Electricity Generation by a Mediator-Less Microbial Fuel Cell Using Mixed Culture

Sevil Aktan
Fatih University
Department of Environmental Engineering
Turkey
sevil@fatih.edu.tr

Ayhan Bozkurt
Fatih University
Department of Chemistry
Turkey
bozkurt@fatih.edu.tr

Emine Ubay Çokgör
ITU Department of Environmental Enginnering
Turkey
ubay@itu.edu.tr

Burcu Irmak Yazicioğlu
Fatih University
Department of Biology
Turkey
iburcu@fatih.edu.tr

Nurullah Arslan
Fatih University
Department Genetic and Bioengineering
Turkey
narslan@fatih.edu.tr

Fahrettin Gücin
Fatih University
Department of Biology
Turkey
fgucin@fatih.edu.tr

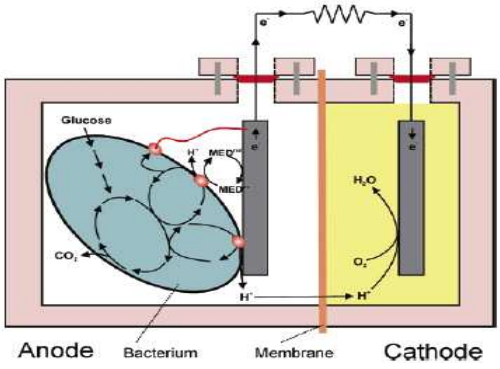
Işılay Ulusoy
Gebze Institute of Technology
Nanotechnology Center
Turkey
iulusoy@gyte.edu.tr

Abstract: A microbial fuel cell (MFC) is a bioreactor that converts chemical energy in the chemical bonds in organic compounds to electrical energy through catalytic reactions of microorganisms under anaerobic conditions. In a MFC, power can be generated from the oxidation of organic matter by bacteria at the anode ,with reduction of oxygen at the cathode. Proton Exchange Membrane (PEM), to allow protons to move across to the cathode while blocking the diffusion of oxygen into the anode. Electrons produced by the bacteria from these substrates are transferred to the anode and flow to the cathode linked by a conductive material containing a resistor, or operated under a load. MFCs have been used to generate electricity from virtually any biodegradable organic matter, including domestic and industrial wastewaters, while at the same time accomplishing wastewater treatment. Using Two Chambered MFC in our laboratory produced 0,8 mW/m2 of anode surface area using pure culture (*S.putrefaciens*) and acetate. Using Single Chambered Flat MFC, we have recently achieved up to 15 mW/m2 using mixed culture and acetate. A MFC-based treatment plant of the future

will likely look a lot like a system built today around a fixed-film system such as a trickling filter. The MFC technology is particularly favored for sustainable long-term power applications.

Introduction

A microbial fuel cell (MFC) is a bioreactor that converts chemical energy in the chemical bonds in organic compounds to electrical energy through catalytic reactions of microorganisms under anaerobic conditions. In a MFC, power can be generated from the oxidation of organic matter by bacteria at the anode, with reduction of oxygen at the cathode. Proton Exchange Membrane (PEM), to allow protons to move across to the cathode while blocking the diffusion of oxygen into the anode (Logan et al., 2005) (Du et al., 2007). Electrons produced by the bacteria from these substrates are transferred to the anode (negative terminal) and flow to the cathode (positive terminal) linked by a conductive material containing a resistor, or operated under a load (Logan et al., 2006). Bacteria can be used in MFCs to generate electricity while accomplishing the biodegradation of organic matters or wastes (Oh and Logan., 2005). Figure 1 shows a schematic diagram of a typical MFC for producing electricity. It consists of anodic and cathodic chambers partitioned by a proton exchange membrane (PEM) (Gil et al., 2003).



Figue 1 Schematic diagram of a typical two-chamber microbial fuel cell

Mediator-less Microbial Fuel Cell

Electrons can be transferred to the anode by electron mediators or shuttles (Rabaey and Verstraete, 2005). But the toxicity and instability of synthetic mediators limit their applications in MFCs. If no exogenous mediators are added to the system, the MFC is classified as a mediator-less MFC even though the mechanism of electron transfer may not be known (Logan, 2004). Some microbes can use naturally occurring compounds including microbial metabolites (Endogenous mediators) as mediators. A real breakthrough was made when some microbes were found to transfer electrons directly to the anode (Kim et al., 1999a, Chaudhuri and Lovley, 2003). These Shewanella putrefaciens (Kim et al., 2002), Geobacteraceae sulferreducens (Bond and Lovley, 2003), Geobacter metallireducens (Min et al., 2005) and Rhodoferax ferrireducens (Chaudhuri and Lovley, 2003) are all bioelectrochemically active and can form a biofilm on the anode surface and transfer electrons directly by conductance through the membrane. When they are used, the anode acts as the final electron acceptor in the dissimilatory respiratory chain of the microbes in the biofilm.

