Obtaining microbial communities with exoelectrogenic activity from anaerobic sludge using a simplified procedure

Short tittle: A simplified procedure to obtain exoelectrogenic activity

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HIGHLIGHTS

- Low-cost increase of exoelectrogenic activity from anaerobic sludge.
- Development of a new sediment-based MFC with simplified configuration.
- The brush inoculated for 30 days in the new MFC achieves 0.9W/m^2 in an AC-MFC.
- The new procedure has comparable performance to more complex techniques.

ABSTRACT

BACKGROUND

Microbial Fuel Cells (MFCs) is a technology used to transform the chemical energy present in substrates into electricity. The starting-up of these systems, i.e. enriching the anodic community in exoelectrogenic bacteria is usually long or requires expensive equipment.

RESULTS

An easy and low-cost procedure based on sediment MFC was developed to select microbial communities with exoelectrogenic activity from anaerobic sludge of a waste water treatment plant (WWTP). The configuration was based on a simple vessel working as a single chamber MFC with a cathode of stainless steel wool in the liquid surface and a submerged graphite fibre brush as anode. In 30 days of operation, a biofilm with remarkable exoelectrogenic activity was grown on the anode of the MFC. This graphite fibre brush anode was able to supply $0.9 W/m^2$ when working in an air-cathode MFC (AC-MFC) during 45 days of operation.

CONCLUSION

The presented procedure was demonstrated as a successful, low-cost and low-maintenance procedure to obtain exoelectrogenic activity and had comparable performances to other more costly and complex inoculation procedures. The Sed-MFC does not require potentiostat, external aeration, stirring, membranes or an enriched inoculum in exoelectrogenic biomass.

Keywords: anaerobic sludge, exoelectrogenic bacteria, microbial fuel cell (MFC), sediment MFC, stainless steel cathode.

INTRODUCTION

Bioelectrochemistry is an emerging technology to transform the chemical energy present in substrates into electricity (in microbial fuel cells, MFC) or other products of interest (in microbial electrolysis cells, MEC) using microorganisms as catalysts.[1-4] These microorganisms, known as exoelectrogens or anode respiring bacteria (ARB), are able to bring electrons out of the microbial cell and transfer them to a solid anode without any external chemical mediator.[5] ARB comprise many bacteria genera, which can be found in different natural environments from marine sediments to anaerobic systems, as *Geobacter*[6-9], *Shewanella*[10-11] or *Rhodoferax*[12].

The starting-up of a bioelectrochemical system, i.e. enriching the anodic community in ARB, usually takes weeks. The most common inoculum consists of using either the liquid effluent or some scrapped biofilm from the anode of an existing bioelectrochemical system. Another common start-up technique is controlling the anode at a fixed potential: i.e. the anode inoculated with anaerobic sludge is immersed into a substrate solution and poised at a certain potential. The choice of the optimal anode potential is a controversial issue[13-14]. An anode with a more positive potential would theoretically result in a higher microbial diversity because different microorganisms would obtain a high yield of energy transferring their electrons to the anode. However, lower anode potential would select those more specialized bacteria able to use a minimal amount of energy to grow releasing electrons to an anode. In any case, working at a fixed anode potential requires an expensive potentiostat, which can be particularly costly if high amounts of ARB are needed. A cheaper option would be using an MFC with a selected external resistor resulting in a desired potential range. Kim et al.[15] studied several inoculation techniques using a classical two-chamber MFC

configuration. They were able to increase the power from 22 to 30 mW/m^2 using ferric iron-coated carbon electrodes. Liu et al.[16] demonstrated that the performance of mixed culture microbial biofilms could be improved by a consecutive, purely electrochemical selection and biofilm acclimatization procedure. Their method was shown to be very efficient but it also required a multipotentiostat.

Despite these advances recently made, MFCs and MECs still face significant challenges for large-scale real-world applications[17]. For example, when moving bioelectrochemical systems into pilot or industrial scale,[18-19] the development of a low-cost and reliable procedure to obtain ARB-enriched biofilms on large anodes will be essential. The selected procedure should not require either ARB-enriched cultures or expensive equipment as for example potentiostats or selective membranes. In this sense, the aim of this study was to develop an efficient (simplified, successful and scalable) technique to select ARB in a graphite fibre brush anode suitable for different bioelectrochemical systems (MFC or MEC). The developed method is based on sediment/benthic MFC and uses anaerobic sludge as inoculum. In short, a benthic MFC harvests energy from natural environments by placing an electrode in the sediment (anode) and connecting it with an electrical circuit to another electrode (cathode) situated on the overlying water layer.[20-24] This work proposes the adaptation of the benthic MFC concept to a simplified lab-configuration (hereafter named Sed-MFC). In short, the Sed-MFC configuration consists of a single chamber MFC where the anode, a brush graphite, is buried into settled anaerobic sludge meanwhile the cathode, a stainless steel wool mesh, floats on the upper layer of the cell, thus in contact with the medium and the atmosphere. Then, the Sed-MFC corresponds to an air cathode configuration.

To the best of our knowledge, this is the first report of a methodology based on benthic MFC to obtain anodes with increased exoelectrogenic activity from raw anaerobic sludge.

