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Bioelectricity Production in Warm Climate Sediment Plant Microbial Fuel Cells Utilizing *Typha domingensis*

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Wetlands are ecosystems rich in organic matter due to the high biomass of vegetation that characterize them. This organic matter could be used as a substrate in devices to generate electricity through Sediment Plant Microbial Fuel Cells (SPMFC). In this study we applied the SPMFC concept adapted to *Typha domingensis* Pers., a typical wetland plant. SPMFCs were constructed with glass cells and graphite electrodes under natural environmental conditions. Electrical current production was $47.9 \pm 10.98 \text{ mA/m}^2$ with $6.12 \pm 2.53 \text{ mW/m}^2$ power (data normalized to plant growth area). Trials were performed using an electrical motor (CEBEK 0.7V and 10 mA) which moved a cardboard propeller for 15 min intervals during 6 days.

Keywords: Constructed Wetland, *Typha domingensis*, renewable energy, green electricity, microbial fuel cell.

INTRODUCTION

Development of renewable self-sustaining energy alternatives must be a priority for the nations of the world in order to reduce dependence on conventional power generation processes, as most of these are combustion processes that increase the concentrations of greenhouse gases in the atmosphere (IPCC, 2007).

An alternative to global energy problems are microbial fuel cells (MFC), devices that use bacteria as catalysts to oxidize both organic and inorganic matter to generate electricity (Logan *et al.*, 2006). From a thermodynamic point of view, these systems have been better defined by Schröder (2007) as devices that perform bioconversion of organic matter into electricity; as the bacteria involved take part of the Gibbs free energy of matter, they should not be considered as catalysts.

MFCs comprise the following elements: an anodic chamber with an anode (preferably graphite) where bioconversions produce electricity, a proton exchange membrane (PEM) to maintain the ion gradient and protons reduced to water with oxygen aerated in the cationic chamber, where the cathode (made of graphite, platinum or a combination thereof) reduces H^+ from the anodic chamber.

Another device that is designed to generate electricity is the sediment microbial fuel cell (SMFC). In an SMFC, the anode is buried in the anoxic sediment and it is connected through a circuit to the cathode, located in the overlaying of the sea water, where the electric potential is higher in the oxidative environment. This connection enables the increase in anode potential so that oxidation of seabed reduced compounds occurs mediated by microbial activity and ions from the environment. The system thus uses a natural electrical potential gradient to generate electricity at sea (Reimers *et al.*, 2006).

The main limitation of an SMFC is that the substrate

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source of bacteria in the sea floor can be used up and not replaced naturally, so the system can have periods without production because of substrate consumption. This has led to the design of devices that constantly feed anode bacterial populations. These devices, sediment microbial fuel cell with plants (SPMFC), thus eliminate the deficiency in mass transport because the plants feed the bacterial populations through rhizodeposition and exudates from the rhizosphere (De Schamphelaire *et al.*, 2008; Kaku *et al.*, 2008).

SPMFCs have been designed using wetland plants, e.g. rice (*Oryza sativa* L.), where the anodic matrix (the anode buried in the soil beneath the rice plant) and a cathode on the surface are the components of a system which does not use a PEM (De Schamphelaire *et al.*, 2008; Kaku *et al.*, 2008). Other similar devices are Plant Microbial Fuel Cells (PMFCs) which use a PEM to separate the chambers in order to maintain the ion gradient and favour the electric potential gradient between the anode and cathode, the anode compartment being test tubes with plants of the species *Glyceria maxima* (Hartm.) Holmb. (Timmers *et al.*, 2012; Strik *et al.*, 2008), *Spartina anglica* C. E. Hubbard, *Arundinella anomala* Steud. and *Arundo donax* L. (Helder *et al.*, 2010).

Wetland plants experience flooded conditions most of the year, this property being the reason why these plants have been used in SMFCs, where dissolved oxygen in water is necessary for the operation of these devices. Due to their poorly drained soil conditions, wetlands are anoxic ecosystems rich in organic matter and they have been considered for potential use as electricity generation systems (Timmers *et al.*, 2012; Helder *et al.*, 2010; Strik *et al.*, 2008).

Wetlands cover approximately 6.54% of the Mexican Republic (Secretaria del Medio Ambiente y Recursos Naturales, SEMARNAT, 2008) 10% of which are in the State of Veracruz (Moreno-Casasola *et al.*, 2008). This pinpoints the location as an area potentially suitable for the development of SPMFCs, an economic sustainable alternative to be implemented in future natural areas and designed environments.

