditions has been described for organic substrates such as glucose (Srivastava et al. 2015) and synthetic wastewater (Oon et al., 2016). Furthermore, sediment and conventional MFCs have been also described to improve anaerobic degradation of organics (Huang et al., 2011; Larrosa-Guerrero et al., 2010).

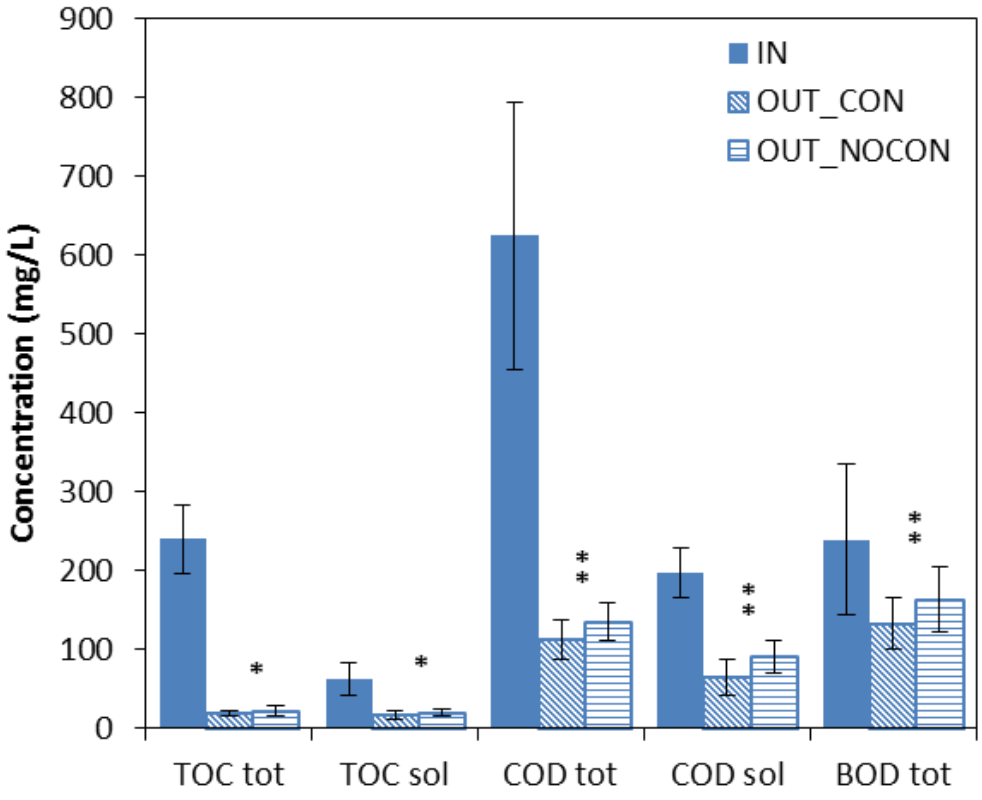

Figure 5. Influent (IN) and effluent (OUT) concentrations in connected (CON) and non-connected (NOCON) gravel based MFCs for the organic matter parameters analyzed: total and soluble organic carbon (TOC_{tot} and TOC_{sol}), total and soluble biochemical oxygen demand (COD_{tot} and COD_{sol}) and biological oxygen demand (BOD_{tot}). Statistical differences between effluent concentrations of the connected systems (CON) and not connected systems (NOCON) are indicated (* : $p_{value}<0.1$; ** : $p_{value}<0.05$). n= from 12 to 27 depending on the parameter.