MATERIALS AND METHODS

Sed-MFC construction and operation

The proposed Sed-MFC consisted of a conventional plastic vessel (1L) with an anode, a cathode and an electrical wire connection (Fig. 1). The anode was a graphite fibre brush (70 mm diameter x 70 mm length) made with fibres of diameter 7.2 µm (type PANEX33 160K, ZOLTEK, Hungary) and titanium wire. The brush was thermally treated at 440ºC for 30 minutes to increase further microbial adhesion.[25] The cathode was commercial SSW placed in the air/liquid interface and connected to a copper wire over the water surface to avoid undesired copper corrosion that could affect MFC performance[26]. This low cost cathode provided high specific area, which balanced the overpotential losses.[27]

Acetate was selected as electron donor and 2-bromoethanesulfonate (BES) was added to prevent methanogenesis. The cell inoculation comprised 500 mL of anaerobic sludge, 125 mL of acetate solution, 0.38 mL of micronutrient solution and 100 mL of phosphate buffer solution (PBS). Then, it was filled up with deionized water up to 1000 mL. The anaerobic sludge was obtained from an anaerobic digester of an urban WWTP (Manresa, Barcelona). The PBS stock solution consisted of (g/L): 80 NaCl, 2 KCl, 14.4 Na_2HPO_4, 2.4 KH_2PO_4 (0.1M, pH 7.4) The acetate solution was (g/L): 11.33 $NaCH_3COO \cdot 3H_2O$, 0.19 $CaCl_2 \cdot 2H_2O$, 1.2 $MgSO_4 \cdot 7H_2O$, 1.02 NH_4Cl and the

micronutrient solution was (g/L): 1.5 $FeCl_3 \cdot 6H_2O$, 0.15 H_3BO_3, 0.03 $CuSO_4 \cdot 5H_2O$, 0.18 KI, 0.12 $MnCl_2 \cdot H2O$, 0.06 $Na_2MoO_4 \cdot 2H_2O$, 0.12 $ZnSO_4 \cdot 7H_2O$, 0.15 $CoCl_2 \cdot 6H_2O$, 0.12 $AlCl_3$, 0.12 $NiCl_3$ and 10 EDTA.[28] The final concentration of acetate in the MFC was 1.4 g/L. 10mM of BES were added to suppress methanogenic activity as in other works.[29-30]

The conductivity and the pH were corrected to be around 10-15 mS/cm and 7.0-7.5 respectively. Cells were kept at room temperature (around 21ºC) during all the operational period. The SSW cathode was immersed 50% in the liquid, and the other 50% exposed to the atmosphere. Then the circuit was closed connecting the titanium wire from the brush and the copper wire from the steel wool through a 560 Ω resistance.

Air cathode MFC (AC-MFC) description

Power and polarisation curves could not be done in Sed-MFCs due to their lack of homogeneity (i.e. the liquid was not stirred). For this reason, when these curves were needed, the brush from the Sed-MFC was slightly rinsed to remove all the non-attached bacteria and was placed in a conventional AC-MFC using a fresh medium with the desired initial acetate concentration.

The AC-MFC (Fig. 1) consisted of a 400 mL glass vessel with a lateral 7 cm diameter aperture where the cathode was assembled. The cathode was made with carbon cloth coated with carbon powder and platinum suspension on the inner side, whereas the outer side was coated with a polytetrafluoroethylene (PTFE, Teflon) solution.[31-32] The anode was the carbon fibre brush coming from the Sed-MFC. Both electrodes were connected through a 560 Ω resistance and voltage evolution was monitored.

Thus, the Sed-MFC has the anode buried in anaerobic sludge and a SSW-based cathode while the AC-MFC has the enriched anode and a Pt-based cathode. The main goal of the Sed-MFC is to enrich the anode in exoelectrogenic bacteria for its posterior use in another MFC.

Chemical analyses and monitoring

Acetate was analysed by gas chromatography (Agilent Technologies, 7820-A) using a flame ionization detector (FID) and helium as carrier gas. The voltage across the external resistance in the Sed-MFC and AC-MFC was monitored using a 16-bit data acquisition card (Advantech PCI-1716, Taiwan) connected to a personal computer with software developed in LabWindows CVI 2010 for data acquisition and monitoring. Cell intensity and power were calculated according Ohm's law (equations 1, 2).

$$I = V / R_e \quad \text{(eq. 1)}$$

$$P = V \cdot I \quad \text{(eq. 2)}$$

where V is the voltage drop in the resistance (V), R_e is the external resistance (Ω), I is the current intensity (A) and P is the power (W). Intensity, as well as power, was normalized with respect to the projected cathode area for comparison purposes for both Sed-MFC and AC-MFC. The cathodic projected areas were around $6.4 \cdot 10^{-3}$ m^2 and $3.9 \cdot 10^{-3}$ m^2 for the stainless steel wool and the platinum-coated carbon cloth, respectively. Power and polarization curves were obtained with a multi-resistance board which allowed changing the external resistance between 25 and 470000 Ω. A 10 minutes period was used for the voltage stabilization at each resistance. Coulombic efficiency (CE), i.e. fraction of electrons recovered as current versus that in the initial organic matter, was calculated as equation 3.

$$CE = \frac{\int Idt}{F \cdot b \cdot \Delta S \cdot V_R} \quad \text{(eq. 3)}$$

where t is time (s), F is Faraday's constant (96485 C/mol-e^-), b is the stoichiometric number of electrons produced per mol of substrate (8 mol-e^-/mol acetate), ΔS is the substrate consumption (mol/L) and V_R the liquid volume (L).

Electrochemical analyses

Low-scan cyclic voltammetry (LSCV) was performed using a μAutolab type II potentiostat in three-electrode mode in the AC-MFC. The anode was used as working electrode and the cathode as the auxiliary one. An Ag/AgCl, KCl 3M electrode (+210 mV vs. SHE) was used as reference electrode. The system was under open circuit conditions for one hour just before the LSCV started. LSCV was recorded at 0.1 mV/s from the anode open circuit potential -0.50 V, to 0.3 V vs. Ag/AgCl.