T. domingensis. is a common hydrophyte species in the wetlands of the State of Veracruz. It has a root system that reaches up to 30 cm below ground level (Vibrans, 2011) and their rhizomes and exudates have a wealth of phytotoxins such as caffeic acid, p-coumaric, gallic acid, linoleic acid and alpha-linolenic acid (Gallardo *et al.*, 2002). These reduced compounds can be metabolized by anaerobic bacteria such as those found in constructed wetlands (Imfeld *et al.*, 2009; Ortega-Clemente and Luna-Pabello, 2012). Bacteria that metabolize both simple and complex organic compounds can generate electricity in devices such as MCFs (Venkata *et al.*, 2008) or SMFCs. The objective of this study was to test the generation of electricity in sediment

plant microbial fuel cells (SPMFC) from rhizodeposits of *T. domingensis*. To demonstrate the capacity of *T. domingensis* rhizosphere to release the reduced compounds which can be oxidized by anaerobic bacteria in the sediment and to generate an electron flow several SPMFCs were set up under the natural climatic conditions of the wetlands of the Port of Veracruz, Veracruz State, Mexico.

MATERIALS AND METHODS

Two experiments were conducted (March to April and May to July 2009) to produce electricity using sediment plant microbial fuel cells (SPMFCs) and *T. domingensis* as the plant species. Two types of graphite material (see: preparation of graphite electrodes) were used for the construction of electrodes to measure electricity generation. The tests were conducted at the facilities of the Veracruz Institute of Technology (ITVER) in the Port of Veracruz, Mexico (19 ° 12 '30" N, 96 ° 05' 00" W, on average 10 meters above sea level, average temperature 25.3 ° C).

Collection and acclimatization of *Typha domingensis*

T. domingensis was collected from the Coastal Research Center of La Mancha (Centro de Investigaciones Costeras La Mancha, CICOLMA) located in a protected area in the State of Veracruz. Same size plants were selected to be able to compare results and for acclimatization placed in glass cells for two weeks under natural environmental conditions in an area where they received direct sunlight without any shadow effect. The plants were irrigated daily with water from the well in the ITVER pump area, enriched with nitrate and phosphate solutions at concentrations of 1.7 mg / L and 0.4 mg / L, respectively (APHA, 1998).

Preparation of graphite electrodes

For the first experiment, electrodes were constructed of graphite leads (STAEDLER, Germany) together with copper wire (cal. 12, operating voltage 300V, CONDUMEX, México) insulated with epoxy resin. The anode had an area of 300 cm² and the cathode 200 cm². For the second experiment, 3 mm diameter wovenwire cathodes were constructed of graphite (ICYTSA, México) with an approximate area of 380 cm². An anode matrix consisting of a layer of graphite granules between 1.5 and 3 mm in diameter (Carbograp S.A., México) at a 15 cm height, and 4 m of 3 mm diameter cable graphite (ICYTSA, México) in contact with the graphite granules.

To support the plants, a mixture of aquarium ornamental

Table 1. Experimental design tracking, from March to April and May to July 2009

First Experiment March-April 2009		Second Experiment May-July 2009	
Experimental Unit SPMFC.	Control	Experimental Unit SPMFC.	Control
BR1, BR2, BR3	BR0	BRa1, BRa2, BRa3, BRb1, BRb2, BRb3	BRa0, BRb0

BR = Bioreactor.