MFCs was also operated using mixed cultures currently achieve substantially greater power densities than those with pure cultures (Rabaey et al., 2004, Rabaey et al., 2005a). Since the cost of a mediator is eliminated, mediator-less MFCs are advantageous in wastewater treatment and power generation (Ieropoulos et al., 2005).

How do Microbial Fuel Cells work?

To understand how an MFC produces electricity, we must understand how bacteria capture and process energy. Bacteria grow by catalyzing chemical reactions and harnessing and storing energy in the form of

adenosine triphosphate (ATP). In some bacteria, reduced substrates are oxidized and electrons are transferred to respiratory enzymes by NADH, the reduced form of nicotinamide adenine dinucleotide (NAD). These electrons flow down a respiratory chain—a series of enzymes that function to move protons across an internal membrane—creating a proton gradient. The protons flow back into the cell through the enzyme ATPase, creating 1 ATP molecule from 1 adenosine diphosphate for every 3–4 protons. The electrons are finally released to a soluble terminal electron acceptor, such as nitrate, sulfate, or oxygen (Logan and Regan, 2006).

Using acetate as substrate, typical electrode reactions are shown below:
Anodic reaction :
$CH_3COO^- + 2H_2O \xrightarrow{microbes} 2CO_2 + 7H^+ + 8e^-$
Cathodic reaction :
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

The overall reaction is the break down of the substrate to carbon dioxide and water with a concomitant production of electricity as a by-product. Based on the electrode reaction pair above, an MFC bioreactor can generate electricity from the electron flow from the anode to cathode in the external circuit (Du et al., 2007).

Types of Microbial Fuel Cell (MFC)

Two-chambered MFC

A typical two compartment MFC has an anodic chamber and a cathodic chamber connected by a Proton Exchange Membrane (PEM), to allow protons to move across to the cathode while blocking the diffusion of oxygen into the anode (Du et al., 2007). The anode chamber contains the bacteria, and it is tightly sealed to prevent oxygen diffusion into the chamber. The headspace can be flushed with nitrogen gas to exclude air from the chamber. The cathode is immersed in water, and the water is bubbled with air (a typical aquarium air pump works well in the laboratory for this purpose). The anode chamber should contain nutrients (nitrogen, phosphorus and trace minerals) and biodegradable substrate (Logan, 2005). As mentioned above, sucrose, lactose, glucose, starch, pyruvate, xylose or wastewaters (domestic ww, animal ww, starch ww) (Logan, 2005) were used as substrate. Figure 2 shows two-chamber H-type system showing anode and cathode chambers equipped for gas sparging (Logan and Regan, 2006).

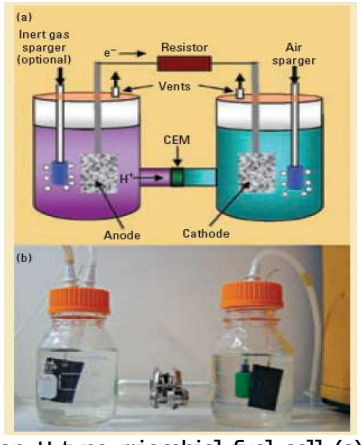
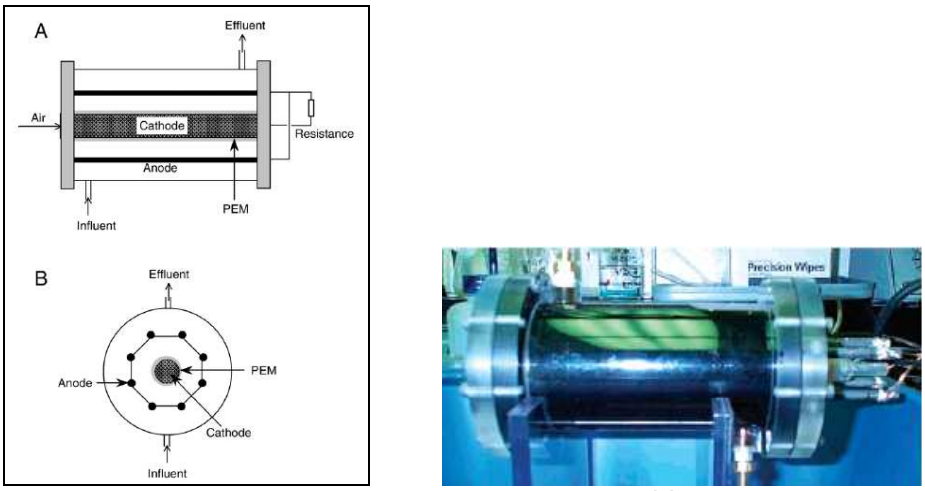


Figure 2. Example of an H-type microbial fuel cell (a) Schematic showing the anode where bacteria form a biofilm on the surface and a cathode, which is exposed to dissolved oxygen. The two chambers are separated by a proton-exchange membrane (PEM). (b) An example of a simple two-chamber system with the PEM clamped between the ends of two tubes, each joined to a bottle.