Scanning Electron Microscopy

Samples of graphite fibre brush were collected and fixed with a solution of 2.5% glutaraldehyde and 2% paraformaldehyde. Samples were treated with osmium tetraoxide, dehydrated with ethanol and dried at critical point with carbon dioxide (BAL-TEC CPD030; Bal-Tec). Then, the samples were coated with few nanometers of Au-C (E5000 Sputter Coater, BIO-RAD, California, USA) to increase signal detection and visualized on a Scanning Electron Microscope (Hitachi S-70, Japan).

RESULTS AND DISCUSSION

Sed-MFC development and performance

Anaerobic WWTP sludge was inoculated in three identical Sed-MFCs with an external resistance of 560 Ω. Fig. 2a shows the voltage profiles obtained during more than 30 days. The initial voltage around 150 mV decreased during the first days of operation due to the acclimatization period. Moreover, residual oxygen presence in the water and the sediments also favoured this initial slow response. After approximately 4 days, the voltage increased linearly (around 0.6 mV/h) which led to an increase in intensity, indicating the development of exoelectrogenic activity. This linear increase period reached fairly high voltage values, up to 300 mV (0.94 A/m^2 and 0.3 W/m^2). A constant water loss was observed due to evaporation, which was detrimental for the Sed-MFC operation, since low water levels prevented the correct contact between the cathode and the medium (i.e. the cathode surface in contact with water decreased). To avoid complete substrate depletion and ensure good contact between the water and the cathode, fresh medium was periodically added causing some oxygen diffusion and partial ARB inhibition. The systems recovered their working voltage some days after the medium addition.

These Sed-MFCs also allow inoculating at different external resistance and thus providing different external conditions that can induce the growth of different microbial communities in the anode. For example, Fig. 2b shows the voltage profiles obtained in another experiment with three cells under the same operational conditions except for the different external resistances used. As can be observed, the potential increases when the external load increases, in agreement with the theoretical background. In this case, the cells with higher external resistances gave similar power results (around 0.17 mW).

The time needed to develop a significant amount of exoelectrogenic biofilm is an essential parameter for the design of Sed-MFCs. To this aim, five graphite fibre brushes

were developed in Sed-MFCs for different time periods. Each brush was transferred directly to an AC-MFC, where power density curves were determined (Figure 3). After these evaluations, it was concluded that a 30-day operational period ensured an acceptable biofilm development.

Another experiment was designed to determine if the Sed-MFC could be further simplified. Ensuring microbial adhesion is essential and thus, a thermal treatment of the graphite fibres was initially performed. Thermal treatments are recommended to enhance microbial adhesion since i) solvents and lubricants (from the anode manufacturing) are washout from the anode surface and ii) active area is increased due to microfractures generation[25], but this treatment increases the construction costs of MFC. Considering that our Sed-MFC architecture was different from other reported MFC (volume, distance between electrodes and electrodes surface are higher), an experiment was performed to study if the positive effect of the thermal treatment was significant in the Sed-MFC configuration. Then, a thermally treated graphite fibre brush and an untreated brush were inoculated in a Sed-MFC for 25 days. After this period, both anodes were placed in two different AC-MFCs. Fig. 4 shows the power and polarisation curves obtained with both brushes. The maximum powers reached by the untreated and treated graphite fibre brush were 312mW/m^2 and 903mW/m^2, respectively. The thermal treatment resulted in not only three times higher power but also in a significant internal resistance decrease: 362Ω for the untreated brush versus 151 Ω for the treated brush. Therefore, these results corroborate the better performance of the thermally treated brush and hence this treatment is recommended for the Sed-MFC. In this sense, the SEM microphotographies (Fig. 4b) for treated fibres corroborate the good colonization of the brush anode.

From Sed-MFC to AC-MFC

The extent of exoelectrogenic activity obtained in the Sed-MFC was evaluated by moving an anodic brush which had been placed in a Sed-MFC for 30 days into a conventional AC-MFC under the same operational conditions (Fig. 5a). The first cycle (from day 0 to 14) corresponds to an acclimation cycle whereas the results from the second cycle (from 14 to 17.5 days) onwards were already promising. A high coulombic efficiency (51%) was achieved, the voltage reached 480 mV and the cycle length was 2.5 days. The experimental voltage ranged between 370 and 450 mV and an average coulombic efficiency of 55% was obtained. Then, only one cycle was needed to adapt the anode brush from the Sed-MFC to an AC-MFC operation. The AC-MFC system performance was very satisfactory, achieving maximum values up to 0.134 A/m^2 (P = 0.07 W/m^2) with a reasonably fair coulombic efficiency.

The exoelectrogenic activity was also evaluated through LSCV by comparing an anodic brush obtained from a Sed-MFC and stabilized in an AC-MFC for 48 hours to a non-inoculated brush (Fig. 5b). The inoculated brush exhibited one order of magnitude higher exoelectrogenic activity than the obtained with the non-inoculated brush, which showed negligible activity. The inoculated anode showed one typical oxidation peak at -0.25 V vs Ag/AgCl. The value of the anode potential giving half of the maximum current density, known as E_{kA}, was around -0.37 V, which is in agreement with the results found for acetate-fed *Geobacter* pure culture systems.[33] The LSCV also showed a high capacitive current for the inoculated anode, indicating the presence of a conductive biofilm attached to the anode surface.[34]

Comparison with other works

The proposed inoculation procedure is based on placing a graphite fibre brush in a Sed-MFC with an anaerobic sludge blanket during 30 days. Anaerobic WWTP sludge is a good candidate for inoculation because it is easy to obtain and contains a high diversity of bacterial communities, including electrochemically active strains of bacteria.[15] The Sed-MFC methodology has several advantages with respect to other MFC configurations. No external aeration is required, as the cathode is directly exposed to air resulting in significant aeration savings. The internal resistance is minimised because the electrodes can be located nearby. The system has low maintenance requirements, as only the level of liquid must be supervised with low periodicity. Neither stirring nor proton exchange membrane (PEM) are required which decreases the operational costs. The main purpose of the PEM is to avoid oxygen entering to the anode. With the proposed configuration, the amount of oxygen in contact with the sludge blanket is negligible, particularly taking into account that the system is not stirred. Moreover, if some oxygen entered, it would be consumed in the upper layer of the blanket, maintaining the lower layer (where the brush is placed) under the required anaerobic conditions.

