XVII[th] World Congress of the International Commission of Agricultural Engineering (CIGR)
Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada June 13-17, 2010

INVESTIGATING DESIGN CRITERIA TO BUILD A PERFORMING MICROBIAL FUEL CELL RUNNING ON SWINE LIQUID MANURE

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CSBE101116 – Presented at Section IV: Rural Electricity and Alternative Energy Sources Conference

ABSTRACT Microbial fuel cell (MFC) is a promising technology for swine liquid manure treatment with a good energy recovery potential. A MFC converts energy available in a bio-convertible substrate directly into electricity while decreasing its chemical oxygen demand (COD). This project is devoted to understand bacterial and electro-chemical phenomena occurring in a microbial fuel cell running on swine liquid manure (MFC_{lm}) as fuel. Many steps have been achieved to obtain these results. Construction of a first single chamber MFC_{lm} generation based on literature has been done. Various support media filling the anodic chamber have been tried to optimize surface area on which bacteria may develop according to the hypothesis that large bacteria concentration will enhance energy production and decrease treatment time. Bacterial communities extracted from selected support media were analyzed using molecular technologies such as DNA extraction, PCR amplification, denaturing gradient gel electrophoresis (DGGE) and cloning and sequencing. These analyses revealed that only a few bacteria species coming from raw liquid manure are responsible for electrical activities. This strategic knowledge could lead to a better understanding of how electricity is produced, through bacteria metabolism and how its production may be boosted. Works have also been done to optimize reaction efficiency. While platinum based "state of the art" direct methanol fuel cell electrode was used as cathode and liquid manure was used as electrolyte, electrode poisoning occurred yielding only weak power outputs. To overcome this problem, a dual chamber MFC_{lm} was developed. Doing this a more efficient electrolytic bridge is created by using a cations exchange membrane and a weak acid electrolyte between anode and cathode. Based on those results, a second MFC_{lm} generation was built. This improved MFC_{lm} is 300 % more powerful than the first one and has long lasting capability, since it could be run non stop for more than 3 months.

Keywords: microbial fuel cell, swine liquid manure, renewable energy.

INTRODUCTION

Microbial fuel cell (MFC) is a new green energy technology (Rabaey and Verstraete, 2005). Through microbial digestion, available energy in a biodegradable substrate is converted directly into electricity. In lowering biological oxygen demand (BOD) and chemical oxygen demand (COD) content of an organic matter loaded influent, MFC may also be seen as a biological wastewater treatment. Like any fuel cell, MFC have an anode, a cathode and an electrolyte. Influent organic content is used as fuel (electron donor) by bacteria in an anaerobic compartment in which anode serves as a final electron acceptor in the digestion process. Electrons flow through an external electrical circuit to the cathode, where they reduce an oxidizing molecule. In many cases, air oxygen is used for this purpose. While organic matter is oxidized by bacteria in anodic chamber, hydrogen protons are released and migrate, through electrolyte, to the cathode. When oxygen is used as oxidizing molecule, water will be generated on the cathode while CO_2 will be released in anodic chamber. MFC maximum open circuit voltage (OCV) may be estimated from half cell redox couples. For instance a MFC running on formate as fuel at the anode and oxygen at the cathode would have a maximum theoretical OCV of about 1200 mV since redox couple formate/CO_2 is -430mV and 820 mV for redox couple ½ O_2/O^{--} (Schroder, 2007). MFC anodic electrons transfer mechanisms are complex and are directly related to bacteria found in anodic chamber and their anaerobic respiratory chain. For iron reducing bacteria, a direct contact between bacteria outer membrane and anode is required to promote electricity (Schroder, 2007). For others, intermediate molecules will often be involved between bacteria and anode to transfer electrons (Schroder, 2007).

Since air is the oxygen cheapest source, many works have been devoted to study single chamber MFC (Min et al., 2007; Min and Logan, 2004; Cheng and Logan, 2006; Logan et al., 2006). By its relative simplicity this air-cathode MFC configuration is economically attractive because anode and cathode share the same compartment so there is no proton exchange membrane between them. Fuel is then the electrolyte and cathode "breath" freely in ambient air. Studies have shown that swine liquid manure (SLM) is a promising fuel for an air-cathode MFC and even odor reduction has been noted in the MFC effluent (Min et al., 2007; Kim et al., 2008). Nevertheless an increase in power output could probably be achieved with a better understanding of bacteria population involved in electrical production when SLM is used. This research project has been devoted to study various air-cathode MFC configurations in order to a) determine best components to achieve maximum power output from a MFC running on SLM and b) identify SLM electrophylic microbial population. To do so, two air-cathode MFC configurations have been tried. The first one had a single chamber while the second had a dual chamber. Dual chamber has been tried in an attempt to improve power output achieved by single-chamber MFC. Long lasting runs were also performed using dual chamber MFC.