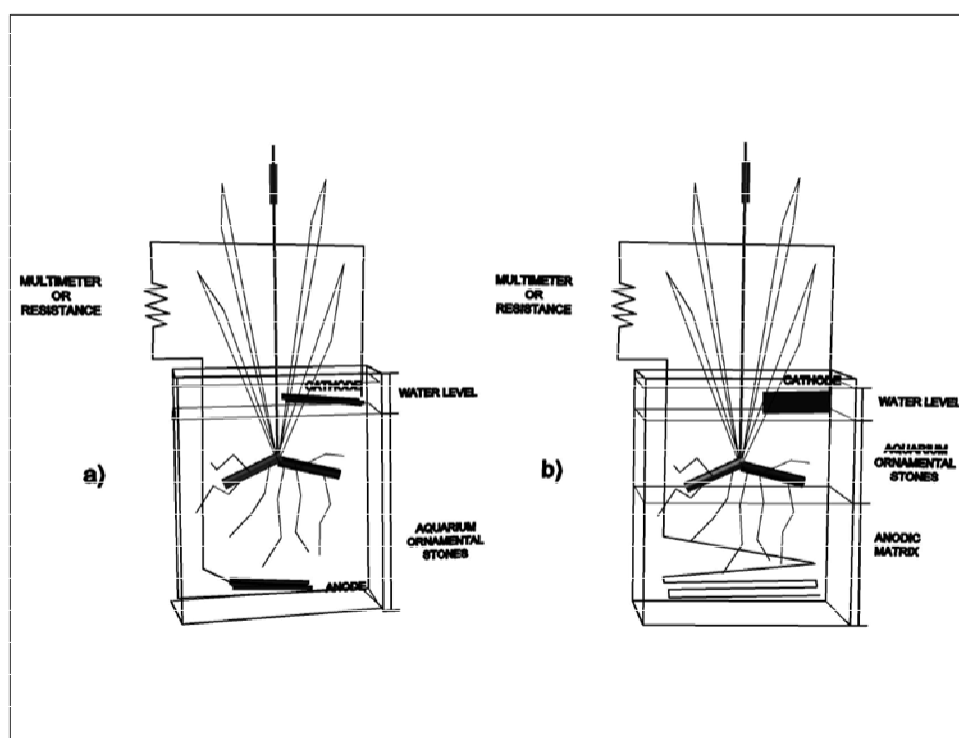


Figure 1. Schematic diagram of SPMFCs a) March-April and b) May-July

stones was chosen.

Preparation of SPMFCs.

For both experiments, glass and silicon cells were built with an area of 300 cm² (12cm wide, 25 cm long) considered as the plant growth area (PGA) also measuring 30 cm high. Fine gravel was used as a support for plant growth.

Experimental design

The experimental designs for both experiments are presented in Table 1. In both experiments all bioreactors (BR) were inoculated with 100 ml sludge from the same place where the plants were collected in order to provide an inoculum to start the devices. The plants underwent

an adaptation period of 2 weeks as in the first trial.

Operation and measurement of the potential differences in SPMFCs

For both experiments, potential difference measurements were taken three times per day at the following intervals:

a) 8 a.m. to 10 a.m., b) 12 pm to 3 pm and c) 6 pm to 8 pm, using a multimeter (Fluke 87V, Fluke Corporation, EE.UU). In the first experiment, the cells were operated in open circuit for 30 days and open circuit voltage and water surface temperature were monitored. In the second experiment (60 days), SPMFCs were operated in open circuit for a few days to reach stabilization; once a potential of 600 mV or more was

reached, an 80 resistance was used for current measurements in closed circuit. Two SPMFCs were operated in open circuit as controls. Once SPMFC

Table 2. Open circuit voltage (OCV) cell average during the 1st and 2nd periods of the second experiment started in May 2009. ADSR (average daily solar radiation), AT (average temperature of surface water).

ADSR	1st Period 34.14±0.06 MJ/m ²		2nd Period 33.8±1.47 MJ/m ²	
	OCV(mV)	AT(°C)	OCV(mV)	AT(°C)
BRa1	147.88 ±329.74	30.84±2.78	490.63± 148.16	35.48 ± 5.96
BRa2	116.37±326.12	30.25±2.86	-4.95 ± 34.86	34.7 ± 5.66
BRa3	132.69±298.45	30.67±2.74	13.18± 71.55	34.28 ± 5.52
BRa0	148.75±202.01	30.87±3.03	185.53± 126.86	35.28 ± 6.18
BRb1	542.47±236.34	30.60±2.42	57.93± 64.02	34.56 ± 5.55
BRb2	515.68±196.33	30.46±2.45	641.24± 250.87	34.48 ± 5.67
BRb3	472.87±172.89	30.34±2.41	59.08 ± 73	34.93 ± 5.46
BRb0	384.27±173.99	30.71±2.61	614.63 ± 101.079	35.16 ± 6.07

