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Review Article

The Effect of Different Substrate Sources Used in Microbial Fuel Cells on Microbial Community

Mehmet Gezginci[1] and Yağmur Uysal[2]*

[1]*Department of Bioengineering and Sciences, Kahramanmaras Sutcu Imam University, Turkey*

[2]*Department of Environmental Engineering, Kahramanmaras Sutcu Imam University, Turkey*

***Corresponding author**

Yağmur UYSAL, Department of Environmental Engineering, KahramanmarasSutcu Imam University, Kahramanmaras, Turkey, Tel: 90 344 280 1684; Fax: 90 344 280 1602; Email: yuysal@ksu.edu.tr

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Abstract

Recently, the increase in the use of fossil fuels triggers a global energy crisis, so renewable bioenergy is viewed as one of the ways to alleviate it. Microbial Fuel Cells Systems (MFCs) that use bacteria converting biochemical energy in organic compounds to electrical energy have a great interest among academic researchers nowadays. Since this technology is renewable and nature-friendly, it could be chosen as one of the alternative sources. However, our knowledge on the microbial ecology of electrochemically active bacterial communities is inadequate; MFC systems are still in their infancy. In spite of the fact that most of researchers have focused on MFCs architecture to optimize power output, the actual microbiological processes taking place in the MFCs are not well defined. In fact, the diversity and eco-physiology of microbial consortia within MFCs are just beginning to be explored. In MFCs, one of the most important parameters affecting power generation is microbes that degrade substrate releasing electrons bringing on electricity generation. In this paper, microbial communities playing important role in the biodegradation of organics in MFCs were reviewed according to substrate sources.

ABBREVIATIONS

MFC: Microbial Fuel Cells; DGGE: Denaturing Gradient Gel Electrophoresis; COD: Chemical Oxygen Demand; BOD: Biochemical Oxygen Demand

INTRODUCTION

Energy shortage in developing industrial world is main problem nowadays. This problem forces scientists to find new alternative energy sources. Microbial Fuel Cells (MFCs) are well accepted as one of the new energy producing methods. MFCs have been used to convert the energy in organic matters presented in wastewater [1-4], aquatic sediments [5], and associated with plants [6] into electrical current. They can convert to chemical energy present in the chemical bonds of organic compounds to electrical energy through catalytic reactions of microorganisms under anaerobic conditions [7]. Therefore MFCs may be an alternative method for the reduction of operational costs of wastewater treatment plants.

MFCs are devices that have generally two chambers, the anode and cathode, which are often separated by an exchange membrane such as proton or cation. This technology includes anodic reactions where electron donors, such as organic compounds and sulfide, are oxidized and cathodic reactions where electron acceptors, such as oxygen, nitrate, nitrite or perchlorate, are reduced [8-11]. In MFCs systems, exoelectrogenic microbes that have the ability to respire through transfer of electrons outside the cell have an important role. Exoelectrogenic microbes in the anode compartment oxidize substrates (electron donors) generating electrons and protons. While electrons are transferred to the cathode through an external circuit, protons are transferred to the cathode through the internal membrane. Electrons and protons are consumed in the cathode reducing an electron acceptor. This electron acceptor is usually oxygen [12]. Figure (1) shows a schematic diagram of a classical two-chambered MFC for producing electricity.

In MFCs, substrate is one of the most important factors affecting electricity generation because of serving as nutrient and energy source for growth of microorganisms involved. To date, in the most of the MFC studies have been used pure compounds such as glucose [13-18], ethanol [19,20] and cysteine as an amino acid [21] for electricity generation. In addition to pure