2. MATERIAL AND METHOD

2.1 Swine liquid manure pre-treatment

Swine liquid manure was collected in a temporary storage pit located under a swine building where hogs growing-finishing occurred. All collected samples were centrifuged in a laboratory centrifuge at 3000 g and supernatant was used to feed MFC. This action was taken to emulate liquid fraction coming out an industrial decanting centrifuge as this type of equipment is becoming popular for its ability to separate swine liquid manure in a solid and liquid fraction (Martin et al., 2006). In addition, swine liquid centrifugation will prevent MFC clogging by large particles.

2.2 Single chamber MFC

This project first generation air-cathode MFC had a single chamber. Figure 1 shows fuel cell exploded view. It consisted mainly in a hollow polypropylene square 125 mm X 125 mm X 25 mm. This frame stood for the anodic chamber and had a 260 mL capacity. Inlet and outlet located on frame sides allowed SLM recirculation (R-SLM) through a peristaltic pump. A gas outlet was also located on frame top. Anodic chamber contained anode and various bacterial supports as described in section 2.3. Anode was a 6mm thick graphite cloth (SGL Carbon). A 100 mm X 100 mm square band was cut to fit anodic chamber inner sizes. A stainless steel screw was driven into the cloth to allow electrical contact. Anode and cathode were separated by a fiberglass cloth to protect cathode from coarse particles migration. For all trials, cathode was a carbon-platinum gas diffusion electrode (ELAT GDE LT 251E-W, BASF) with 0.5 mg cm-^{2} platinum loading. Cathode working area is 100 cm^{2}. Stainless steel wire mesh (22 meshes cm^{-2}) 0.6 mm thick (SS316, Grillage Major Inc.) was positioned over a cathode as current collector. Neoprene gaskets prevented MFC leakage and 8 screws and nuts tightened assembly.

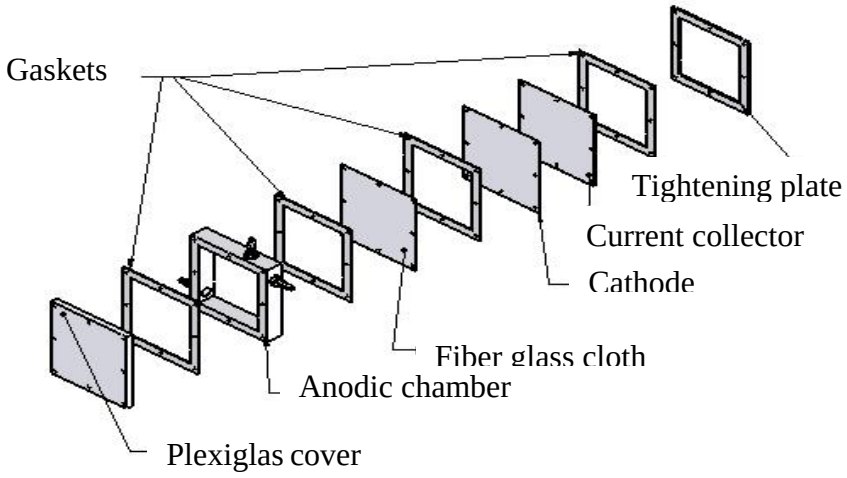

Figure 1. Single chamber MFC exploded view.

2.3 Bacterial growth support media

To allow all possible electrophylic microbial populations growth, various support media have been tried inside the anodic chamber. Doing that, bacteria requiring direct electrons transfer from cytoplasm to electrode may grow on the graphite cloth anode while others using intermediate molecule as electron shuttle may grow on an anode external media. Therefore five various support media have been tried. First support media (S1) was made of 6 mm micro porous quartz spheres (Substrat Pro, Eheim) with 450 m^2 L^{-1} working area. Second support (S2) was made of coal particles having 3 to 4 mm in diameter (Fluval, Hagen). Third support (S3) was made of 5 to 8 mm diameter activated coal particles (C2764, Sigma-Aldrich) with specific surface area of 600 m^2 g^{-1}. Fourth support (S4) was activated coal powder (C4386, Sigma-Aldrich) with specific surface area of 1000 m^2 g^{-1}. Finally, fifth support (S5) was commercial activated coal particles (GC8X30S, Culligam), 2mm in diameter, used by our research institute for water demineralization.