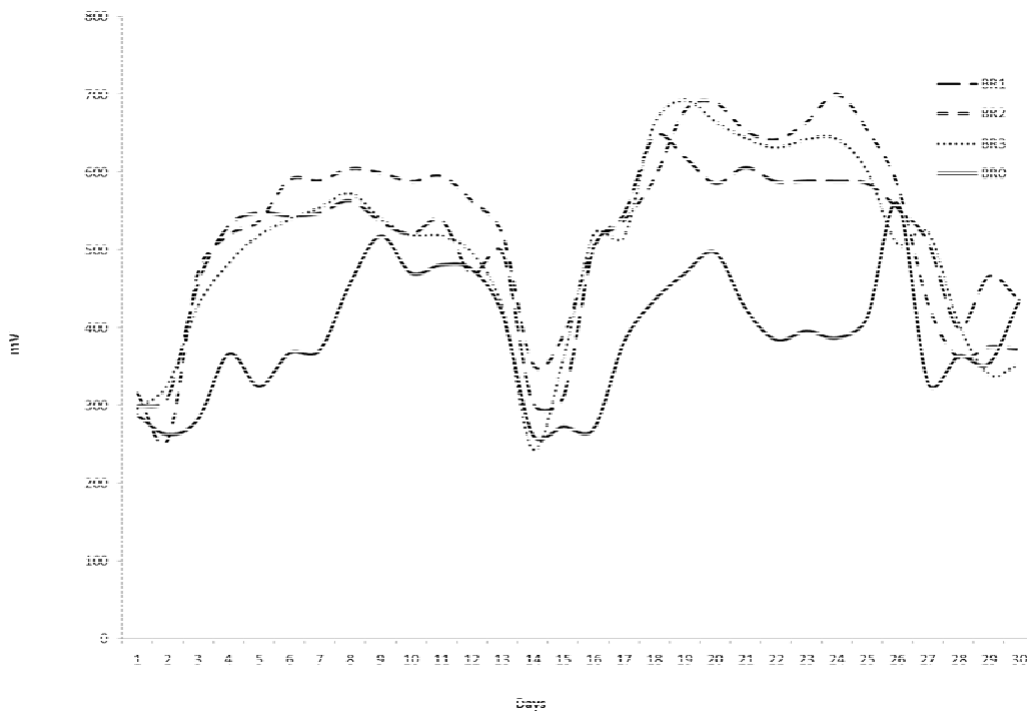


Figure 2. OCVs for the experiment from March 23 to April 23 (31.81 ± 2.35 MJ/m² average daily solar radiation).

current generation stability was observed in closed circuit, the control SPMFCs were operated in closed circuit in order to test electrical energy production. After 5 days, 3 pairs of current producing SPMFCs were connected in series and then these three pairs were connected in parallel. An electric motor (CEBEK, Spain) with a resistance of 70 (with power requirements of at least 700 mV and 10 mA) was used to test the ability of these current generation devices.

In the SPMFCs, potential difference (voltage) was measured and current was calculated by Ohm's law, Eq (1).

$$I = V/R \dots\dots\dots \text{Eq. (1)}$$

Power was obtained as the product of current times voltage, Eq (2).

$$P = VI \dots\dots\dots \text{Eq. (2)}$$

Current and power data obtained were normalized to plant growth area (300 cm²) to obtain measurements in mA/m² and mW/m², respectively, as proposed by Logan *et al.* (2006) to facilitate result comparison. The average daily solar radiation during the experiment was calculated using the algorithm of Yang *et al.* (2006) and meteorological variables (pressure, relative humidity and sunshine hours) provided by the Forecast Center of the Gulf of Mexico, part of the National Water Commission (Comision Nacional del Agua, CNA).