Furthermore, our results indicate that the equivalent organic matter removed by the exoelectrogenic pathway in connected MFC (estimated from the electrons transferred through the external circuit) is much higher (25.2 ±11.1 mg O_2) than the actual difference between the connected and the unconnected MFCs (average difference being 11.7 ±11.3 mg O_2). Authors suggest two hypotheses for this fact (Figure A.1 - Supplementary Material). In one hand, this result may suggest that there is some inorganic electro-active species that is interacting with the electrode but cannot be detected by means of the COD analysis. The second hypothesis is based on the fact that bacteria performing the

anaerobic degradation of organic matter such as methane producing bacteria) are being outcompeted by exolectrogenic bacteria; therefore, under active MFC systems the anaerobic route is of lesser extent than in unconnected MFC. According to current literature, bacteria that are able to reduce an electrode would gain more energy per mol of organic matter oxidized than anaerobic bacteria (Li and Yu, 2015)). Therefore, if exoelectrogenic bacteria are actually outcompeting anaerobic bacteria, the conventional anaerobic pathways for organic matter oxidation in MFC^+ would decrease because of the existence of a more efficient degradation pathway (exoelectrogenesis). According to this second hypothesis, the fact that the COD equivalent to the current generated is higher than the actual difference between the COD removed by MFC^+ and MFC^- is just an artifact. This artifact is caused by the assumption that anaerobic organic matter removal pathways in MFC^+ are of equal extent than those of MFC^- and that the increase of treatment efficiency in MFC^+ is the sum of the COD removed by conventional anaerobic routes plus the exoelectrogenic route. However, if this second hypothesis is true, the actual increase of organic matter treatment efficiency in MFC^+ shall be estimated taking into account that anaerobic routes in active systems are of lesser extent than in MFC^- (Figure A.1 – Supplementary material). Unfortunately, our experimental design does not allow us to differentiate the relative contribution of each one of the two suggested pathways. Therefore, it remains still unknown the main route responsible for the improvement of MFC treatment efficiency here reported. Further studies dealing with a thorough characterization of microbial populations activity would be of use to elucidate the actual changes on the overall ecosystem due to the presence of an electroactive degradation pathway.

3.4 Nutrient removal: nitrogen, suphate and phosphorus

Although the main purpose of constructed wetlands is the removal of organic matter from wastewater, other contaminants such as nitrogen or phosphorus are also of importance given current figures of environmental pollution and environmental policy restrictions.

391 In subsurface flow constructed wetlands nitrogen is mainly removed by means of
392 nitrification and denitrification processes (García et al., 2010). While the nitrification
393 requires oxygen and low carbon contents, denitrification requires carbon and an anoxic
394 environment. In horizontal subsurface flow constructed wetlands, nitrification is the main
395 limiting process for overall nitrogen removal due to restricted oxygen availability within
396 the treatment bed. However, depending on environmental conditions other nitrogen
397 removal pathways (such as anaerobic ammonia oxidation) can occur (Pelissari et al.,
398 2016; Saeed and Sun, 2012). Ammonia removal in our experiment was, in average, that
399 of 66±14% and 53±17% for MFC^+ and MFC^-, respectively (Table 1). These values are
400 within the range of those reported in the current literature for the treatment of swine slurry
401 with MFCs (Doherty et al., 2014). Nitrite and nitrate species were also found in MFC^+
402 and MFC^- outlet samples, confirming that nitrification was being carried out within the
403 systems as has been already described for wetlands operated under batch hydraulic
404 regime (Pedescoll et al. 2011). However, nitrification is an aerobic process that requires
405 4.3 g of O_2 per gram of NH_3-N oxidized (Metcalf and Eddy Inc., 1991). Oxygen
406 concentrations within the anodic chamber ranged from <0.1 to 0.4 mg O_2/L. Nitrification
407 has been described to be a possible pathway at low oxygen concentrations (Fitzgerald
408 et al., 2015). However, in such high carbon and low oxygen content environment the
409 aerobic heterotrophic pathway would predominate over the nitrification process when
410 competing for oxygen. Under these conditions, nitrification probably occurred within the
411 cathodic chamber where wastewater was in direct contact with the atmosphere.
412 Recirculation of wastewater within the anodic chamber probably enabled the subsequent
413 denitrification within the anodic compartment (Oon et al., 2016).