2.4 Dual chamber MFC

Even though single chamber MFC is attractive by its relative construction simplicity, it is probably not suited for liquid manure. SLM may reduce oxygen reduction potential at the cathode because its neutral pH, may cause electrode poisoning with its sulfur content and may clog cathode with various sticking materials. A dual chamber air-cathode was then designed to improve single chamber performances and to create a more stable fuel cell through time. In this design, anode and cathode chambers will be separated by a cations exchange membrane (CMI-7000, Membranes International Inc) and cathodic chamber will be filled with a clean electrolyte. It was decided that a natural weak acid should play this role. Citric acid ($C_6H_8O_7$) was retained because this organic acid is totally biodegradable and non-toxic for man and environment and can be produce naturally and cheaply. Figure 2 shows dual chamber MFC exploded view. MFC main body is a 600 mL hollow Plexiglas cylinder. SLM was re-circulated (R-SLM) by a peristaltic pump from inlet and outlet ports located respectively at each cylinder end. Anode was made from stainless steel wire mesh to form a cylindrical basket which could be slide inside the main body. This hollow anode held bacteria media support. To allow maximum contact area between SLM and media support, a smaller stainless steel hollow cylinder was placed inside anode larger cylinder and was not filled with media support in order to let R-SLM flow freely into anode central part. As shown on figure 2, cathode was placed at one end of the main body. This electrode was a circular carbon-platinum gas diffusion electrode (ELAT GDE LT 251E-W, BASF) with 0.5 mg cm^{-2} platinum loading. Cathode working area is 25 cm^2. Close to the cathode, citric acid layer was created in adding a thin gasket in which two hypodermic needles were inserted. These two needles are used to fill and circulate citric acid into the small chamber created by gasket free inner space. Stainless steel circular wire meshes covering the cathode act as current collector.

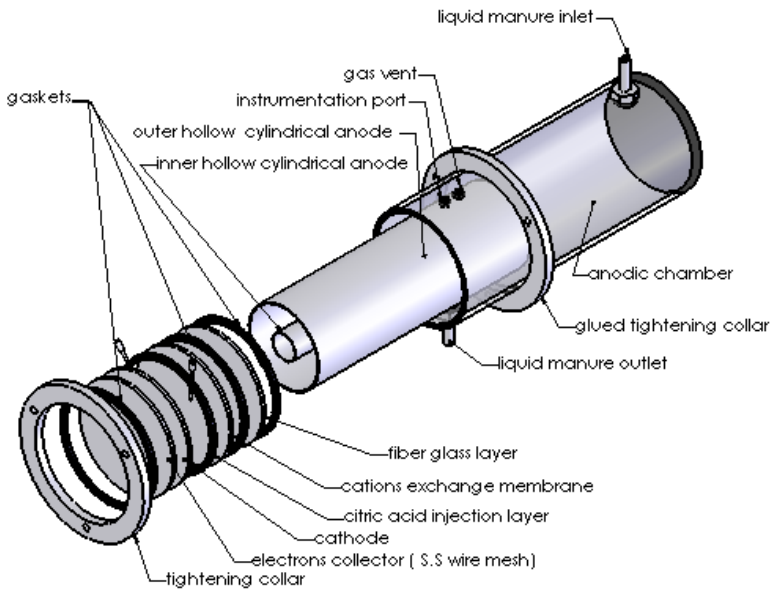

Figure 2. Dual chamber MFC exploded view.

2.5 Voltage measurements

In closed electrical circuits, all MFC have been connected to a 100 ohms external resistance. Data logging (21X, Campbell Scientific) was designed to monitor continuous voltage and current outputs during MFC runs. MFCs open circuit voltages from unconnected cells to external load were measured when steady states conditions were reached (at least 15 minutes). Half-cell voltages were measured according to an Ag/AgCl reference probe. For commodity in this text, half-cell voltage will be expressed according to standard hydrogen electrode (SHE) (SHE= Ag/AgCl voltage + 202 mV)

2.6 Sampling and DNA extraction

Raw and re-circulated SLM samples were collected from operating dual chamber MFC. In the same time, samples of activated coal inside dual chamber MFC were collected in 4 different areas between inlet and outlet (figure 3). Samples were collected in two types of tubes (11.5 mL and 2 mL) and sample replicates were washed before freezing the resuspended pellets at -20 °C. Microbial DNA from 3 coal grains and from aliquots of SLM and R-SLM were extracted using the CTAB extraction method (Lee and Taylor, 1990) with some modifications. A volume of 100 uL of silica, 2 balls of copper (0:1mm diameter) and 600 uL of CTAB were used for lysis. Lysates were then purified on Qiaquick mini columns (Qiagen) For 3.5 mL activated coal samples, the same technique was applied using 3 balls of copper and an initial volume of CTAB of 3.5 mL.

2.7 PCR-DGGE detection of bacteria

2.7.1 PCR amplification conditions:

Specific primers 984F and 1378R (Heuer et al., 1997) were selected to amplify the total bacteria population. A GC loop is used on the primer 984F (Muyzer et al., 1993) to allow a good separation in the DGGE (Denaturing Gradient Gel Electrophoresis) electrophoretic migration. The PCR reaction is performed in a final volume of 25 uL containing 5 uL of DNA, 2.5 uL of 10X amplification buffer with 15 mM $MgCl_2$ (Qiagen), 2.5 uL dNTP 2 mM (Invitrogen), 0.2 uL of TaqADN polymerase (5 U / uL) (Qiagen) and 0.25 uM of each primer. The samples were amplified in a PTC200 thermocycler (MJ-Research). The PCR program starts with activation of the TaqADN polymerase at 95 °C for 15 min and continued with 35 cycles (denaturation 95 °C 30 sec, annealing 60 °C 30 sec, elongation 72 °C 30 sec) and ended with an elongation final step 5 min at 72 °C.