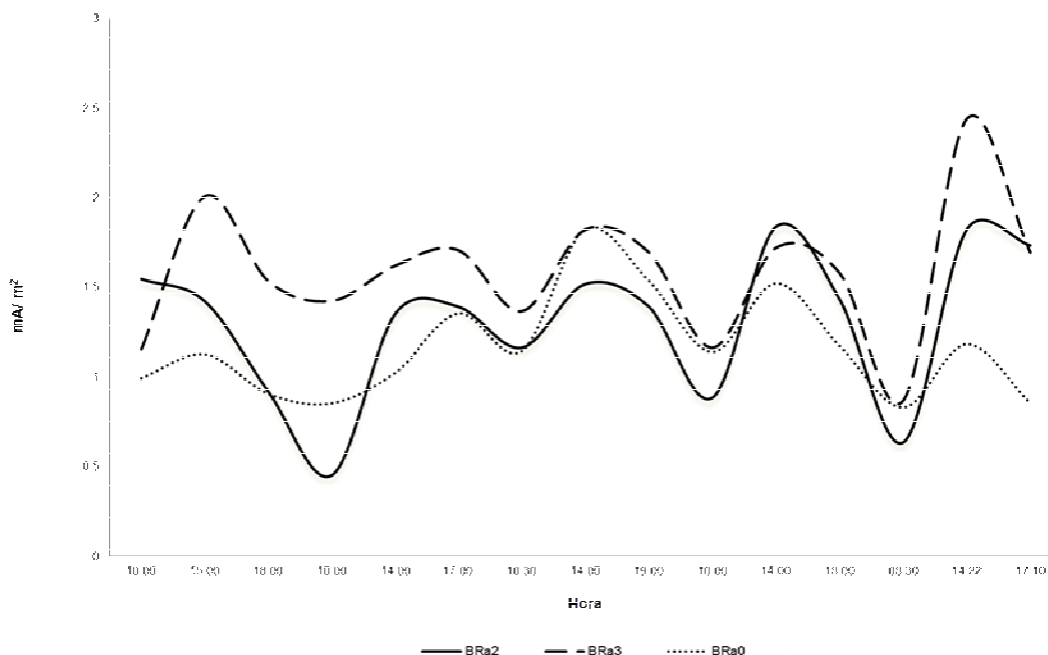


Figure 3. Behavior of three bioreactors (BRa2, BRa3, BRa0) for five days (From 4 to 8 June). The measurement interval times correspond to: morning (m) = 8-10 am; afternoon (a) =12-3pm and night (n) = 6-8 pm.

Statistical analysis

A normality test of open circuit voltages (OCVs) was conducted with the Anderson-Darling test. With the normalized power results, a Mann Whitney test was performed to establish differences between cell performance (BRa0, BRa1, BRa2, BRa3, BRb0, BRb1, BRb2, and BRb3). Both tests used the Minitab 14 program. Cell OCVs for the different periods are summarized in Table 2 with their means and standard deviations.

RESULTS AND DISCUSSION

In the first series of experiments (March-April 2009), there was an increase in SPMFC voltage from 0 to 600 mV during the first 5 days. By day 14, there was a decay in voltages coinciding with adverse weather conditions on those days (winds from the North). Later, from day 18, measurements in the three SPMFCs remained above 600 mV and the experiment was stopped on day 26 due to the observed decreasing trend of SPMFC voltage. During this period, the cells with plants exhibited voltages higher than the control bioreactor (BR0) (Figure 2).

In the May-June 2009 experiments, the SPMFCs remained in open circuit during an initial period from 24 May to 3 June. During this first period, the cells attained

750 mV OCVs, indicating anaerobic conditions at the anode (De Schamphelaire *et al.*, 2008) and they also showed algal growth on BRb2 and BRb1 cathodes (Table 1). From 4 to 8 June, SPMFCs were maintained in closed circuit, a maximum voltage of 200 mV (81.3 mA/m^2 , 16.26 mW/m^2) being achieved. This period was characterized by constant solar radiation of 34.21 MJ/m^2 . Voltage measurements in closed circuit at noon were higher than in the morning or afternoon. In general, cells with plants exhibited better performance than controls (Figure 3).

During the period in closed circuit, the current produced by the cells with plants was observed to be on average 18% higher than controls and the average cell power with plants was 42% higher than in controls. In both cases, the water surface temperature was similar (Table 3).

Having tracked the closed circuit SPMFCs for 6 days, tests were made to start the CEBEK engine. 3 pairs of current producing SPMFCs were connected in series and then these three pairs were connected in parallel to achieve periods of 15 minutes a day for 6 days. A second follow-up period with open circuit was held from June 24 to July 19. During the last 15 days of this period, the OCVs of most SPMFCs reversed polarity, however BRa1, BRb2 and BRb0 showed no decline in potential difference. During this period, decay and death of the BRb2 cell plant became evident, that cell having achieved up to 899 mV in open circuit (Table 2).

A Mann Whitney test was used to compare the power generated between SMFCs and SPMFCs in May 2009.

Table 3. Average cell measurements in closed circuit during May.

