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Operational, design and microbial aspects related to power production with microbial fuel cells implemented in constructed wetlands

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ABSTRACT

This work aimed at determining the amount of energy that can be harvested by implementing microbial fuel cells (MFC) in horizontal subsurface constructed wetlands (HSSF CWs) during the treatment of real domestic wastewater. To this aim, MFC were implemented in a pilot plant based on two HSSF CW, one fed with primary settled wastewater (Settler line) and the other fed with the effluent of a hydrolytic up-flow sludge blanket reactor (HUSB line). The eubacterial and archaeal community was profiled on wetland gravel, MFC electrodes and primary treated wastewater by means of 16S rRNA gene-based 454-pyrosequencing and qPCR of *16S rRNA* and *mcrA* genes. Maximum current (219 mA/m^2) and power (36 mW/m^2) densities were obtained for the HUSB line. Power production pattern correlated well with water level fluctuations within the wetlands, whereas the type of primary treatment implemented had a significant impact on the diversity and relative abundance of eubacteria communities colonizing MFC. It is worth noticing the high predominance (13–16% of relative abundance) of one OTU belonging to *Geobacter* on active MFC of the HUSB line that was absent for the settler line MFC. Hence, MFC show promise for power production in constructed wetlands receiving the effluent of a HUSB reactor.

1. Introduction

Microbial Fuel Cells (MFC) are bioelectrochemical systems that generate current by means of electrochemically active microorganisms as catalysts. In a MFC, organic and inorganic substrates are oxidized by bacteria and the electrons are transferred to the anode from where they flow through a conductive material and a resistor to a higher redox electron acceptor, such as oxygen, at the cathode (Logan et al., 2006; Rabaey et al., 2007). Different extracellular electron transfer (EET) mechanisms proposed can be divided in two main mechanisms; a) direct electron transfer (DET) and b) indirect electron transfer (IET). DET is based on the physical contact between the microbial outer membrane (OM) proteins, such as c-type cytochromes (Reguera et al., 2005; Holmes et al., 2006), or a conductive nanowires or pili (Gorby et al., 2006; Reguera et al., 2005), and the anode electrode surface. In IET, direct contact between the microbes and the electrode surface is not required, and in this case soluble electron shuttles or electron mediator compounds are involved in this process. A range of electron mediators produced by bacteria have been reported, such as melanin, phenazines (Rabaey et al., 2005), flavins and quinones (Freguia et al., 2009). So far, there are two well-known bacterial genera which present exoelectrogenic activity in pure culture, i.e., *Shewanella* (Ringeisen et al., 2006) and *Geobacter* (Richter et al., 2008; Kiely et al., 2011). To date, a high diversity of microorganisms has been described to perform anode respiration to a certain degree (Logan, 2009). Over 20 different exoelectrogenic bacteria have been reported in the last decade, belonging to diverse phylogenetic groups: alpha-proteobacteria (*Rhodopseudomonas*, *Ochrobactrum* and *Acidiphilium*), beta-proteobacteria (*Rhodoferax*, *Comamonas*), gamma-proteobacteria (*Shewanella*, *Pseudomonas*, *Klebsiella*, *Enterobacter and Aeromonas*, *Citrobacter*), delta-proteobacteria (*Geobacter*, *Geopsychrobacter*, *Desulfuromonas* and *Desulfobulbus*), Epsilon-proteobacteria (Arcobacter), Firmicutes (*Clostridium* and *Thermincola*), Acidobacteria (*Geothrix*) and Actinobacteria (*Propionibacterium*) (Logan, 2009; Xing et al., 2010). However, the power density achieved in most of the experiments working with mixed cultures is

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higher than in pure cultures (Rabaey and Verstraete, 2005; Rabaey et al., 2004; Nevin et al., 2008). These results reinforce the idea that increased electricity generation could be attributed to synergistic interactions within the microbial community. Namely, there could be microorganisms that do not exchange directly electrons with the electrode, but could be settling up interactions among other members of the microbial community playing a crucial role not only in the operation of a MFC but also on its performance improvement (specially under the presence of complex organic substrates such as wastewater) (Borole et al., 2011 and references therein). Methanogens such as *Methanosaeta* and *Methanosarcina* are, for example, routinely detected in mixed species, anode biofilms of MFC, where they presumably promote syntrophic interactions with exoelectrogenic eubacteria in the anode biofilm (Chung and Okabe, 2009; Rotaru et al., 2014a, 2014b; Sotres et al., 2015).