Single Chambered MFC (SCMFC)

A simpler and more efficient MFC can be made by omitting the cathode chamber and placing the cathode electrode directly onto the PEM. This set up avoids the need to aerate water because the oxygen in air can be directly transferred to the cathode. Several designs are possible for this system. In the first design used in Prof.

Logan's Laboratory in Penn University, used to demonstrate electricity generation from wastewater, the cathode was placed in the center of a cylinder, so that the anode chamber formed a concentric cylinder around the cathode (large SCMFC; Liu et al., 2004) (Figure 3). Graphite rods were placed inside the anode chamber, and these rods extended outside of the anode chamber and were connected to the cathode via an external circuit containing a resistor. Air was able to passively flow through the center tube so that it could react at the cathode. The Nafion membrane was hot-pressed onto the cathode, which was wrapped around a perforated plastic tube to provide support, with the membrane in contact with the solution in the anode chamber.



(C)

Figure 3. Schematics of a cylindrical SC-MFC containing eight graphite rods as an anode in a concentric arrangement surrounding a single cathode. ((A) drawn with modifications after Liu et al., 2004. (B) drawn to illustrate a photo in Liu et al., 2004.) (C) Photo of laboratory-scale prototype of the SCMFC used to generate electricity from wastewater

It is not essential to place the cathode in water or in a separate chamber when using oxygen at the cathode. The cathode can be placed in direct contact with air (Liu and Logan, 2004). Much larger power densities have been achieved using oxygen as the electron acceptor when aqueous-cathodes are replaced with air-cathodes. The second type of SCMFC was a single tube, with the two circular electrodes placed on opposite ends of the tube (small SCMFC; Liu and Logan, 2004). The end containing the anode is capped in order to prevent oxygen diffusion into the chamber, while the other end is open so that one side of the cathode faces air, while the other is bonded to the PEM and faces the solution in the anode chamber. Two platinum wires extend from the top for electrical connections (Figure 4).

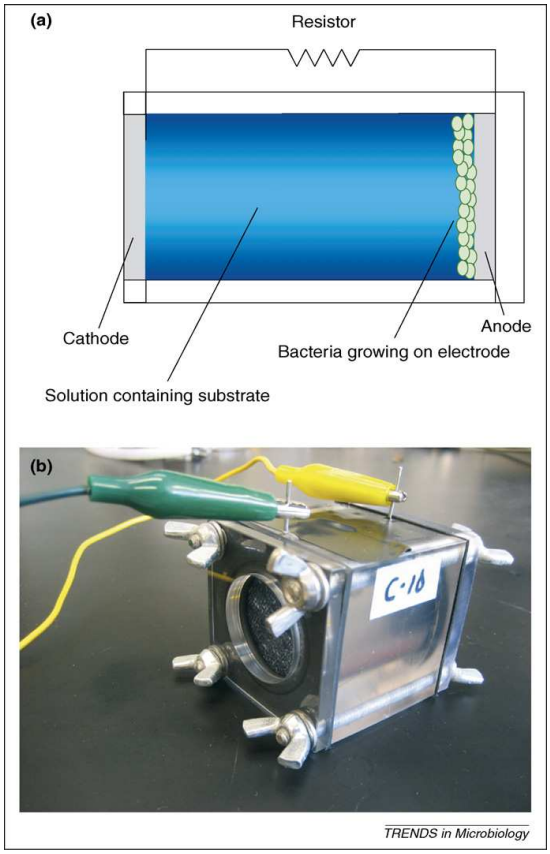


Figure 4. (a) A schematic and (b) a photograph of a single-chamber microbial fuel cell. The cathode is exposed to air on one side and the solution containing the biodegradable substrate is on the other side. The anode chamber containing the exoelectrogenic bacteria is sealed off from oxygen (Logan and Regan 2006)

Materials and Methods

Construction of Two Chambered Microbial Fuel Cell (TCMFC)

Two Chambered MFCs were constructed using two glass bottles in our laboratory. The fuel cells have electrode compartments of approximately 200 ml capacity. Each cell compartment had three ports at the top, for electrode wire, the addition and sampling of solutions, and gassing. The two compartments of each cell was separated by a Proton exchange membrane (PEM) (Nafion 117 (Dupont Co., USA)). The anode compartment was loaded with freshly prepared bacterial suspension (suspended in 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 M NaCl), vitamin and mineral solution and substrate. The cathode compartment was loaded with 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 M NaCl. Nitrogen and air were continuously purged through anode and cathode compartments to maintain anoxic and aerobic conditions, respectively. (flow rate: approximately 15 ml per min). The microbial fuel cell was immersed in a water bath to maintain temperature (25°C) (for summer conditions, Julabo FT 200-for winter conditions Julabo heater). Our two chambered MFC system can be seen