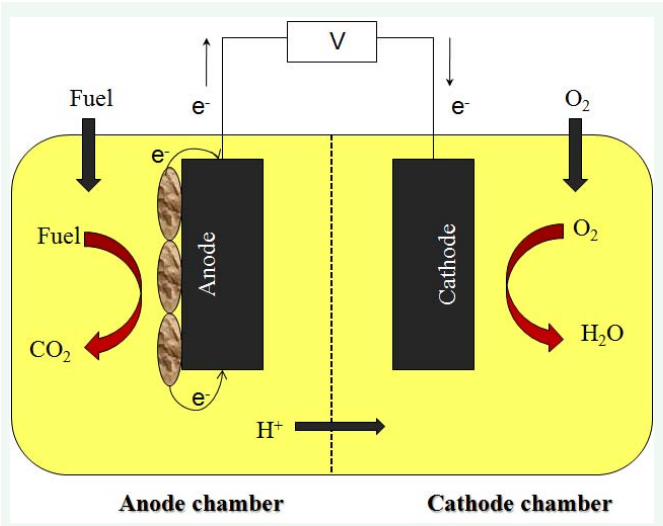

Figure 1 Schematic diagram of a classical two-chambered MFC.

compounds, MFCs can generate electricity directly from various complex substrates such as domestic wastewater [22,23], ocean sediments [24] and various industrial wastewater such as starch processing wastewater [12,25,26], beer brewery wastewater [27].

In order to capture electrical energy from wastewater, a better understanding is needed on how the operating conditions of the system affect microbial communities (particularly exoelectrogenic populations), current densities, and recovery of the substrates as current. The complex mixture of organics presented in most wastewater streams suggests that diverse microbial communities are needed to oxidize the organic matter, since many exoelectrogenic bacteria can only utilize a limited range of substrates [28]. In addition, it is also necessary to understand the bacterial community and dominant species contributing to exoelectron transfers in the anode biofilmin order to obtain better performance from a MFC. The power production in the MFCs can be also dependent on the presence of specific strains. For example, *Shewanella oneidensis* consistently produces power densities that are much lower than mixed culture communities in MFCs [29]. Thus, based on 16S rDNA sequencing analysis, it is important that the bacterial consortia and pre-dominant species vary with operational conditions, such as inoculum and substrate type [19,21,25,30-33].

There is little information on how the operational environment affects community structure and system performance for this bioelectrochemical system. For example, diffusion of oxygen into the anode chamber can affect power generation in an MFC [34], and presumably the microbial community structure. Community analysis of MFC biofilms shows that there is no single emergent microorganism or 'winner' in the bacterial communities developing on the anode. Because, there are several different bacteria capable of electricity production in respect to range of operating conditions, system architectures, electron donors and electron acceptors at the cathode [31].

The analysis of the bacterial community that developed over time using denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA gene fragments and sequencing of dominant bands showed great phylogenetic diversity with the identification of sequences derived from bacteria of the taxa *Firmicutes*, ɣ-, β- and α-*Proteobacteria* [35]. On the basis of the sequences of cloned PCR-derived 16S rDNA fragments with unique restriction fragment length polymorphism (RFLP) patterns, a river sediment evolved into a community dominated by β-Proteobacteria (related to *Leptothrix* spp.) when fed river water, and predominantly α-Proteobacteria (mainly *Actinobacteria*) emerged when the reactor was fed a glucose–glutamic acid mixture [30]. Sequences from a DGGE-screened 16S rDNA clone library showed that a marine sediment used to inoculate an MFC fed with cysteine resulted in a bacterial community in which 97% of the sequences detected belong to the ɣ-*Proteobacteria* but were similar to *Shewanella affinis* KMM 3686 (40% of clones), with *Vibrio* spp. and *Pseudoalteromonas* spp. being the next most frequently detected [21]. Here, we review the substrates used in the MFC studies and their effects on microorganisms that are active in MFCs.

Microbial aspects of MFCs

As power generation is catalyzed by microorganisms, extensive attention has been paid to characterize the microbial communities in MFCs powered by various fuels. The microbial communities identified in the various studies were very diverse [33,36,37]. It was found that the type of substrate fed to an MFC had potentially an impact on the structure and composition of the microbial community which subsequently influenced the efficiency of the MFCs[32,36]. On a microbiological point of view, still little is known regarding the nature of the 'anodophilic' microbial consortium, also known as electrochemically active bacteria [38]. It is still suggested that different microbial sources should be experimented for getting better-performing community [39]

The choice of the inoculum source is a key parameter in the MFC design. Most bacteria have capacity to transfer electrons released from oxidation of organic matter to electrode. The most common sources of electro-active microorganisms have been domestic wastewater, activated and anaerobic sludge and marine sediments [40-42]. Alternative sources have been also reported as heat treated soils [43], garden compost [44], manure [45] and rumen [46]. However, very little research has investigated electro-active native microflora in agro-industrial wastes although numerous agro-industrial wastes are rich in mixed populations [47]. Acclimation or adaptation of the inoculum has sometimes been performed in a specific phase or observed during MFC running. The electro-activity of a microbial consortium can also be obtained by re-cultivating biofilm collected from a running MFC [48].