Compounds oxidized at the anode are mainly simple carbohydrates such as glucose or acetate that can be already present in the environment or obtained from the microbial degradation of complex organic substrates such as organic sediments or wastewater (Min and Logan, 2004; Reimers et al., 2001; Rabaey and Verstraete, 2005). MFC are, therefore, an alternative technology to harvest energy directly from wastewater in the form of electricity (Du et al., 2007). In order to ensure the use of the anode as the final electron acceptor by electrochemical active microorganisms, no acceptor with higher redox potential shall be present in their vicinity (Lefebvre et al., 2011). Consequently, the electromotive force of the cell will depend on the redox gradient between the anode and the cathode (Logan et al., 2006; Rabaey and Verstraete, 2005).

To generate the redox gradient between electrodes, MFC require two separated areas that contain the anode (anaerobic area) and the cathode (aerobic area). In some aquatic environments there is the existence of natural redox gradients that can be exploited to produce energy via MFC implementation. So far, MFC have been mostly implemented in rice paddy fields (De Schamphelaire et al., 2008; Kaku et al., 2008) or marine sediments (Reimers et al., 2001; Rezaei et al., 2007). Furthermore, horizontal subsurface flow constructed wetlands (HSSF CWs) are engineered systems used for wastewater treatment that are subjected to great spatial redox variations (especially in depth) (García et al., 2003). Although the system is mainly anaerobic (Baptista et al., 2003), the very upper part of the wetland remains under aerobic conditions because its close contact with the atmosphere giving redox gradients of about 0.5 V vs SHE (García et al., 2003; Dusek et al., 2008; Pedescoll et al., 2013; Corbella et al., 2014). As a result, natural redox gradients in HSSF CWs could be exploited to produce energy via MFC implementation, though only laboratory or small-scale based experiments with synthetic wastewater are currently available (Yadav et al., 2012; Villaseñor et al., 2013; Fang et al., 2013; Zhao et al., 2013). Furthermore, one of the main problems of constructed wetlands is clogging (Pedescoll et al., 2011a). To prevent it, primary treatments are applied to wastewater. Generally, physical treatments such as settlers or Imhoff tanks are used. However, recently other technologies such as hydrolytic upflow sludge blanket (HUSB) reactors are being considered (Pedescoll et al., 2011b). A HUSB reactor prevents methane formation during organic matter hydrolysis due to a low HRT when compared to conventional anaerobic digesters (Ligero et al., 2001). Moreover, HUSB reactors have the advantage over conventional settling of providing a higher concentration of biodegradable substrates (such as acetate) (Gonçalves et al., 1994) that can be easily removed in HSSF CWs. HUSB reactors as a primary treatment are of special interest in the context of MFC implemented in HSSF CW. Accordingly, HUSB reactors will provide a higher concentration of rapidly biodegradable substrate when compared to conventional settling, thus providing higher amount of fuel for MFC. This work aimed at determining the amount of energy that can be harvested by implementing MFC in HSSF CW during the treatment of real domestic wastewater. The effect of the type of primary treatment on power production, the daily and seasonal pattern of power production and the assessment of microbial populations associated to wastewater, electrodes (graphite) and CW materials (gravel) are also addressed.

2. Material and methods

2.1. Pilot plant

The pilot plant used in this study consisted of two horizontal subsurface flow constructed wetland. The wetlands were set up in March 2011 and had 0.4 m^2 of surface (70 cm length × 55 cm width). Wetlands were filled up with gravel (D_{60} = 7.3; C_u = 0.8) giving an initial porosity of 40%. Water level within the wetlands was kept at 30 cm (5 cm below the gravel surface). Both wetlands were planted with common reed (*Phragmites australis*), which were very mature at the moment this study was conducted (2.5 years after wetlands construction). Each wetland had a PVC cylinder of 20 cm diameter placed at the middle of the wetland that served not only to sample extraction but also to allocate the MFC.

The pilot plant was fed with urban wastewater pumped directly from the municipal sewer. Initially, wastewater was coarsely screened and after that it was pumped to a homogenisation tank of 1.2 m^3 where wastewater was continuously stirred in order to avoid solids sedimentation. After the homogenisation tank, wastewater was conveyed to the primary treatment. The primary treatment consisted of conventional settling for one of the wetlands and an anaerobic treatment based on a hydrolytic up-flow sludge blanket reactor (HUSB reactor) for the other. The HUSB reactor consisted of a PVC cylinder of 115 L of volume that was operated at 4 h of HRT and at 10 g VSS/L. The settler consisted of two PVC cones of 14 L volume each that were operated in parallel. After the primary treatment, wastewater was pumped to the wetlands at a flow rate of 21 L/day, giving a design HRT of 2.6 days and an organic loading rate of 7.2 $g.BOD_5.m^{-2}.day^{-1}$ and 6 $g.BOD_5.m^{-2}.day^{-1}$ to the HUSB and Settler line, respectively.

2.2. Microbial fuel cells

Six MFC were set up for the purposes of the present work. Three of them were placed within the wetland fed by a HUSB reactor

Fig. 1. Microbial fuel cells implemented within the wetland at the beginning of the experiment.