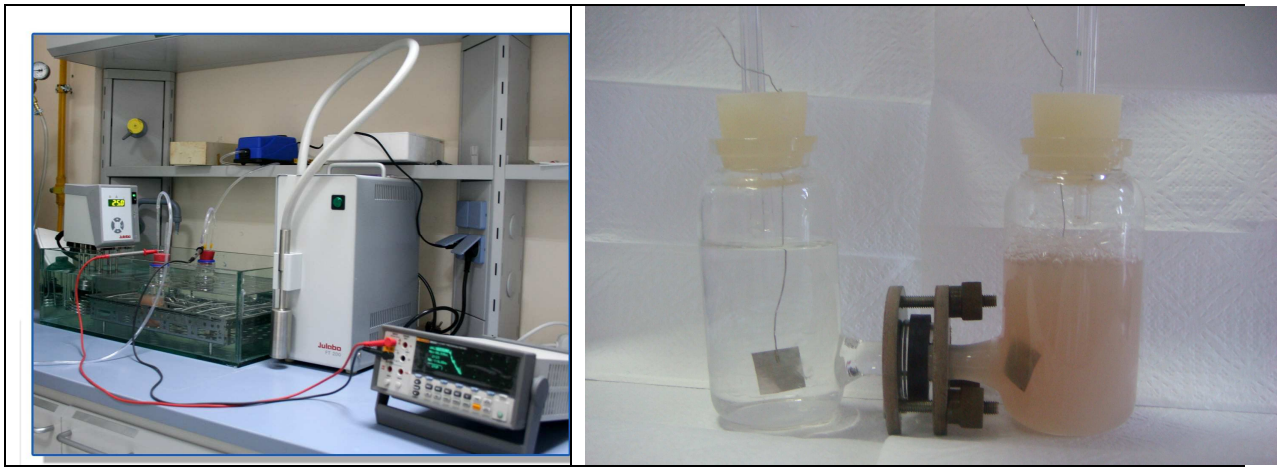


Figure 5. Two Chambered MFC in Fatih University (FU) Laboratory

Construction of Single Chambered Flat Microbial Fuel Cell (SCFMFC)

The MFC consisted of an anode and cathode placed on opposite sides in a plastic (Plexiglas) cylindrical chamber 1,6 cm long by 3 cm in diameter (empty bed volume of 12 mL; anode surface area per volume of 62,5 m^2/m^3). The anode electrodes were made of Ballart carbon paper (without wet proofing) and did not contain a catalyst. The carbon electrode/PEM cathode (CE-PEM) was manufactured by bonding the PEM directly onto a flexible carbon-cloth electrode containing 0.4 mg/cm2 of Pt catalyst (Vulcan). The PEM (Nafion 115, Dupont) was sequentially boiled in H_2O_2 (30%), deionized water, 1M H_2SO_4, and deionized water (each time for 1 h). The PEM was then hot-pressed directly onto the cathode by heating it to 100 °C at 100 Bar for 4 min. Platinum wire was used to connect the circuit (100 ohm). The SCFMFC can be seen in Figure 6. The anode and cathode are placed on either side of a tube, with the anode sealed against a flat plate and the cathode exposed to air on one side, and water on the other. When a membrane is used in this air-cathode system, it serves primarily to keep water from leaking through the cathode, although it also reduces oxygen diffusion into the anode chamber.

Figure 6. SCMFC in Fatih University Laboratory

Construction of Single Chambered Tubular Microbial Fuel Cell (SCTMFC)

The SCTMFC consisted of a single cylindrical plexiglass chamber (10 cm long by 2,5 cm diameter; empty bed volume of appr. 63 mL). The anode electrodes were made of Ballart carbon paper (without wet proofing) and did not contain a catalyst (Figure 7). The air-porous cathode consisted of a carbon/platinum catalyst/proton exchange membrane (PEM) layer fused to a plastic support tube. The cathode/PEM was placed onto a 1 cm diameter plastic (Plexiglas) tube containing 2 mm diameter pores at 2 mm intervals (cathode tube). Air flow through the tube was passive oxygen transfer (no forced air flow). Platin wire was used to connect the circuit.