Reported configurations in the literature[14] propose an initial polarisation period where a certain potential is applied to the cell in order to enhance ARB growth on the anode. This external voltage is reported to increase the ARB growth at the expense of increasing the cost. However, the proposed Sed-MFC does not consider the polarisation period since the objective is to develop an efficient (i.e. simplified, successful and scalable) procedure to obtain anodic microbial communities with exoelectrogenic activity using anaerobic sludge. The total cost of the cell materials is practically due to

the titanium wire (around 166€/m, 0.5 mm diameter titanium) used to build the anode brush. Table 1 compares the performance of the presented procedure with other reported works. This comparison is not a straightforward issue since a wide range of reactor types, volumes, inoculum sources and substrates are found in the literature. In our case, we compare the experimental results obtained in the first batch with the AC-MFC when the anodic brush was transferred. In this study, a maximum power of 0.9 W/m^2 (Fig. 4) was reached, which is a fairly good result for a reactor volume of 400mL. Wang et al.[35] presented a selection strategy able to reach half the power output of this study in about the same time, 35 days, and using a similar reactor volume, 480 mL. However they used a potentiostat, what increases considerably the cost of the inoculation process. Other studies where inoculation time was high, such as Logan et al.[25], obtained very high power output, nevertheless the volume was much lower, which obviously reduces power losses. Kim et al.,[15] who worked with a similar reactor volume of 620mL, stated that 50 hours were needed for inoculation when anaerobic sludge was used as inoculum; however, power output was thirty times lower than the one observed in this study. Finally, Wang et al.[36] also presented a work where inoculation time was very fast (60 hours) and power output was of the same order of magnitude as ours. However, the inoculum was coming from a previous working MEC with an already enriched exoelectrogenic environment that could have expedited the inoculation process.

Thus, our system, in comparison with others, seems to provide a fair amount of exoelectrogenic activity in a relatively high reactor volume when starting up from a poor ARB environment like anaerobic sludge from an anaerobic digester in a reasonable time frame.

CONCLUSIONS

A simplified and efficient procedure to increase the exoelectrogenic activity of anodic microbial communities from anaerobic WWTP sludge was developed. The Sed-MFC configuration was demonstrated as a successful, low-cost and low-maintenance procedure to obtain exoelectrogenic activity. The anode graphite fibre brush developed in a Sed-MFC for 30 days provided good results and showed comparable performances to other more costly and complex inoculation procedures. The Sed-MFC does not require potentiostat, external aeration, stirring or membranes. The electrodes can be located nearby decreasing the internal resistance and the anaerobic sludge blanket allows maintaining strict anaerobic conditions in the anode.

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REFERENCES

1. Bennetto HP, Microbes come to power. *New Sci* **114**:36-40 (1987).

2. Bennetto HP, Electricity generation by microorganisms. *Biotechnol Educ* **1**:163-168 (1990).

3. Liu H, Cheng S, Logan BE, Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. *Environ Sci Technol* **39**:5488-5493 (2005).

4. Rozendal RA, Hamelers HVM, Euverink GJW, Metz SJ, Buisman CJN, Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *Int J Hydrogen Energ* **31**:1632-1640 (2006).

5. Logan BE, Exoelectrogenic bacteria that power microbial fuel cells. *Nat Rev Microbiol* **7**:375-381 (2009).

6. Dumas C, Basseguy R, Bergel A, Microbial electrocatalysis with Geobacter sulfurreducens biofilm on stainless steel cathodes. *Electrochim Acta* **53**:2494-2500 (2008).

7. Kim M, Lee Y, Optimization of culture conditions and electricity generation using Geobacter sulfurreducens in a dual-chambered microbial fuel-cell. *Int J Hydrogen Energ* **35**:13028-13034 (2010).

8. Lovley DR, Microbial fuel cells: novel microbial physiologies and engineering approaches. *Curr Opin Biotech* **17**:327-332 (2006).

9. Yi, H., Nevin, K.P., Kim, B.-., Franks, A.E., Klimes, A., Tender, L.M., Lovley, D.R., 2009. Selection of a variant of Geobacter sulfurreducens with enhanced capacity for current production in microbial fuel cells. Biosens. Bioelectron. 24, 3498-3503.

10. Biffinger JC, Byrd JN, Dudley BL, Ringeisen BR, Oxygen exposure promotes fuel diversity for Shewanella oneidensis microbial fuel cells. *Biosens Bioelectron* **23**:820-826 (2008).

11. Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH, A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. *Enzyme Microb Technol* **30**:145-152 (2002).

12. Liu Z, Li H, Effects of bio- and abio-factors on electricity production in a mediatorless microbial fuel cell. *Biochem Eng J* **36**:209-214 (2007).

13. Torres CI, Marcus AK, Parameswaran P, Rittmann BE, Kinetic experiments for evaluating the nernst-monod model for anode-respiring bacteria (ARB) in a biofilm anode. *Environ Sci Technol* **42**:6593-6597 (2008).

14. Wagner RC, Call DF, Logan BE, Optimal set anode potentials vary in bioelectrochemical systems. *Environ Sci Technol* **44**:6036-6041 (2010).

15. Kim JR, Min B, Logan BE, Evaluation of procedures to acclimate a microbial fuel cell for electricity production. *Appl Microbiol Biotechnol* **68**:23-30 (2005).