2.7.2 DGGE analysis and standardization of DGGE profiles:

The DGGE analysis were performed using a device DCode Universal Mutation Detection System (Biorad). A volume of 4.5 uL of PCR product is added to 4.5 uL of 2X sample buffer (0.05 % (w/v) Bromophenol blue, 0.05 % (w/v) Xylene cyanol and 70 % (v/v) glycerol. Samples were loaded into a gel 6 % (w/v) Acrylamide (Sigma) / Bis-acrylamide (Biorad) (37.5:1) containing a denaturant gradient of urea (Sigma), formamide (LabMat), 40 % to 60 % (v/v). For DGGE analysis of total bacterial populations, electrophoresis was performed with 1X TAE buffer (140 mL 50X TAE buffer, 2 M Tris base, 1 M glacial acetic acid, 50 mM EDTA pH 8.0, in 7 L final) at a constant temperature of 60 °C for 15.5 h at 50 V. The gels were then stained with SYBR gold (Invitrogen) for 10 min, then scanned under UV light (Transilluminator, UVP) by a CCD camera using the software AlphaImager (AlphaInotech). Standardization of the DGGE profiles is obtained by the analysis of scanned gels using the software Phoretix 1D (Nonlinear) and control DGGE markers analyzed in parallel wells in the DGGE gels.

2.7.3 Identification of molecular bands sampled from DGGE profiles:

A putative bacterial population can be associated with each band of a DGGE profile. The method involved direct cloning of all amplified products of bacterial populations amplified by PCR with the primer pair 63F/1387R (Marchesi et al., 1998). Amplified products are cloned with the Strataclone kit (Stratagene). The resulting clones are selected by PCR amplification with the primer pair GC-984R/1378R (Heuer et al., 1997) and then analyzed by DGGE. The clones whose amplified product corresponds to a DGGE profile band of interest are then sequenced (CRCHUL, Canada). The sequences obtained were analyzed by the software Geneious (Biomatters) to compare the sequences with those in 16S rRNA banks Collection from NCBI (NCBI, 2010).

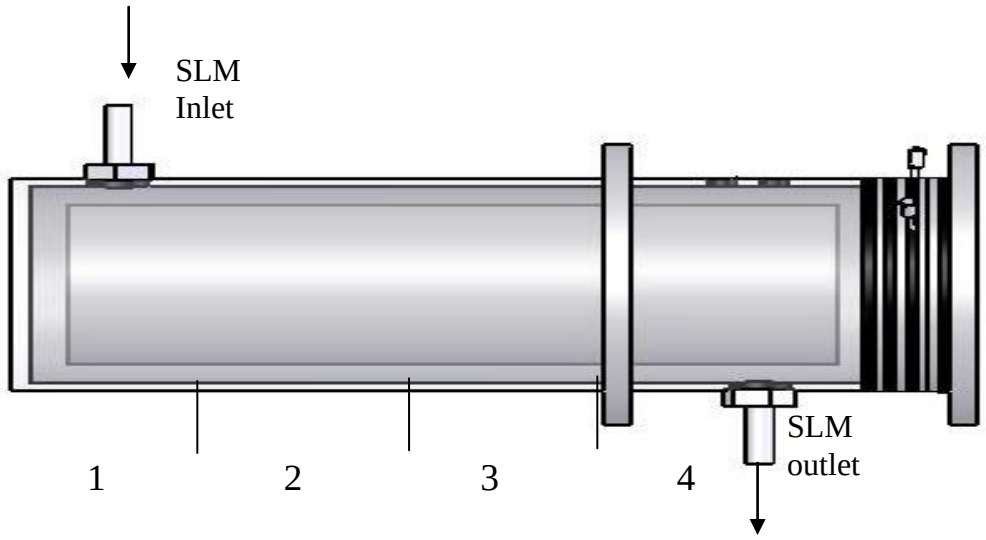

Figure 3. Dual chamber MFC sampling zones

3. RESULTS

3.1 Single chamber MFC with various growth support media

Six single chamber MFCs were created using one of five supports media described in section 2.3 whereas one MFC had none (control). Support media load was 35 g for MFCs with S2 to S5 while MFC with S1 was filled up since large voids are created by this support. All cells contained at least 100 mL liquid manure. Figure 4 shows MFC closed circuit voltage readings for 18 days. MFC with no support media shows the weakest voltage (160 mV) whereas highest voltage (380 - 400 mV) was reached by MFCs having coal (S2 to S5) as support media. MFC voltage with S1 was between these 2 values (300 mV). Polarization curves and power curves are shown on figures 5 and 6. Current is expressed in current density (I/A) which is the ratio between courant (I) and cathode surface area (A). Power (P) is estimated by the relation P = VI where V is the voltage. Power density is the calculated ratio P/A.