	Temperature of surface water	Current	Current density	Power	Power density
Bioreactors	°C	mA	mA/m ²	mW	mW/m ²
Bra2	37.00 ±4.12	1.30 ±0.42	43.27 ±13.83	0.15 ±0.08	5.05 ±2.69
Bra3	36.93±4.08	1.59± 0.38	52.88±12.53	0.22±0.10	7.24±3.40
Bra0	37.13±4.19	1.16±0.29	38.79±9.61	0.12 ± 0.06	3.91±2.03
Brb1	36.93±3.53	1.20 ±0.41	40.01±13.72	0.13±0.09	4.37±3.07
Brb2	36.80 ±3.49	1.66±0.31	55.44±10.43	0.23 ± 0.08	7.81±2.73
Brb0	36.73±3.84	1.25±0.39	41.81±13.05	0.14±0.08	4.69±2.67
Average BR.	36.92±3.77	1.44±0.33	47.90 ±10.98	0.18±0.08	6.12±2.53
Average BraO-Brb0	36.93±3.86	1.21±0.31	40.30 ±10.41	0.13±0.07	4.30±2.20

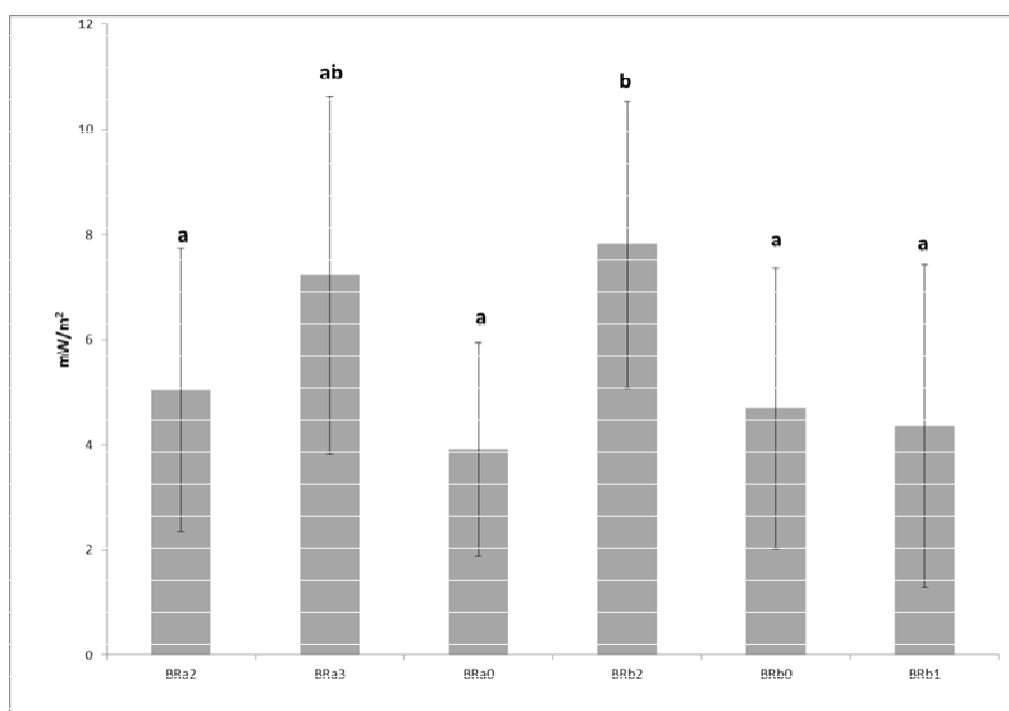


Figure 4. Comparison of power generated by SMFCs and SPMFCs using the Mann-Whitney test in May-June 2009 (different letters indicate significant differences).

The results of this test showed no statistically significant difference between BRb2 and BRa3 ($p > 0.05$). However, BRa3 was similar to other SPMFCs (Bra2, Bra0, Brb0, Brb1), but not to BRb2 (Figure 4).

At the end of each experiment a reddish color was observed at the roots, probably due to the presence of iron. A considerable increase in root volume was also observed.

In both experiments, the SPMFCs reached OCVs of up to 700 mV, potentials characteristic of these systems in other environments and with other plants (Lowy *et al.*, 2006; Kaku *et al.*, 2008; De Schamphelaire *et al.*, 2008). This demonstrates the good performance that

wetland plants typical of the Port of Veracruz may exhibit in SMFCs.