Figure 7.Single Chambered Tubular MFC in Fatih University Laboratory

Scanning electron micrograph (SEM) Analysis

The wide diversity of bacteria that exist in MFC reactors, driven in part by a variety of operating conditions, demonstrates the versatility of bacteria that can either transfer electrons to the electrode or can exist in the reactor as a result of symbiotic relationships with electricity-producing bacteria. Electrochemically

active bacteria seem to be abundant in a variety of samples used to inoculate MFCs, including wastewaters, sludges, river and marine sediments. Rapid acclimation of an MFC can be seen when using domestic wastewater. Bacteria on the anode were examined using a scanning electron microscope (SEM) (Figure 8,)

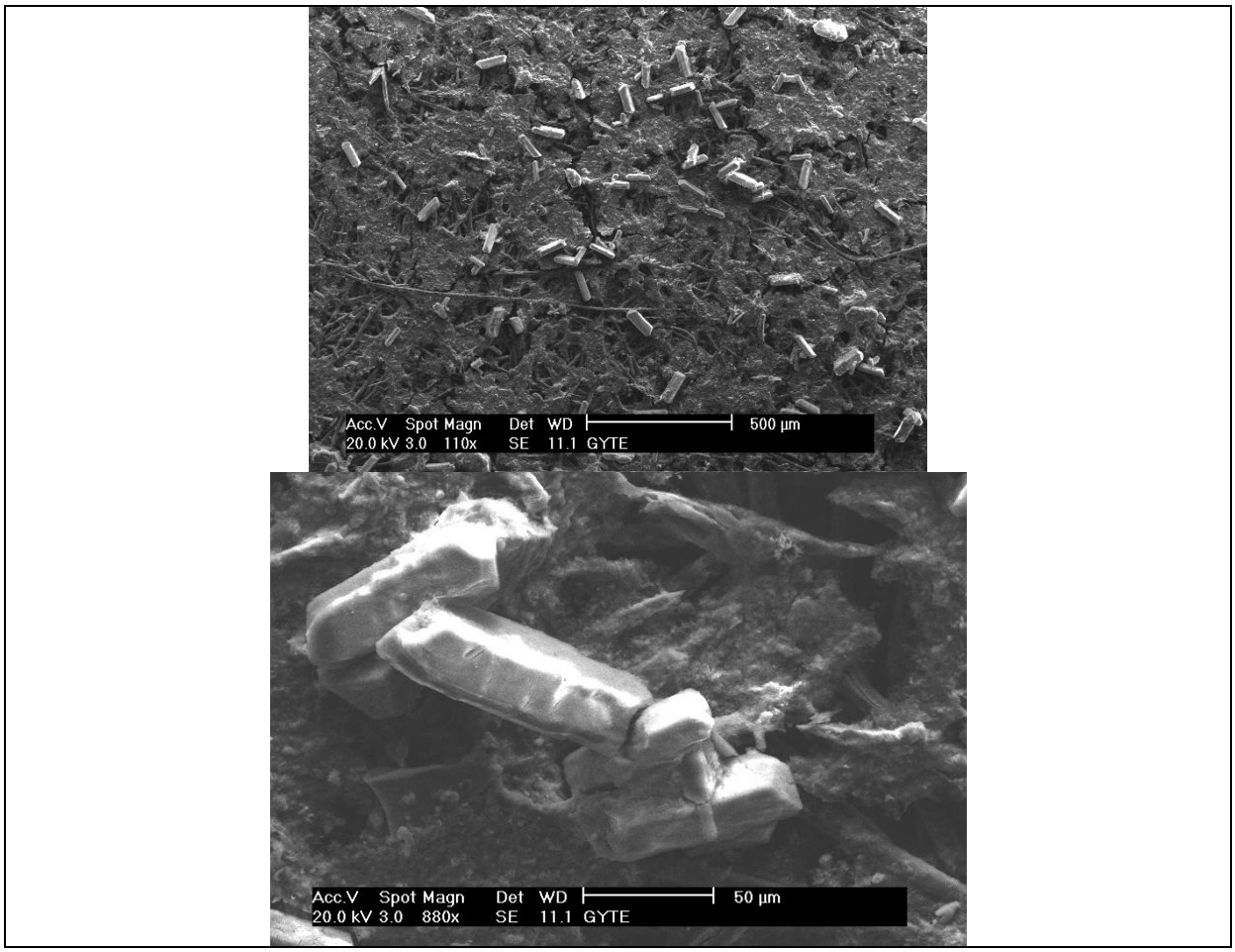


Figure 8. A scanning electron micrograph (SEM) of bacteria of domestic

Potential and Current Measurements of the Microbial Fuel Cell

The system was monitored (15 minutes intervals) using a multimeter(Fluke 8846A Digit precision Multimeter) connected to a personal computer. The circuit was completed with external resistances. Cell voltages were measured at various external resistances. Current (i) was calculated at a resistance (R) from the voltage(V) by i= V/R. Power (P) was calculated as $P=i^2V$.