16. Liu Y, Harnisch F, Fricke K, Sietmann R, Schröder U, Improvement of the anodic bioelectrocatalytic activity of mixed culture biofilms by a simple consecutive electrochemical selection procedure. *Biosens Bioelectron* **24**:1006-1011 (2008).

17. Zhou M, Wang H, Hassett DJ, Guc T, Recent advances in microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for wastewater treatment, bioenergy and bioproducts. *J Chem Technol Biot*, **88**:508-518, (2013).

18. Cusick RD, Bryan B, Parker DS, Merrill MD, Mehanna M, Kiely PD, Liu G, Logan BE, Performance of a pilot-scale continuous flow microbial electrolysis cell fed winery wastewater. *Appl Microbiol Biotechnol* **89**:2053–2063 (2011).

19. Logan BE, Scaling up microbial fuel cells and other bioelectrochemical systems. *Appl Microbiol Biotechnol* **85**:1665–1671 (2010).

20. Dumas C, Mollica A, Féron D, Basséguy R, Etcheverry L, Bergel A, Marine microbial fuel cell: Use of stainless steel electrodes as anode and cathode materials. *Electrochim Acta* **53**:468-473 (2007).

21. Hong SW, Chang IS, Choi YS, Chung TH, Experimental evaluation of influential factors for electricity harvesting from sediment using microbial fuel cell. *Bioresource Technol* **100**:3029-3035 (2009).

22. Seok WH, Hyung JK, Yong SC, Tai HC, Field experiments on bioelectricity production from lake sediment using microbial fuel cell technology. *B Kor Chem Soc* **29**:2189-2194 (2008).

23. Zhao J, Li XF, Ren YP, Wang XH, Jian C, Electricity generation from Taihu Lake cyanobacteria by sediment microbial fuel cells. *J Chem Technol Biotechnol* **87**:1567-1573 (2012).

24. Ren Y, Pan D, Li X, Fu F, Zhao Y, Wang X, Effect of polyaniline-graphene nanosheets modified cathode on the performance of sediment microbial fuel cell. *J Chem Technol Biot*, DOI: 10.1002/jctb.4146 (2013).

25. Logan B, Cheng S, Watson V, Estadt G, Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ Sci Technol* **41**:3341-3346 (2007).

26. Zhu X, Logan BE, Copper anode corrosion affects power generation in microbial fuel cells. *J Chem Technol Biot*, DOI 10.1002/jctb.4156 (2013).

27. Ribot-Llobet E, Nam JY, Tokash JC, Guisasola A, Logan BE, Assessment of four different cathode materials at different initial pHs using unbuffered catholytes in microbial electrolysis cells. *Int J Hydrogen Energ* **38**:2951-2956 (2013).

28. Lovley DR, Phillips EJP, Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl Environ Microbiol* **54**:1472-1480 (1988).

29. Chidthaisong A, Conrad R, Specificity of chloroform, 2-bromoethanesulfonate and fluoroacetate to inhibit methanogenesis and other anaerobic processes in anoxic rice field soil. *Soil Biol Biochem* **32**:977-988 (2000).

30. Nielsen JL, Juretschko S, Wagner M, Nielsen PH, Abundance and phylogenetic affiliation of iron reducers in activated sludge as assessed by fluorescence in situ hybridization and microautoradiography. *Appl Environ Microbiol* **68**:4629-4636 (2002).

31. Cheng S, Liu H, Logan BE, Increased performance of single-chamber microbial fuel cells using an improved cathode structure. *Electrochem Commun* **8**:489-494 (2006).

32. Cheng S, Liu H, Logan BE, Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. *Environ Sci Technol* **40**:364-369 (2006).

33. Torres CI, Krajmalnik-Brown R, Parameswaran P, Marcus AK, Wanger G, Gorby YA, Rittmann BE, Selecting anode-respiring bacteria based on anode potential: Phylogenetic, electrochemical, and microscopic characterization. *Environ Sci Technol* **43**:9519-9524 (2009).

34. Srikanth S, Marsili E, Flickinger MC, Bond DR, Electrochemical characterization of Geobacter sulfurreducens cells immobilized on graphite paper electrodes. *Biotechnol Bioeng* **99**:1065-1073 (2008).

35. Wang X, Feng Y, Ren N, Wang H, Lee H, Li N, Zhao Q, Accelerated start-up of two-chambered microbial fuel cells: Effect of anodic positive poised potential. *Electrochim Acta* **54**:1109-1114 (2009).

36. Wang A, Sun D, Ren N, Liu C, Liu W, Logan BE, Wu W, A rapid selection strategy for an anodophilic consortium for microbial fuel cells. *Bioresource Technol* **101**:5733-5735 (2010).

Tables

Table 1. Comparison of different procedures aiming at increased exoelectrogenic activity.

References	**[36]**	**[28]**	**[16]**	**[14]**	**This study**
Volume (mL)	420	26	620	480	400
Internal resistance (Ω)	N.D.	8	N.D.	91.84	133
Maximum power (W/m^2)	0.23	2.4	0.008-0.03	0.45	0.9
Reactor type	H-type	Cube air cathode	H-type	Cube-type	Air cathode
Inoculum origin	Previous MEC	Previous MFC	Anaerobic sludge	Anaerobic sludge	Anaerobic sludge
Cathode catalyst	Platinum	CoTMMPP	Platinum	Ferricyanide	Platinum
Substrate	Acetate	Acetate	Acetate	Glucose	Acetate
Polarization period	No	No	No	Yes	No
Inoculation time	60 hours	>6 month	50 hours	35 days	30 days