Figure 5 shows that OCV is close to 500 mV for all cells, while maximum current density is achieved with activated coal as support medium (S3, S4, S5). Figure 6 power curves reveals dramatic change between control and MFCs with various support media. As control MFC maximum power density was 30 mW m^{-2}, maximum power density achieved by MFC with activated coal was 190 mW m^{-2}. For MFC with support media from S3 to S5, cathode open circuit voltage with respect to reference probe was 300 mV (SHE) while anode OCV was -250 mV (SHE).

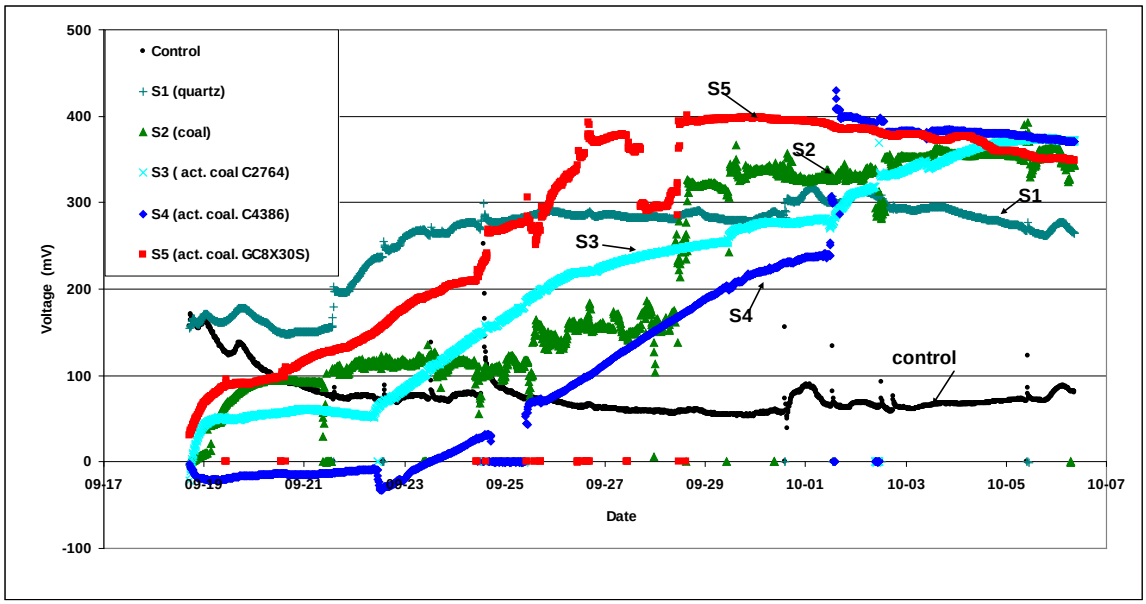

Figure 4. MFCs voltage evolution through time.

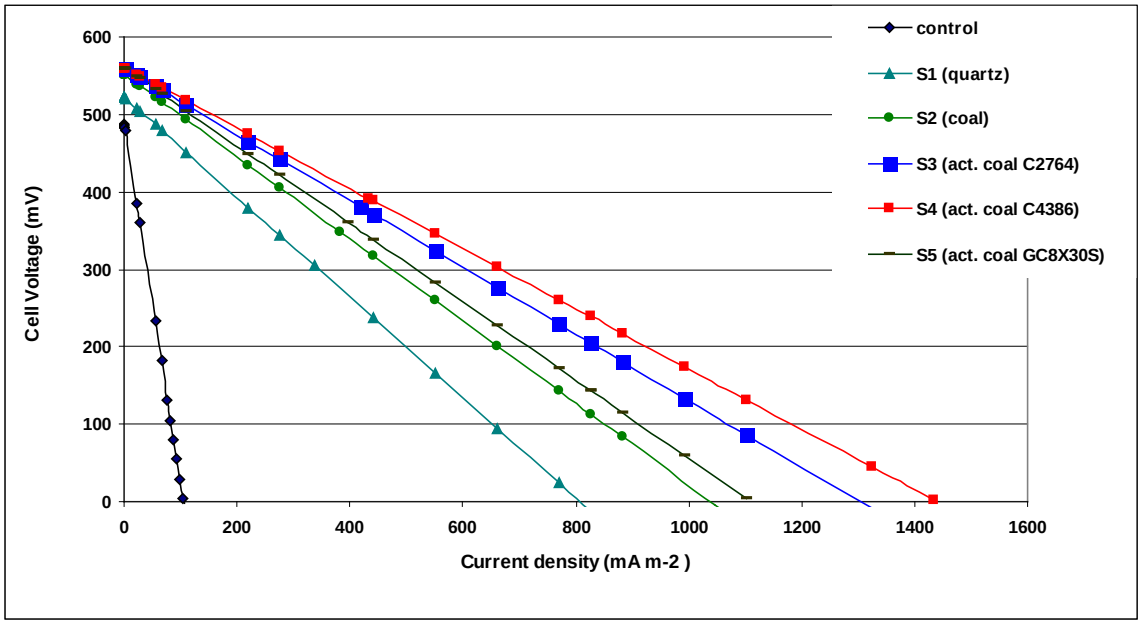

Figure 5. MFCs polarization curves.