Cyclic voltammogram

The cyclic voltammograms of the cell suspensions were obtained using a potentiostat (Voltalab, PGZ402 Potentiostat 30V-1A)

Results

Cyclic Voltammograms

In Cyclic Voltammograms (CV) tests the potential is gradually increased in this case from (-1 V) to (1.2V) for

SCFMFC and SCTMFC, respectively. The current is monitored to see if there is peak in current due to oxidation or reduction of chemicals in solution. The voltage is then reversed back to the original potential. CV results can be seen figure 9 for SCFMFC and figure 10 for SCTMFC.
The scan rate of 50mV/s was employed. Figure 9 shows the cyclic voltammogram (CV) of whole mixed culture cell suspensions of SCFMFC. The CVs show that the bacterial cell suspensions have a redox potential of around –0.2V, current density appr. 1mA/cm^2. CV of SCTMFC results can be seen Figure 10. The redox potential of the cell is around -0.5V, current density appr. 0.05 mA/cm^2.

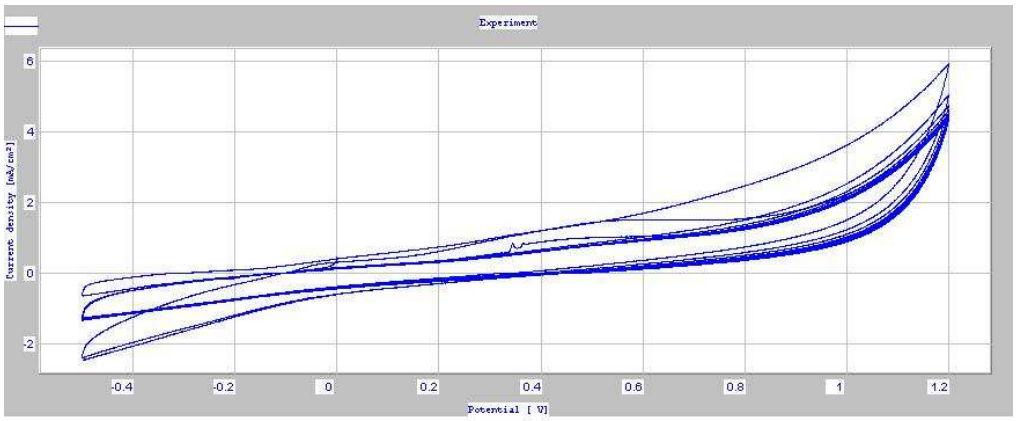


Figure 9. Cyclic voltammograms for anode with biofilm and 500 mg/L acetate for SCFMFC. .

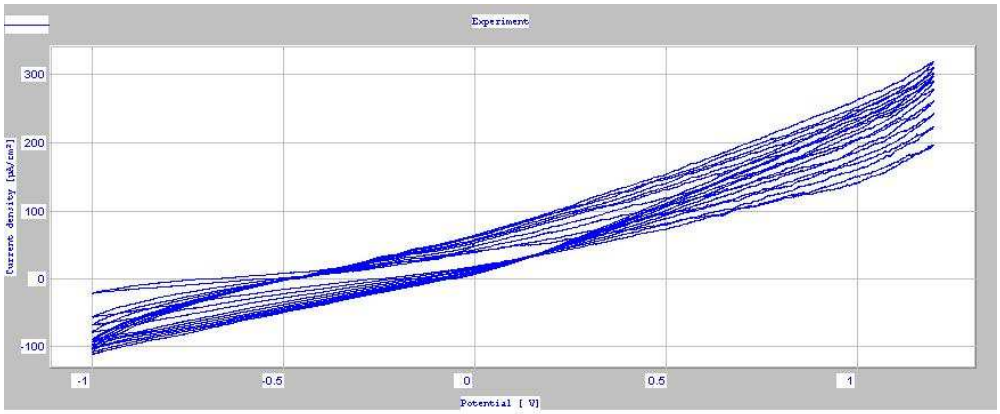


Figure 10. Cyclic voltammograms for anode with biofilm and 500 mg/L acetate for SCFMFC

Power Generation from TCMFC system

A membrane MFC inoculated with S.putrefaciens and acetate produced 0,8 mW/m^2. . The circuit was completed with a fixed load of 5kΩ were used to determine the power generation as function of load. Current (i) was calculated 4μA. Potential(V)=iR, Power (P) was calculated as P=iV. P=i^2.R=(4*10^{-6})^2.(5*10^3)=0,08 μW/cm^2=0,8 mW/m^2

Power Generation from SCFMFC system

Single Chambered Flat MFC inoculated with domestic wastewater(5000 mg/L) and 2000 mg/L acetate then 500 mg/L produced 15,3 mW/m^2. . The circuit was completed with a fixed load of 5,1Ω were used to determine the power generation as function of load. After 50 hours, current (i) neasurement was calculated 7μA.(Figure 11). Figure 12 shows that maximum power density of SCFMFC was 15.3mW/m^2