Since electricity could be generated in MFCs from various pure and complex substrates including sugars, acetate, butyrate, propionate, alcohols, proteins and wastewater streams, mixed-culture microorganisms are generally preferred to degrade these various sources in MFCs. In addition to this, mixed-culture bacteria are obtained easily from nature such as activated sludge and soil, and have high substrate consumption rate as well as low substrate specificity [49]. Electrochemically active bacteria are enriched mostly activated sludge or marine sediment [50,51]. Additionally, since these bacteria generate more power density compared to pure-culture bacteria, they are much more desirable inoculum.

Metal-reducing bacteria that have natural ability to transform diverse metal ions could be used to generate electricity and treat wastewater in MFCs. These bacteria importantly affect nonenzimatic-reduction of Fe (III) and Mn (IV) elements separately each other. *Geobacter sulfurreducens*, *Geobacter metallireducens*, *Geobacter psychrophilus*, *Desulfuromonas acetoxidans* and *Geopsychrobacter electrodiphilus* species found marine sediments and belonging to *Geobacteracea* family directly reduce these elements [52].These species also are important ones due to direct electron transfer capability to anode without using any mediators that are generally chemical and toxic compounds to human [53,54]. In MFCs, since generated electron numbers are related to proportionally electricity generation, these bacteria assimilating acetate to carbon dioxide also have a significant species. Although they have high electron transfer rate, they are some disadvantages such as low growth rate and high substrate specificity. Besides, these bacteria inoculate as a pure culture, they could contaminate from undesirable microorganism during operating of MFCs [14,55].

Besides microorganisms mentioned above as a result of using molecular genetics tools, researchers reported that Proteobacteria α, β, γ, δ, Firmicutes, Bacteroidetes, Actinobacter, Cyanobacter, Spirochaetes, Cloroflexi, Verrucomicrobia, Acidobacter, Clostodia, Sfingobacter, Flavobacter were found using on anode electrode operating under different conditions [56-59].

The effect of different substrates on microbial community in MFCs

Enriched bacterial communities used in MFC systems have range diversity such as Proteobacteria, Firmicutes, Acidobacteria, Clostridia phylaand undefined colonies [60,61]. Generally microbial diversity is affected by substrate type using in the enrichment as well as used system types such as batch systems or continuous systems. With sequencing of dominant bands using amplified 16S rDNA region in DGGE has revealed enormous phylogenetic diversities belonging to α-, β-, γ- Proteobacteria and Firmicutes (Table 1). However, no typical electricity-producing consortium has yet been observed to develop. Although qualitative temporal changes in the composition of microbial communities in MFCs have been reported [35,62-64], the power output of mixed-community MFCs has not been shown to correlate with the abundance of any specific species so far [65].

Recently, MFCs populated by mixed microbial communities have garnered a lot of attention because of their stability and high power production [33,35,66,67]. Compared to pure cultures, enriched microbial communities in these studies were more stable and robust due to nutrient adaptability and resistance to stresses. While *Geobacter* [33,67,68] or *Shewanella* [21] strains were most abundant in some enriched MFC systems, several reports have documented that bacterial communities in other MFCs were more diverse, with α-, β-, γ-, and δ-Proteobacteria, Firmicutes, and Bacteroidetes being found in large abundances among the bacterial populations enriched at MFC anodes [30,35,64,66,69,70].