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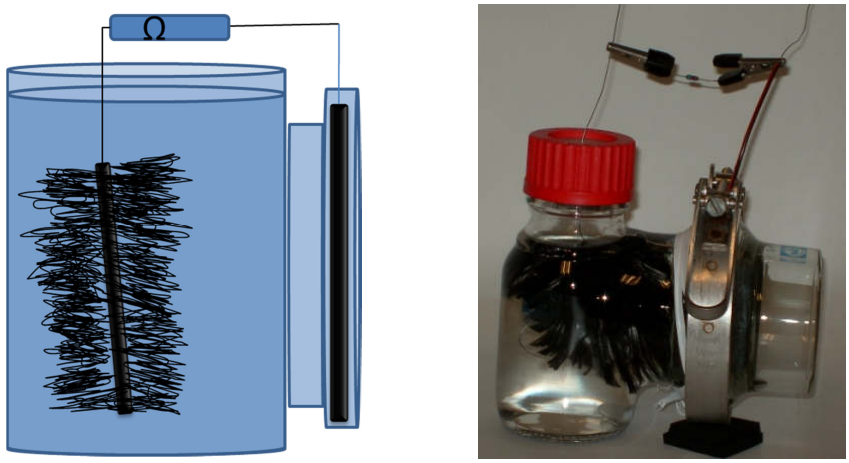
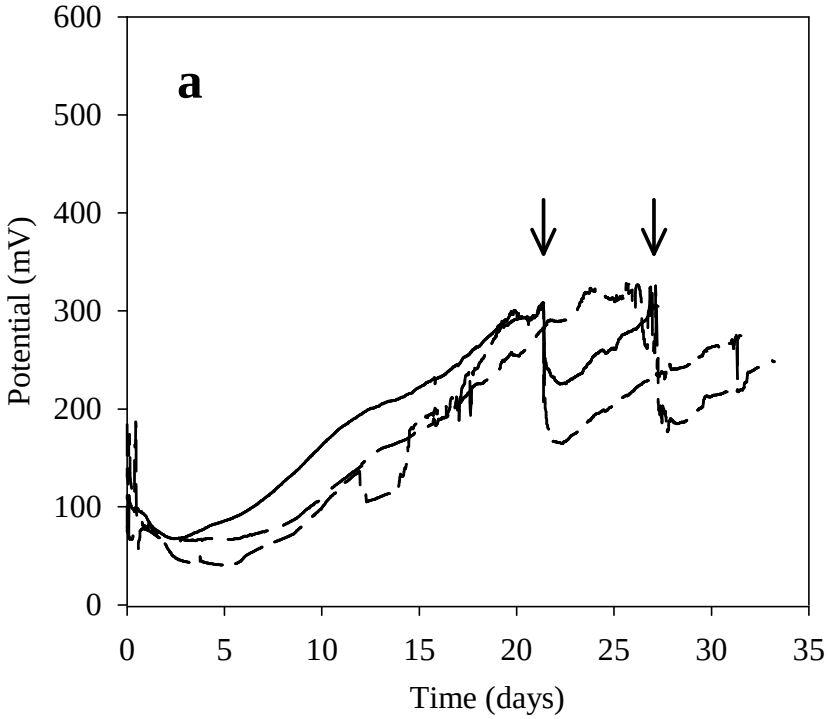


3 **Fig. 1.** Schematic representations (Left) and pictures (Right) of the Sed-MFC (Top) and

4 the AC-MFC (Bottom)

5



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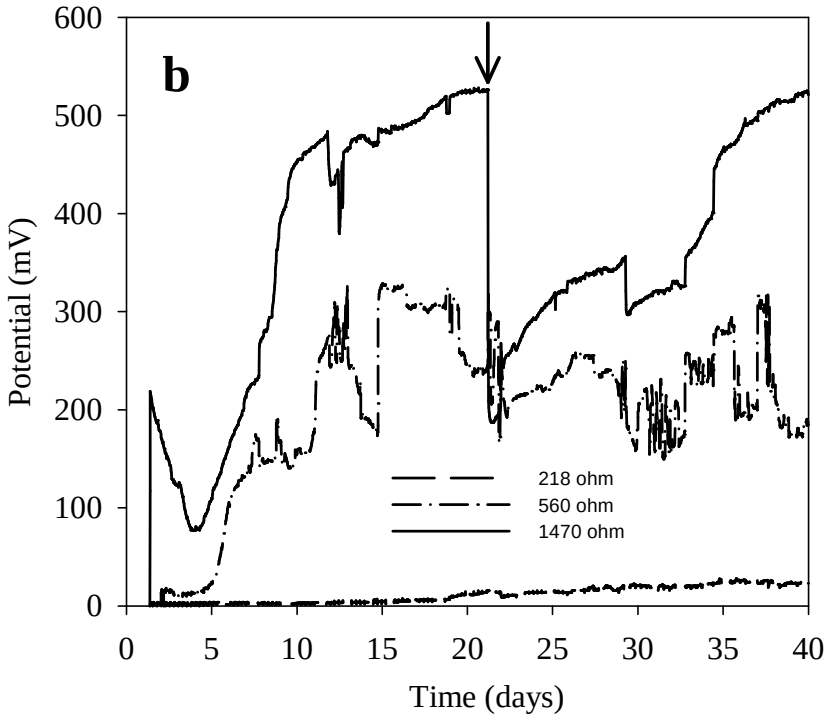


2

3 **Fig. 2.** a) Monitored voltage across 560Ω resistance for three different Sed-MFC with
4 identical inoculation. b) Experimental profiles for three different Sed-MFC with
5 different external resistances. Arrows indicate substrate addition.