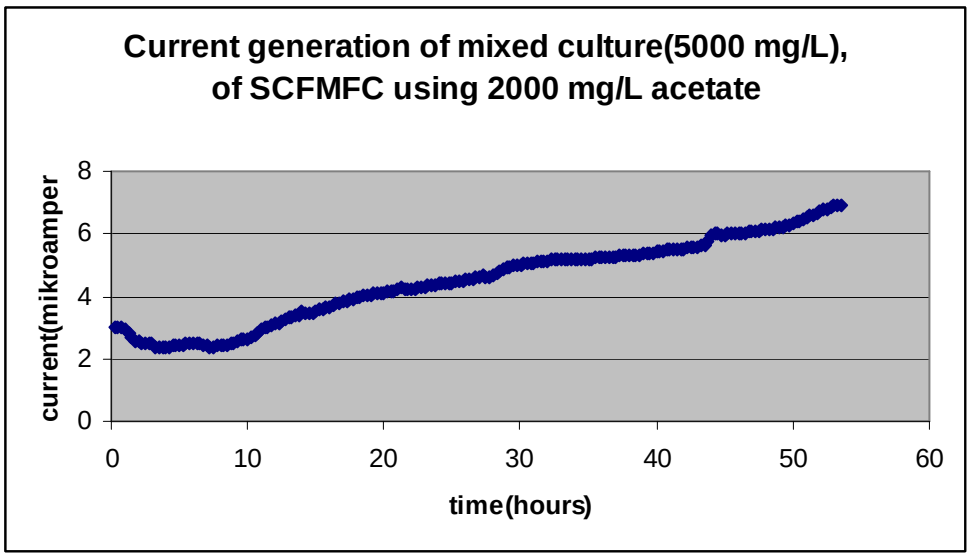


Figure 11. Current generation as a function of anode

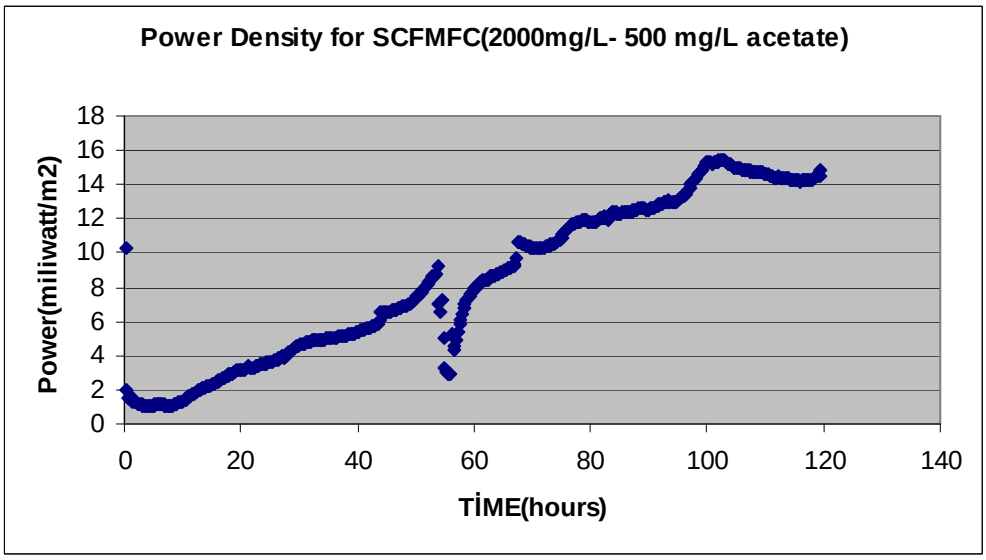


Figure 12. Power density for SCFMFC

Discussion

MFCs typically produce power at a density of less than 50 mW/m2 (normalized to anode projected surface area) (Bond et al, 2003, Tender et al 2002 and Kim et al 1999). Power generation using a membrane(Nafion) MFC inoculated with G. Metallireducens was 37 to 40 mW/m2 which was similar to that found by others using Geobacter spp. and other pure cultures in two chambered MFCs. Bond et al. (2002) obtained 14mW/m2 using a two chambered fuel cell, while Bond and Lovley (2003) achieved 49 mW/m2 using G. Sulfurreducens and acetate-fed membrane fuel cells. These levels of power are higher than those reported for MFCs with S.putrefaciens IR-1 and lactate(0,6 mW/m2)(Kim et al,2002) or Rhodoferax ferrireducens and glucose (8mW/m2) (Chaudhuri and Lovley, 2003). Mixed cultures in the same membrane MFC inoculated with wastewater generated a same power density(38 mW/m^2).,

In this study, power generation using Nafion MFC inoculated S.putrefaciens using 6M acetate was 0,8 mW/m^2 for TCMFC and power generation of SCFMFC inoculated with domestic wastewater using 2000 mg/L acetate and 500 mg/L acetate) was 15,3 mW/m2. The observation that power density is much larger using the single chambered than a two-chambered MFC is consistent with previous studies.

A critical factor in the power density achieved in a two chambered system was the system internal resistance, which was primarily a function of the proton exchange system (Min. B, 2005). We believe that more useful

mediator-less microbial fuel cell system will be obtained by modifying and improving the fuel cell format, the fuel itself, concentration of bacteria, electrode surface area, electrode material, membranes, contact time, and environmental conditions.