Although the substrate type influences the bacterial diversity in the anode biofilm of MFCs and cell power, there has been few studies reported. Previous studies were generally carried out with a single substrate or each study used a different inoculum, which has made it difficult to collectively compare studies. A predominance of *β*-Proteobacteria clones in the phylogenetically diverse anode community was reported by Kim [19] using a two-chamber MFC inoculated with anaerobic sludge and fed SPW, and by Phung [30] using a river water fed-MFC with a river sediment inoculum. However, *δ*-Proteobacteria was dominant in the MFC inoculated with aquatic sediments [67]. Logan [21] reported a significant abundance of γ-Proteobacteria in a cysteine-enriched MFC inoculated with sediments.

Lee [59] inoculated their MFC systems where acetate was used as a substrate source with activated sludge. Class of Proteobacteria, Firmicutes and undefined colonies were found as a result of phylogenetic analyses. 16S rDNA gen analysis demonstrated that *δ*-Proteobacteria has 21% of all population. Electron microscope studies of biofilm showed a difference compared to one that was used complex substrate. To be able to degrade complex compounds, it has to be fermented. Due to fermentation, it is needed more bacterial diversity, such as fermentative bacteria.

Nguyet [56] reported that oligotrophic bacteria that are found in limited substrate source as inoculum was used to analyze bacterial diversity in two-chambered MFCs when they used different substrates sources that were synthetic wastewater including surface water, 10 ppm glucose or glutamate as a substrate, DGGE results done with amplified 16S rDNA showed each system that has different substrate source was different each other. While the most dominant class for surface water was β-Proteobacteria, α-Proteobacteriawas found dominant the system where synthetic wastewater was used. On the other study that used high concentration of glucose and glutamate with COD of 200 mg/L, γ-Proteobacteria (36.5%), Firmicutes (27%) and δ-Proteobacteria (15%) were found as dominant population in continuous MFC systems[58]. These differences in the dominant

Table 1: The Effect of Some Different Substrates on the Microbial Population Dynamics (%) in MFCs.

Class (%)							
Substrate	***α-P****	***β-P***	***γ-P***	***δ-P***	**Firmicutes**	**Other**	**Reference**
Glucose/Glutamate (copiotrophic)	1.4	6.8	36.5	14.9	27.0	13.4	Choo et al. [88]
Glucose/Glutamate (oligotrophic)	64.4	21.1	3.3	0.0	0.0	11.1	Phung et al. [30]
SurfaceWater	10.8	46.2	12.9	12.9	0.0	17.2	Phung et al. [30]
Acetate	7.0	1.7	17.3	68.8	1.0	3.8	Phung et al. [25]
Propionate	0.0	19.4	22.4	10.2	0.0	41.8	Jang et al. [56]

bacteria between these studies showed that effect of substrate concentration had an effect on the microbial diversity as well as different substrate sources.

Formate is a fatty acid that is directly metabolized and non-fermentable. Additionally, formate is known as both byproduct and precursor in acetate synthesis by acetogenic bacteria. Ha et al.[36], were investigated bacterial diversity obtained four different biofilm that were close to influent of anode, close to effluent of anode, activated sludge fed with formate and bacteria fed with acetate in MFCs. When these biofilms were compared by DGGE, similar band numbers and band densities were found between one close to influent of anode and one close to effluent of anode. Activated sludge fed with acetate had fewer bands than one fed with formate. It was probably due to acetil-CoA that is byproduct in formate degradation. Acetogenic bacteria that caused more bands were necessary to degrade acetil-CoA [36].

Ethanol and methanol that have low molecular weight were examined for electricity generation using two different, one and two-chambered, MFC systems. There was not sufficient electricity generation in methanol studies. When ethanol was used as a substrate, β-Proteobacteria, *Azoarcus sp*. and *Desulfuromonas sp.M76* bacteria were found dominant species as 33.3%, 17.4%, 15.9%, respectively, with result of analysis of anolyte and biofilm using 16S rDNA-based molecular techniques [19].

Ammonium that is the most essential inorganic compounds in wastewater and agricultural waste was examined to generate electricity using rotated-cathode MFC systems. DGGE analysis indicated that bacteria enriched using ammonium as a substrate were demonstrated differences compared to original inoculation culture without feeding ammonium. While β-Proteobacteria and Firmicutes were dominant in original inoculation bacteria, β-Proteobacteria*, Nitrosomonas europaea* that are ammonium-oxidizing bacteria, and *Comamonas sp. IA-30*, *Bacterium CYCU-0215*, *Diaphorobacter nitroreducens* that are denitrification bacteria were dominant in MFCs used ammonium as a substrate [71].