414 Furthermore, results showed that ammonia outlet concentration was always lower in
415 MFC^+ when compared to the MFC^- ($P<0.1$), indicating a stimulation of the ammonia
416 removal in connected systems (Figure 6). This result is in accordance to that described
417 in current literature (Jung et al., 2008; Lu et al., 2009). Higher ammonium removal in

418 active MFCs has been mainly attributed to an increase of pH near the cathode as a
419 consequence of the consumption of protons which results in direct volatilization of
420 ammonia. However, our pH measurements within the bulk liquid surrounding the cathode
421 ranged between 6.5 and 7.2, regardless the experimental system considered (MFC^+ vs
422 MFC^-); therefore, we can hardly think of ammonia volatilization being the predominant
423 process for higher ammonia removal in our systems. Furthermore, active microbial fuel
424 cells have been also described to enhance the presence of anaerobic ammonia oxidation
425 bacteria (ANAMMOX) (Di Domenico et al., 2015). Anammox process has much lower
426 oxygen requirements than the conventional nitrification/denitrification process.
427 Accordingly, in our experimental systems oxygen concentration was very low which may
428 have resulted in an adequate environment for the anammox process to take place.
429 Unfortunately, no microbial population studies were performed in our experimental
430 systems. Therefore, still remains unknown whether the overall ammonia treatment
431 improvement observed in our active MFC was related to and enhancement of anammox
432 populations.

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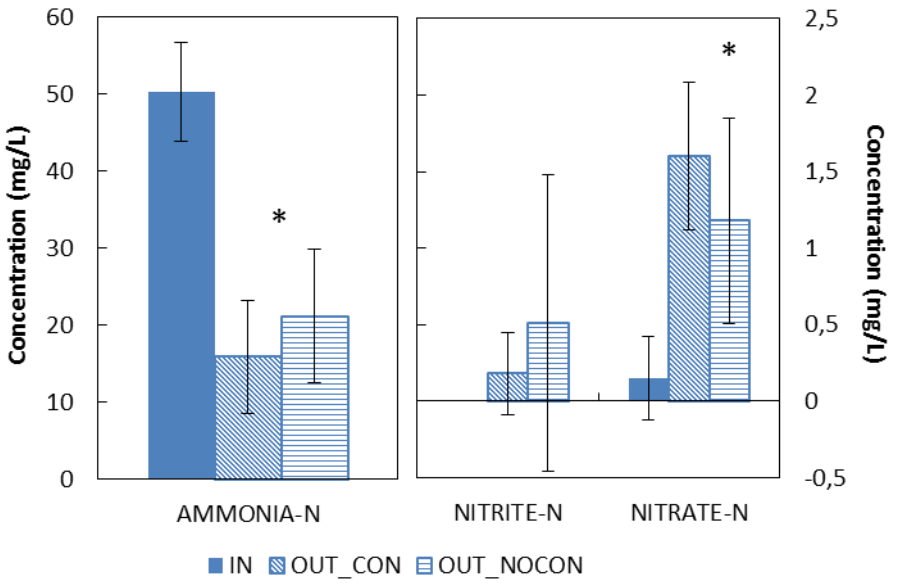
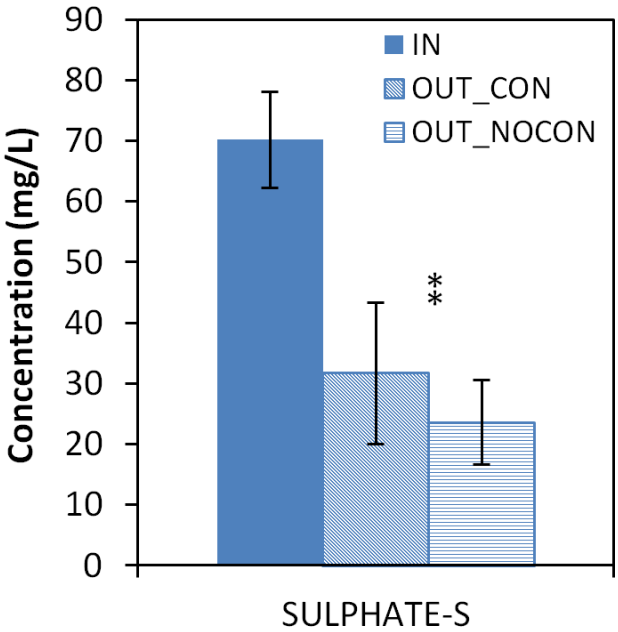