In the second stage of the experiments, the SPMFCs generated current during the 5 days of tracking in closed circuit and during the 6 days of testing with the electric motor, an average of 6.12 ± 2.53 mW/m² (Table 2), which corresponds to 42 % more than the SMFC control; the average power generated by SPMFCs is twice that reported in a field of rice (*O. sativa*) under environmental conditions, that is to say 3 mW/m² (Kaku *et al.*, 2008).

In the Mann-Whitney test, BRa3 and BRb2 showed a statistically significant difference in power generation with respect to other cells, indicating that the generation of

power in cells with plants turned out to be similar in at least two reactors. However the BRa3 cell is similar to the BRa2, BRa0, BRb0, BRb1 group of cells (Figure 4). It is important to note that better results were obtained in generating power ($6.12 \pm 2.53 \text{ mW/m}^2$) compared to a similar work reported by Kaku et al. (2008) in temperate climates (3 mW/m^2). Although they did not perform better than control systems in the greenhouse ($21 \pm 6 \text{ mW/m}^2$) as reported by De Schamphelaire et al. (2008), they were successful in proving that power generation devices can be improved using natural wetland plants from the State of Veracruz.

During the last 15 days of both experiments, most SPMFCs were characterized by decay in OCVs except BRb2, which maintained its polarity with $641.24 \pm 250.87 \text{ mV}$. This observed difference in BRb2 could be due to death of the plant which served as a nutrient for the population of anaerobic bacteria in cell sediments, allowing the device to maintain high OCVs. Furthermore, the decrease in OCVs in SPMFCs could be attributed to the possible oxygenation of the sediment and thus of the anode. This is because oxygenation is a common mechanism of the *T. domingensis* and *T. latifolia* species (Ortega-Clemente and Luna-Pabello, 2012; Chabbi et al., 2000). This mechanism changes the redox potential of the sediment and the latter's bacterial populations. Chabbi et al. (2000) report changes in the redox potential of -300 mV to $\approx 300 \text{ mV}$ after an hour with 24 cm long *T. domingensis* roots, dimensions similar to those achieved by the plant roots in this experiment.

In the area of La Mancha Veracruz, positive redox potentials in sediments inhabited by communities of *T. domingensis* have been identified (Moreno-Casasola et al., 2008). The large number of roots and organic richness of the soil lend themselves to the idea of designing SPMFCs to take advantage of these conditions. However, the design of these devices in confined cells enables their plant roots to expand and oxygenates the anode matrix, as happened in these experiments in which plant filled cells with roots increased the amount of dissolved oxygen and avoided anaerobiosis. This situation is unfavorable for the production of electricity for a prolonged period, so this represents a challenge for the design of this type of cell on a larger scale. It is therefore important to consider plant adaptations to mitigate the anaerobic effects of continuous soil flooding. In the case of *T. domingensis*, the aeration conditions it imposes on the soil change the system conditions at the rhizosphere, a limiting factor for the electricity production process considering that this is an anaerobic process. Plants such as *O. sativa* provide anaerobic respiration mechanisms such as the Pasteur effect (when anaerobic CO_2 produced exceeds CO_2 produced by aerobic metabolism) (Gibbs et al., 2000; Cronk and Fennessy., 2001).

De Schamphelaire et al. (2008). Considered aer-ation effects in roots but not for a prolonged period. The

Pasteur effect is the strategy used by this plant to mitigate the lack of oxygen, not excessive aeration as in the case of *T. domingensis*. *O. sativa* is the most appropriate for the design of SMFCs that use plants, but other wetland plants of Veracruz and other designs could be studied that allow this promising technology to be taken advantage of.

CONCLUSIONS

In both experiments, SPMFCs achieved OCVs of up to 700 mV in open circuit, indicating the good performance that the typical plants of wetlands of Veracruz can achieve in SPMFCs. In the second stage of the experiments, the current generated by SPMFCs during the 5 days of closed-circuit monitoring and the 6 days of testing with the electric motor, an average of $6.12 \pm 2.53 \text{ mW/m}^2$ was generated corresponding to 42% more than the SMFC control.

With these results, the implementation of other wetland plant species that do not exhibit the drawbacks presented by *T. domingensis* could be envisaged to favor electricity production by prolonged continuous systems.

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