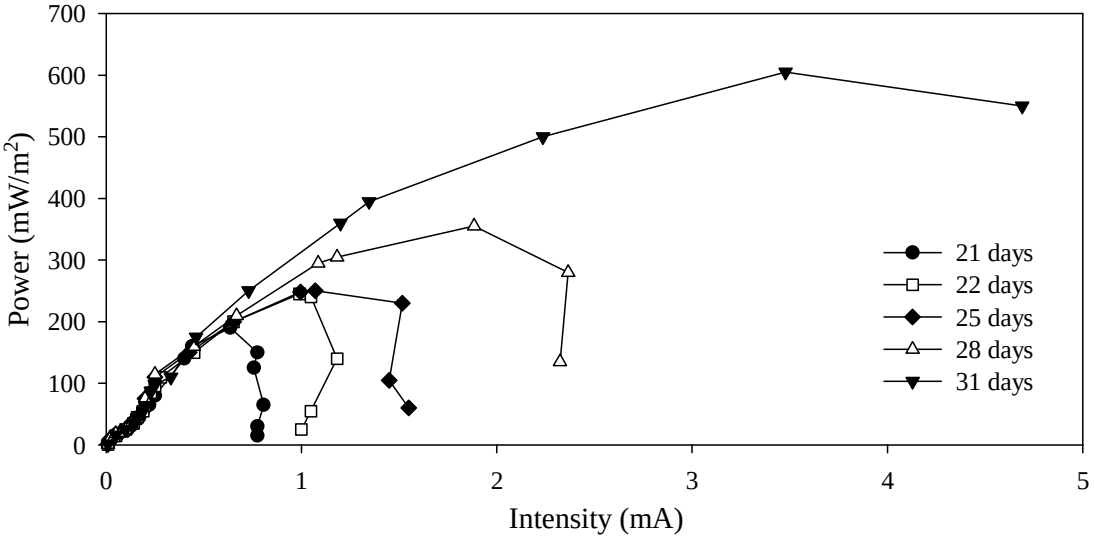
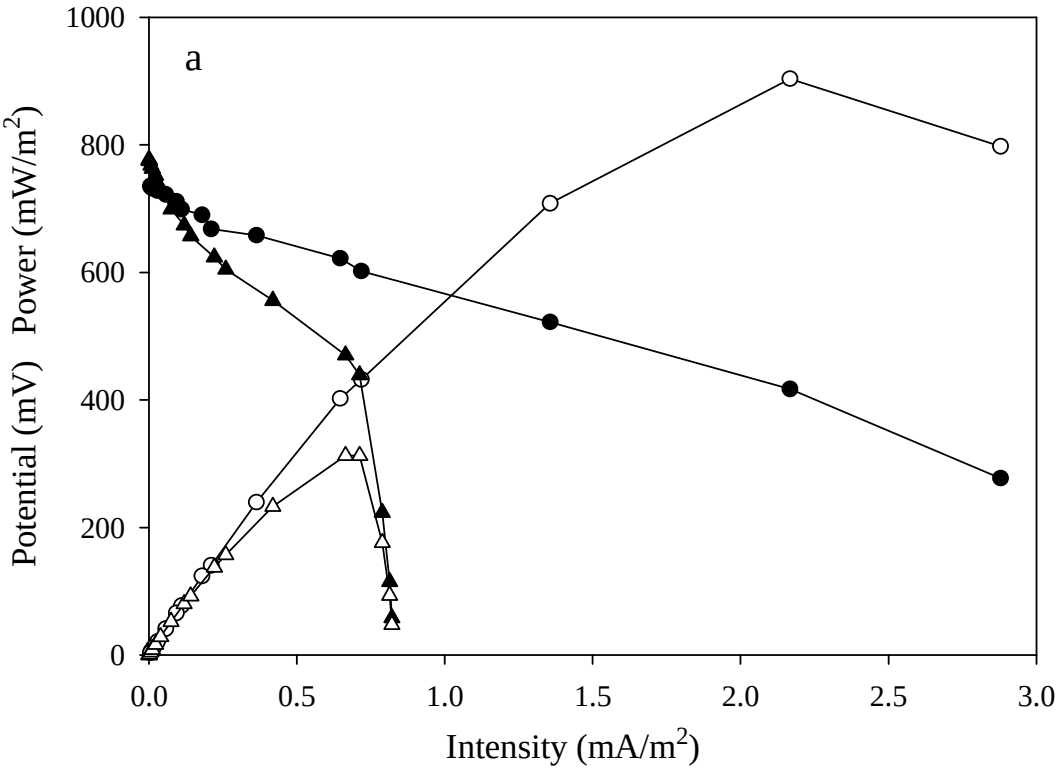


Fig. 3. Power curves in AC-MFC of anodes developed in Sed-MFCs with different inoculation periods.