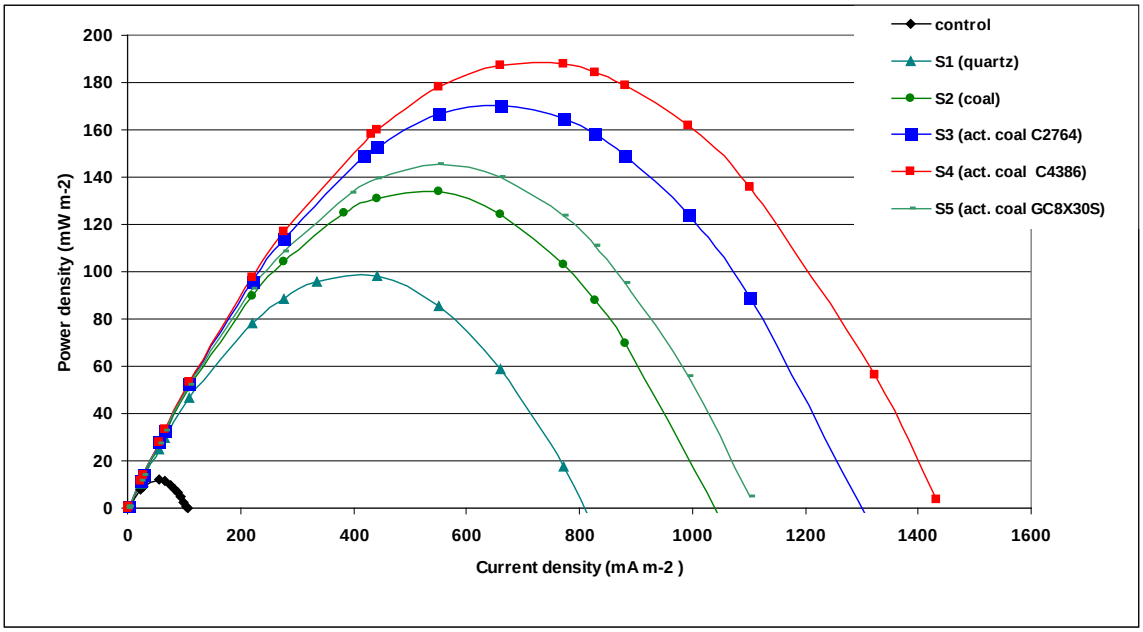

Figure 6. MFCs power curves.

3.2 Dual chamber MFC

Following MFC single chamber experiments with various support media, several trials (data not shown) have been done to enhance MFC power output. Cathodes seem to be very ineffective as the measured redox potential was very far from the theoretical 800 mV (SHE). Suspecting that oxygen reduction was inhibited by SLM, a cathodic chamber was included into the MFC single chamber with weak organic acid as electrolyte. Preliminary tests showed a positive shift toward the theoretical value using this configuration plus low citric acid concentration as electrolyte. But long term runs were not possible to achieve, probably due to insufficient design. Figure 2 shows an improved design. This design showed long term runs capability so MFC micro flora evolution and analysis were allowed. To run dual chamber MFC, 80 g activated coal S3 was incorporated into anodic chamber and 0.015 M citric acid was used as electrolyte. Dual chamber MFC ran for a period of 3 months. Figure 7 shows MFC closed circuit voltage covering one typical month period. On average, 400 mV was measured while MFC was running on 100 ohms external resistance. Figure 8 shows polarization and power curves for typical observed OCV and closed circuit values. Typical OCV values were over 1000 mV. Cathode open circuit voltage with respect to reference probe was 770 mV (SHE) while anode OCV was -260 mV (SHE). Maximum power density was close to 800 mW m^{-2}.