In another study, propionate as a substrate was used to enrich electrochemically active microbial community in MFCs. Propionate is a desirable byproduct in anaerobic ecosystems where fatty acids are metabolized under methanogenic conditions especially as a result of syntrophic cooperation. DGGE of 16S rDNA and FISH (Fluorescence in Situ Hybridization) that are culture-independent techniques were used to identify microbial community. DGGE results showed that undefined bacteria were dominant as 42% of total population. Identified bacteria were followed by γ-Proteobacteria, β-Proteobacteria and δ-Proteobacteria, 22%, 19%, and 10%, respectively. Addition to DGGE results, FISH results confirmed that undefined bacteria were dominant [57].

Bacterial communities enriched from wastewater sludge with lactate, succinate, N-acetyl-D-glucosamine (NAG), acetate, formate, and uridine in two-chambered MFC were analyzed by DGGE. BLAST (Basic Local Alignment Search Tool) and Phylogenetic analyses using DGGE band patterns (band absence or presence with total 22 bands detected) showed that the original sludge inoculums contained mainly β-Proteobacteria, γ-Proteobacteria, Firmicutes, and Bacteroidetes. Uncultured *Geobactersp*. and *Burkholderia* -like bacterium TP243 were major groups enriched by lactate, NAG, and uridine while another distinct *Geobacter sp.* (closely related to *G. sulfurreducens*), *Azonexus sp*., uncultured β-Proteobacteria, and *Aquaspirillum sp*. were found in fuel cells enriched by succinate, acetate, and formate [72].

Potato processing wastewater collected from the primary clarifier of the wastewater treatment system, and diluted as 10x with ultrapure water in order to lower the organic loading rate were used as both substrate and inoculum. It that had the relatively high concentrations of volatile fatty acids (VFAs), and a relatively high solution conductivity was suitable substrate for MFCs. Microbial communities on the anode of the MFCs were dominated by *Geobacteraceae* (>60% of all clones). *G. lovleyi* populations were present, representing 14% of the clones sequenced. Strains with significant similarity to *G. sulfurreducens* were identified and represented 37% of the total bacterial community [73].

Phylogenetic analysis in the MFCs where anaerobic sludge collected from a biosolids mesophilic digester as inoculum and sucrose as substrate were used revealed a diverse bacterial community both in the anodic biofilm and in the suspended culture. This diverse bacterial community consisted mainly of the phyla Firmicutes and Bacteroidetes and different classes of the phylum Proteobacteria. The dominant bacterial species obtained from the DGGE profiles varied over time in the anodic biofilm and the suspension of all MFCs [65].

In another study to evaluate the bioenergy generation and the microbial community structure from palm oil mill effluent using MFC observed seven distinct bands and several weak bands from the inoculums by DGGE. The nucleotide sequences of the strongest bands revealed that major microbial species were affiliated with *Acetanaerobacter* sp. Iso-W4, *Petrotoga olearia*, iron-reducing enrichment clone CL-W2, *Porphyromonadaceae bacterium* NML 060648, uncultured anaerobic bacterium clone C-99, uncultured bacterium and *Clostridiaceae bacterium* JN18_V56_P [74].

The effect of substrate changes on the performance and microbial community of two-chambered MFCs using glucose, lactate and butyrate as substrates was investigated by Zhang et al.,[75]. It was observed that microbial community was also changed when the substrate was changed. The MFCs enriched with mixture of acetate and glucose showed more diverse bacterial community compared with only acetate-enriched and only glucose-enriched MFCs. Moreover, the microbial community was different from those in glucose-enriched MFCs after switching to acetate, and acetate-enriched MFCs after switching to glucose, although glucose and acetate were all used in these three MFCs. In the results, it was reported that *Clostridium* and *Bacilli* of phylum Firmicutes were detected in acetate-enriched MFCs after switching to glucose. By contrast, Firmicutes completely disappeared and *Geobacter*-like species were specifically enriched in glucose-enriched MFCs after feeding acetate to the reactor. This study demonstrated that the anodic microbial community, enriched for a specific substrate, had different capability to acclimate to substrate changes depending on the substrate type.