434

435 **Figure 6**. Influent (IN) and effluent (OUT) concentrations at connected (CON) and non-
436 connected (NOCON) gravel based CW-MFCs for the nitrogen compounds analyzed:

437 ammonia (NH_4^+-N), nitrite (NO_3^--N) and nitrate (NO_2^--N). Statistical differences between
438 effluent concentrations of the CON and the NOCON systems are indicated (* : p_{value}<0.1;
439 ** : p_{value}<0.05; n=12).
440
441 Sulfate concentration was also monitored along the study period, and results are
442 presented in Figure 7. Inlet wastewater used in this experiment constituted a sulphate-
443 rich media. However, although not connected systems were able to remove up to 70%
444 of the inlet sulphate, it was less efficiently removed in connected systems (ca. 57%).
445 According to current literature, sulfides (that are the product of the sulfate reduction
446 through the oxidation of the organic matter by means of sulfate reducing bacteria) can
447 be partially re-oxidized o sulfur using the anode as the electron acceptor, thus generating
448 electrical current;thensulfur is described to be potentially further oxidized into sulfates in
449 a cyclical sulfate-regenerating process (Lovley, 2006). Therefore, if sulfates are being
450 regenerated in our system, their concentration should be higher in connected than in
451 unconnected MFCs. Our results support this hypothesis since the concentration of
452 sulfates in the anode chamber of connected MFCs was always significantly higher than
453 those measured in unconnected MFC (Figure 7).



454

455 **Figure 7**. Influent (IN) and effluent (OUT) sulphate (SO_4^{2-} - S) concentrations in
456 connected (CON) and not connected (NOCON) gravel based MFCs. Statistical
457 differences between effluent concentrations of the CON and the NOCON systems are
458 indicated (* : p_{value}<0.1; ** : p_{value}<0.05; n=12).
459
460 Phosphorus removal in our active MFC was significantly increased when compared to
461 unconnected MFC, and accounted for about the 20% of the phosphorus influent
462 concentration (Figure 8). The enhancement of ortophosphates removal in MFC^+ systems
463 has been reported in previous studies and the main mechanism described is the
464 formation of phosphorus precipitates around the cathode in the form of struvite that can
465 account for up to the 27% of the influent phosphorus concentration (Ichihashi and
466 Hirooka, 2012). In our experiment we also found a white precipitate around the cathode
467 (Figure A.2 – Supplementary material). However, X-ray diffraction analysis technique did
468 not reveal any phosphorus-based compound, and the precipitates observed at the
469 cathode were mostly Calcite ($CaCO_3$) and Halite (NaCl), regardless the experimental
470 condition considered (Figure A.3 – Suplementary material). It has been also described
471 that biological phosphorus uptake can be enhanced in bioelectrochemical systems
472 working under low current conditions (<10 A) (Zhang et al., 2012). The average current
473 generated in our systems was very low (ca. 1.45 mA and 1.6 mA to the gravel and
474 graphite-based MFC, respectively), which might have enhanced the phosphorus removal
475 through biological uptake rather than chemical precipitation at the cathode. Overall,
476 authors believe that future work on the characterization of biological phosphorus uptake
477 in membrane-less microbial fuel cells working under low current generation conditions
478 shall be addressed to confirm the findings here reported.
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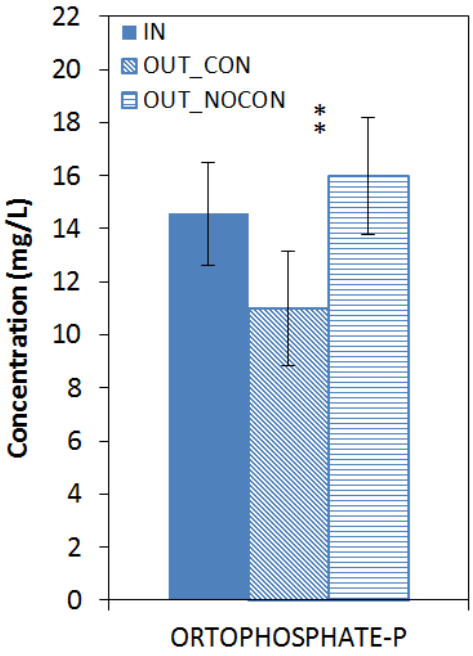