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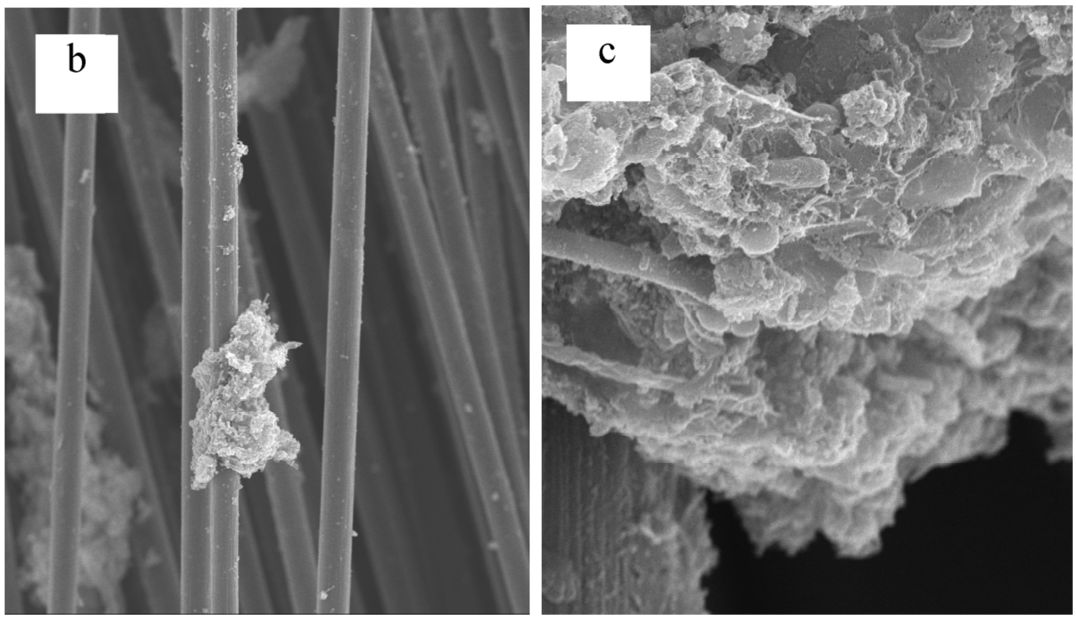
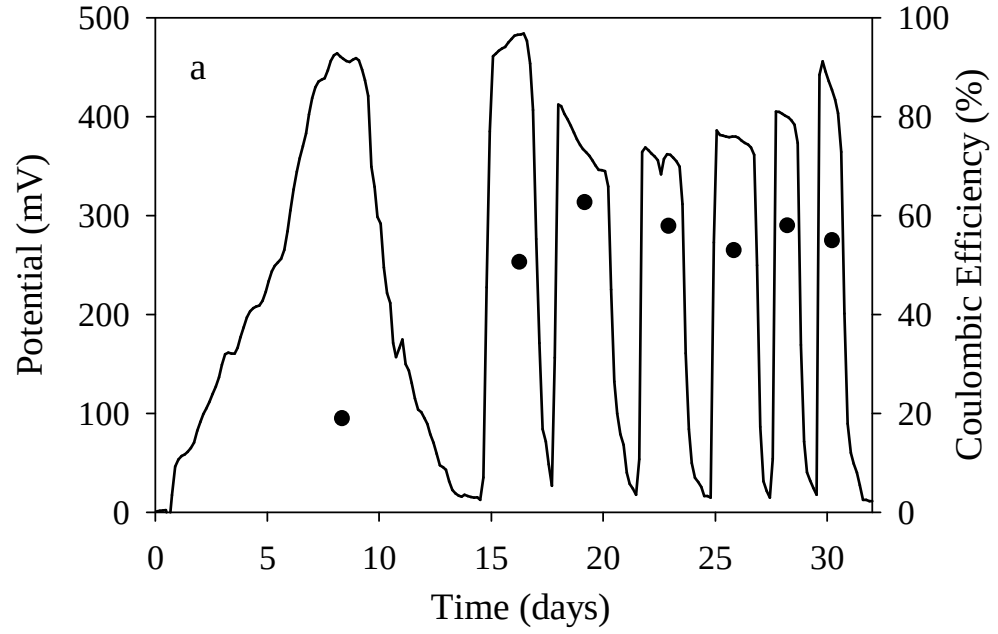


2 .

3 **Fig. 4.** a) Power (white symbols) and polarization (black symbols) curves for thermally
4 treated (circles) and untreated (triangles) graphite fibre brush. b) and c) SEM photos of
5 a colonized treated fibre.



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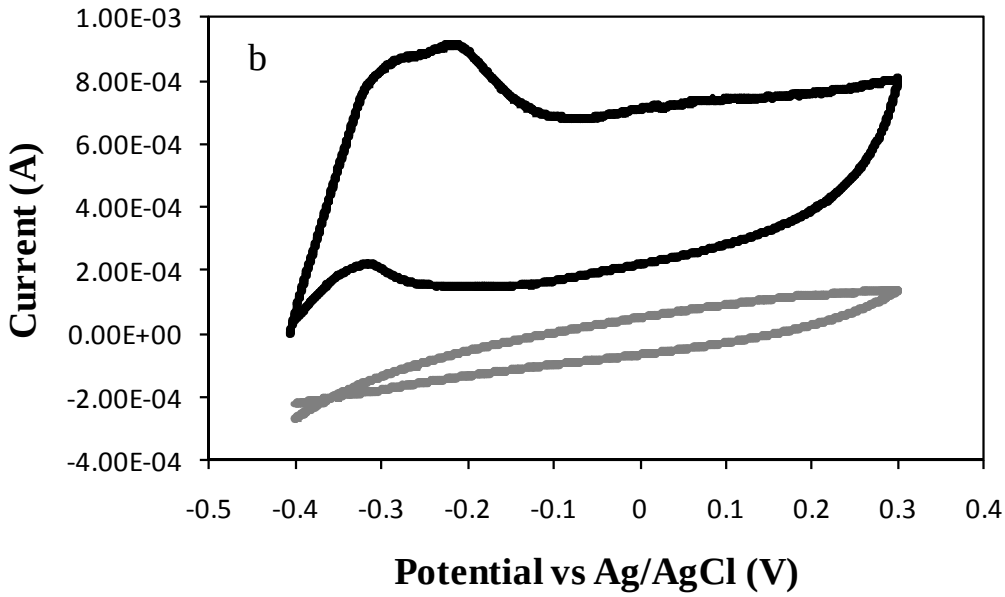

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3 **Fig. 5.** a) Experimental voltage profiles (line) and Coulombic efficiencies (dots) for
4 each batch cycle of an AC-MFC using an anode brush inoculated for 30 days in a Sed-
5 MFC. b) LSCV of an anode brush in an AC-MFC, 48 hours after being removed from a
6 Sed-MFC (black) and anode brush without bacteria (grey).

1