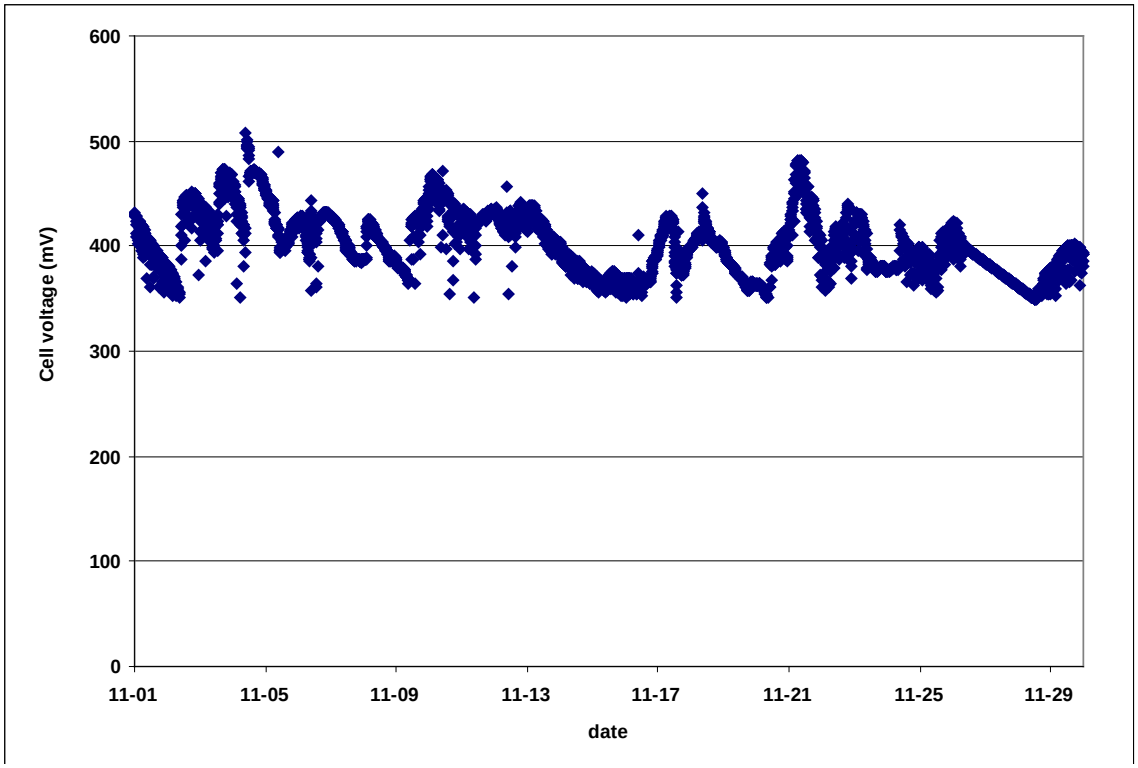

Figure 7. Dual chamber MFC voltage through time.

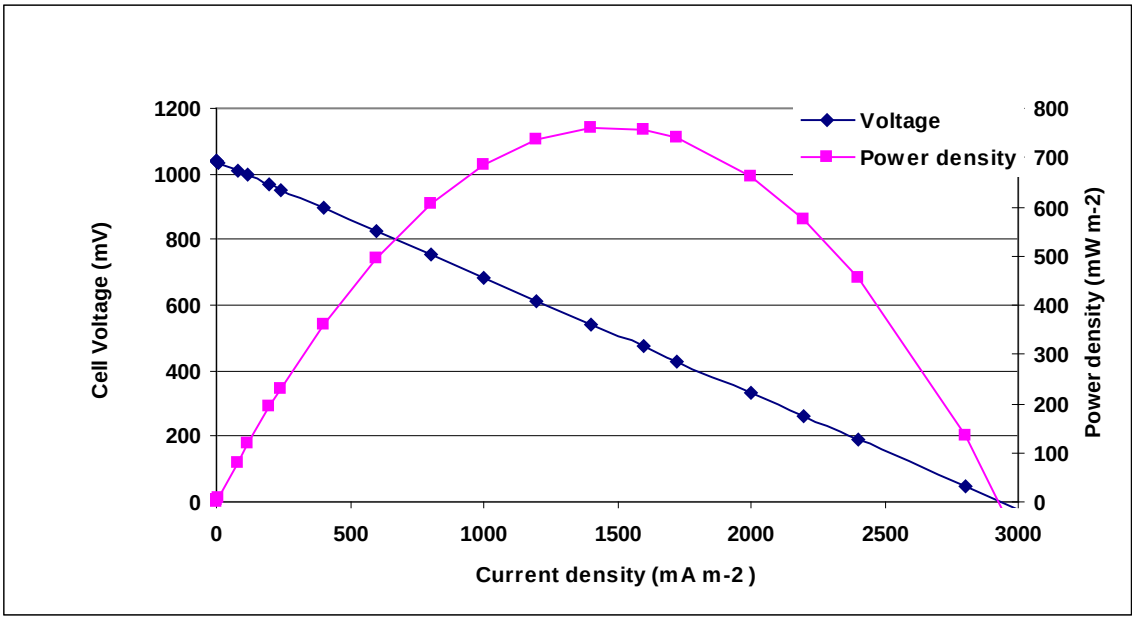

Figure 8. Dual chamber MFC polarization and power curves.