Figure 8. Influent (IN) and effluent (OUT) orthophosphate (PO_4^- - P) concentrations at connected (CON) and non-connected (NOCON) gravel based MFCs. Statistical differences between effluent concentrations of the CON and the NOCON systems are indicated (* : p_{value}<0.1; ** : p_{value}<0.05; n=12).

3.5 Significance of results reported in the context of constructed wetlands

Results here reported strongly suggest that the operation of constructed wetlands as MFCs would increase domestic wastewater treatment performance. Constructed wetlands (CWs) is a robust and efficient technology that properly designed is able to meet the limits established in current environmental policy (Kadlec and Wallace, 2009). However, CWs are suitable technologies only for small communities (Puigagut et al., 2007) due to their large surface requirements when compared to conventional wastewater treatment technologies (Kadlec and Wallace, 2009). Accordingly, current research efforts on wetland technology have focused on the design and implementation of intensive strategies (such as wetlands aeration, reciprocation, specific filter media, etc..) to improve nutrients and organic matter treatment efficiency and reduce surface requirements. However, most of the current available intensified wetlands configurations

500 result in higher costs of operation (mostly due to higher energy consumption rates). In
501 this context, results here reported point out MFCs as an alternative to current available
502 intensive HF CWs strategies to increase their removal rates with energy generation
503 instead of high energy consumption. Furthermore, higher organic matter degradation
504 rates in HF CWs operated as MFCs may result in a reduction of wetlands' surface
505 requirements, thus enabling more compact designs and reducing the cost for land
506 acquisition (Srivastava et al., 2015).

507 Overall, our results suggest that HF CWs operated as MFCs is a promising strategy to
508 improve domestic wastewater treatment efficiency (either in terms of organic matter
509 removal or nutrients). However, further studies at pilot scale under more realistic
510 conditions (such as planted systems operated under continuous flow) shall be performed
511 to confirm the findings here reported.

512 **4. Conclusions**

513 220 ohms is the best external resistance out of those here tested to optimize the
514 treatment of domestic wastewater with constructed wetlands membrane-less microbial
515 fuel cells.

516 For the purpose of improving domestic wastewater treatment efficiency, gravel or
517 graphite-based anodes coupled to an electron collector (stain-less steel mesh) appear
518 to be equally suitable.

519 Gravel-based anode membrane-less MFCs operated at closed circuit showed ca. 21%,
520 18%, 15%, 31% and 25% lower effluent concentration than unconnected MFCs to the
521 BOD_5, COD, TOC, PO_4^{-3} and NH_4^+-N, respectively.

522 Constructed wetlands operated as MFCs is a promising strategy to improve domestic
523 wastewater treatment efficiency. However, further studies at pilot scale under more

524 realistic conditions (such as planted systems operated under continuous mode) shall be
525 performed to confirm the findings here reported.

526

527 **5. Acknowledgments**

528 This study was funded by the Spanish Ministry of Science and Innovation (MICINN)
529 (project CTM2010-17750). Clara Corbella kindly acknowledges her PhD scholarship
530 (2014 FI_AGAUR, Generalitat de Catalunya). Authors are also grateful to Laura
531 Martinez, Justine Boudou and Noemie Devesa for their contribution to the experimental
532 work of this study.

533

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