Applications

Microbial fuel cells are not new – the concept of using microorganisms as catalysts in fuel cells was explored from the 1970s and microbial fuel cells treating domestic wastewater were presented in 1991. However, it is only recently that microbial fuel cells with an enhanced power output have been developed providing possible opportunities for practical applications (Rabaey and Verstraete, 2005).

One of the first applications could be the development of pilot-scale reactors at industrial locations where a high quality and reliable influent is available. Food processing wastewaters and digester effluents are good candidates. Moreover, decreased sludge production could substantially decrease the payback time. In the long term more dilute substrates, such as domestic sewage, could be treated with MFCs, decreasing society's need to invest substantial amounts of energy in their treatment. The growing pressure on our environment, and the call for renewable energy sources will further stimulate development of this technology. MFCs have been proposed as a method to treat wastewater, and thus it is important to evaluate the overall performance in terms of (BOD), (COD), or (TOC) removal (Logan et al., 2006).

However, MFC power generation is still very low (Tender et al., 2002; Delong and Chandler, 2002), that is the rate of electron abstraction is very low. One feasible way to solve this problem is to store the electricity in rechargeable devices and then distribute the electricity to end-users (Ieropoulos et al., 2003). The MFC technology is particularly favored for sustainable long-term power applications (Du et al, 2007). A MFC-based treatment plant of the future will likely look a lot like a system built today around a fixed-film system such as a trickling filter. The important difference is that this future system could produce not only enough electricity to run the plant, but to help run the town-transforming your local wastewater treatment plant into a power plant.

References

Bond, D. R.; Holmes, D. E.; Tender, L. M.; Lovley, D. R. (2002), Electrodereducing microorganisms that harvest energy from marine sediments. Science, *295*, 483-485.

Bond DR, LovleyDR. (2003). Electricity production by Geobacter sulfurreducens attached to electrodes. Appl Environ Microbiol;69:1548–55.

Du Z, Li H, Gu T. (2007). A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy Biotechnology Advances 25, 464–482

Gil GC, Chang IS, Kim BH, Kim M, Jang JY, Park HS. (2003). Operational parameters affecting the performance of a mediatorless microbial fuel cell. Biosens Bioelectron;18:327–34.

Ieropoulos I, Greenman J, Melhuish C. (2003). Imitation metabolism: energy autonomy in biologically inspired robots. Proceedings of the 2nd international symposium on imitation of animals and artifacts;. p. 191–4.

Ieropoulos IA, Greenman J, Melhuish C, Hart J. (2005). Comparative study of three types of microbial fuel cell. Enzyme Microb Tech;37:238–45.

Kim, B. H.; Park, D. H.; Shin, P. K.; Chang, I. S.; Kim, H. J. (1999). Mediatorless biofuel cell. U.S. Patent 5976719.

Kim BH, Kim HJ, Hyun MS, Park DH. (1999). Direct electrode reaction of Fe (III)-reducing bacterium, Shewanella putrifaciens. J Microbiol Biotechnol;9:127–31.

Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH. (2002). A mediatorless microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzyme Microb Tech. 30:145–52.

Liu, H.; Logan, B. E. (2004). Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane. Environ. Sci. Technol. 38, 4040–4046.

Logan BE, Murano C, Scott K, Gray ND, Head IM. (2005)Electricity generation from cysteine in a microbial fuel cell. Water Research, , 39: 942–952

Logan BE, Hamelers B, Rozendal R, Schroder U, Keller J, Freguia S, et al. (2006). Microbial fuel cells: methodology and technology. Environ Sci Technol. 40:5181–92.

Logan BE, Regan JM (2006). Electricity producing bacterial communities in microbial fuel cells Trends in Microbiology, Vol.14 No.12

Min B, Cheng S, Logan BE. (2005). Electricity generation using membrane and salt bridge microbial fuel cells. Water Res 39:1675–86.

Oh SE, Logan BE. (2005). Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. Water Res 39:4673–82.

Rabaey K, Lissens G, Siciliano S, Verstraete W. (2003). A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Lett;25:1531–5

Rabaey K, Boon N, Siciliano SD, Verhaege M, Verstraete W. (2004). Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl Environ Microb 70:5373–82

Rabaey, K.; Boon, N.; Hofte, M.; Verstraete, W.(2005a), Microbial phenazine production enhances electron transfer in biofuel cells. Environ. Sci. Technol. 39, 3401-3408.

Rabaey K and Verstraete W, 2005(b). Microbial fuel cells: novel biotechnology for energy generation, Trends in Biotechnology, 23 No:6: 291-298

Tender LM, Reimers CE, Stecher HA, Holmes DE, Bond DR, Lowy DA, et al. (2002). Harnessing microbially generated power on the seafloor. Nat Biotechnol 20:821–5.