3.3 MFC microbial population

Figure 9 shows comparison of PCR-DGGE bands profiles of bacterial DNA extracted from samples of raw SLM and R-SLM, and from unwashed (U-) or washed (W-) activated coal (AC) collected from a dual chamber MFC that ran for three months. The universal PCR primers 984F+GC/1378R were used to amplify a 394 bp sequence of the bacterial 16S rRNA gene. Amplified DNA sequences were analysed by DGGE, fluorescently stained with SYBR-gold and detected with a CCD camera. The distance of migration of the bands from top to bottom of the DGGE gel is given by the Rf values. Up to 18 reproducible bands were detected in a typical DGGE profile and each band may represent one or more bacterial populations. The digital image of the gel was processed using Phototrix 1D software. The relative fluorescent intensity of each band varied from 0 to 17000 units and was proportional to the quantity of DNA amplified sequences. The analysis of DGGE profiles from SLM and R-SLM samples showed up to 9 major bands; band 0,458 was not detected in AC samples, bands 0,257, 0,299, 0,342 and 0,446 showed higher intensity in SLM sample, while bands 0.510, 0.540, 0.659 and 0.717 were detected in all tested samples but with higher intensity in SLM and R-SLM samples. The analysis of DGGE profiles of AC samples showed that 2 major bands (0.400 and 0.475) and up to 7 less intense bands (0.279, 0.326, 0.529, 0.580, 0.606, and 0.644) were detected in both AC samples but not detected in SLM and R-SLM samples. These 9 bands may represent bacterial populations that found an ecological niche in AC. The band (0.400) was equally detected in both AC samples, while the band 0.475 was mostly detected in washed AC sample. The unwashed AC sample represents the complex matrix of AC fragments embedded in R-SLM while washed AC sample represents AC fragments that were suspended in saline buffer before being centrifuged to obtain a pellet enriched with AC fragments that were coated by bacterial biofilms. Total DNA extract of a representative W-AC sample was prepared, and the amplified 16S rRNA complete sequences were cloned, selected and sequenced to identify bacterial populations associated with W-AC fragments that represent MFC strategic component. While the bacterial composition of these biofilms are still under investigation, genus *Clostridium sp*. has been linked to major band 0.400, while 4 other genera *Desulfuromonas sp.*, *Pseudomonas sp.*, *Bacteroidetes sp.* and *Alcaligenes sp.* have been associated with the major band 0.475.

Rf	SLM	R-SLM	U-AC	W-AC
0.257	2221	0	748	211
0.279	0	0	1954	615
0.299	9094	562	0	247
0.326	0	0	3305	450
0.342	7643	2536	839	551
0.400	0	0	16678	16978
0.446	9351	4059	1158	1927
0.458	11788	12469	0	0
0.475	0	0	5701	16487
0.510	13362	16544	5966	2638
0.529	0	0	388	118
0.540	5568	6041	77	48
0.580	0	291	2836	2640
0.606	0	0	2482	2317
0.625	0	0	1914	1859
0.644	0	0	448	171
0.659	1609	1189	131	454
0.677	553	433	321	89
0.717	1122	575	0	105
0.800	0	968	4087	3410

Figure 9. Comparison of PCR-DGGE bands profiles of bacterial DNA extracted from samples.

4 CONCLUSION

Using single chamber air-cathode MFCs, this project has revealed that power output may be significantly enhanced when a growth support media is included into anodic chamber. This observation shows that electrons transfer from bacteria to anode is mainly achieved by an intermediate molecule and not by direct contact between bacteria outer membrane and anode. Therefore MFC anode main role is to oxidize electrons carrier molecules and this electrode does not play a major role as a bacterial biofilm site development. These observations are strategic in developing an efficient and powerful MFC fuelled by SLM. Among various support media tested, activated coal has produced best results. Even though direct single chamber MFC is a quite simple design, it is not appropriated with SLM. Measured half-cell OCV potential for oxygen reduction is low (300 mV (SHE)). Long term operating cell is also prohibited by cathode poisoning. Therefore dual chamber MFC was developed interfacing anode and cathode with a cations exchange membrane and low concentration citric acid as electrolyte. In this design, anode is a stainless steel wire mesh since no bacteria are presumed to develop on it. Results from this new MFC generation showed great shift toward positivity for half-cell OCV potential oxygen reduction (770 mV (SHE)). Whereas MFC single chamber OCV was close to 500 mV, dual chamber MFC OCV is slightly higher than 1000 mV (1030 mV), which is close to

theoretical 1200 mV. Power density is four times higher with dual than single chambers MFC, achieving respectively 750 and 190 mW m^{-2}.

Long term runs were realized with dual chamber MFC. These long term trials allowed full bacteria development and stable microbial population. Bacteria population analysis into support media, raw SLM and circulating SLM were conducted through time. Specific well adapted bacterial populations were identified in activated coal while they were undetectable in SLM and R-SLM. Because an intense electronic activity is noted with AC loaded MFC, it is most probable that this activity is linked with the identified bacteria. First thing to investigate will be their specific anaerobic metabolism and find how it could be linked to each other. One clue could be given by *Desulfuromonas sp*, detection in AC. Since they are sulphur reducing and acetate oxidizing bacteria (Euzéby, 2010) sulphur cycle could be involved and explain, at least in part, electrons transfer mechanisms in a MFC running on SLM.

Acknowledgements. Authors wish to thank Quebec ministry of Agriculture, Fisheries and Foods for its financial support and IRDA laboratory team for its technical expertise.

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