In recent studies MFC were used for denitrification. In a one experiment done by Li et al., Anammox biomass was inoculated to obtain electrons using sodium acetate with COD 1000 mg/L. In order to maintain the ammonia and nitrite concentration $NaNO_2$ and $NH_4(SO_4)_2$ were added to cathode compartment. To investigate how microorganisms were affected, 16S genes for quantification of the total eubacteria amount, *nirK* genes for quantification of denitrifying bacteria and *amx* genes for quantification of Anammox bacteria were performed with three pairs of primers. Microbial community analyses showed that *Candidatus Brocadiasinica* was the main anammox community, and *Rhodopseudomonas palustris* with electrochemical driven denitrifying ability [76]. In the other one done by Vilajeliu-Pons et al., MFC configuration treating swine manure were evaluated at long-term. Not only nitrogen but also organic matters were removed from swine manure. MFC was operated with 300 mg COD L^{-1} of acetate. Cathode was inoculated with effluent from a denitrifying MFC treating nitrate-contaminated groundwater. With FISH results, members of Firmicutes and alpha-, gamma- and delta-Proteobacteria (*Geobacter sulfurreducens*) were detected on the anode compartment. DGGE profiles of cathode biofilm showed the presence of a more diverse microbial community compared to that found in the anode. The nitrifying community at the cathode was composed of Betaproteobacterium *Nitrosospira sp.* as the main ammonia-oxidizing bacteria and Alphaproteobacterium *Nitrobacter alkalicus* as the main nitrite-oxidizing bacteria [77]. The study assessed with pig slurry in terms of microbial community. MFC was operated under continuous two-chambered one for a year. Result showed that MFC with PG were predominant by Flavobacteriaceae, Chitinophagaceae, Comamonadaceae and Nitrosomonadaceae.

Two chambered tubular upflow microbial fuel cell consisting activated carbon fibre felt was evaluated to removese a food processing watewater. The microbial communities used in the experiment was a mixture of sludge from 6 monthspre-acclimatized MFC and activated sludge from secondary clarifier of waste water treatment plant. After generating power for 205days, DGGE was done to find out which microbial genus was dominant in the reactor. Results showed that Stenotrophomonas genus was the predominatone [78].

Collected pigs lurry collected from pigfarm (Calldetenes, Catalonia) having 6908 mg O_2 kg^{-1} total COD was used to treat the pig waste and generate power on two-chambered MFC. The inocula used in the system was the anode biofilm from the MFC fed with2- bromoethanesulfonate (BES-Inh) inoculated with biomass taken from a mesophilic anaerobic digester fed with slaughter house waste after one year in operation. 454-Pyrosequencing and DGGE results showed that Archaeal communities showed much less diversity than Eubacterial communities. Although hydrogen otrophic methanogens (Methanomicrobium sp. and Methanoculleussp) were initially the most abundant, at latestages, a cetoclastic methanogens (Methanoseata sp.) were predominant in theanode [79].

CONCLUSION

The fact that energy sources that have been known is running out day by day is obvious. To overcome with the problem, scientists are still trying to find new alternative sources. MFCs renewable and nature-friendly are one of the alternative sources. But it is still in its infancy. To get more efficient electricity from MFCs, it still needs to be studied more. Our knowledge on the microbial ecology of electrochemically active bacterial communities is inadequate. In fact, the diversity and eco-physiology of microbial consortia within MFCs are just beginning to be explored. Microbial community inoculated MFCs is one of major compounds to have to be examined. Because electrons that are important for electricity generation released by microbial community affected by some parameters such as substrate sources, MFCs type. In this paper, the effect of different substrates on microbial community was mentioned. It was clear that the dominant group of microbial community was changed depending upon substrate type. They identified using molecular techniques such as PCR, DGGE, sequencing and FISH. After finding microbial community that generates more power density, they should be examined more detailed, improved their genes responsible for electricity generation, and if it is possible these genes should be transferred to another microbial communities to be able to obtain more electricity.

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