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Microbiology of a Mediator-less Microbial Fuel Cell

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Introduction

A microbial cell (MFC) is a device converting chemical energy into the electric energy with the catalytic reactions of electrochemically active microbes [1]. In an MFC microbes oxidize electron donors, and electrons are transferred to the anode, which is separated from cathode by a cation exchange membrane. The cathode is aerated. Due to the potential developed between the two electrodes, electrons move from anode to cathode when they are connected through a circuit.

The electron transport chains are located in the inner membrane of mitochondria in eukaryotes and in the cytoplasmic membrane of prokaryotes, and they are enclosed by electrically non-conductive cell wall and other surface structures. To facilitate the electron transfer from the electrochemically inactive microbial cell to an electrode, mediators have been used in the conventional MFCs. The mediators are toxic phenolic compounds. For this reason MFCs have been applied in very limited areas [http://www.ncbe.reading.ac.uk/].

It has been shown that an Fe(III) reducing bacterium, *Shewanella putrefaciens* is electrochemically active, and metabolizes lactate in a fuel cell-type electrochemical cell in the absence of electron acceptors with concomitant current generation [2, 3]. Based on this finding similar electrochemical cells were used to enrich electrochemically active microbes with different nutritional characteristics [4-8]. They are copiotrophic cultures enriched with wastewater, or artificial wastewater containing glucose and glutamate or acetate, and oligotrophic cultures with artificial wastewater or river water.

Enrichment Culture

A fuel cell-type electrochemical device was inoculated with activate sludge collected from a local sewage work and continuously fed with artificial wastewater containing mineral salt solution added with 5 mM acetate. Immediately after the inoculation an open circuit potential of about 0.5 volt was developed between the anode and cathode. Within 3 weeks after the circuit was closed connecting the electrodes through a resistance of 500 Ω, a current of around 1.5mA was generated stably with the consumption of acetate. These results show that the inoculum contains electrochemically active particles, and that the particles multiply converting acetate into electricity. Similar MFCs were developed using wastewater from a starch processing factory, artificial wastewater containing 200 mg l^{-1} glucose and glutamate as BOD. Oligotrophic MFCs were also maintained

using artificial wastewater containing 10 mg l^{-1} glucose and glutamate as BOD or river water as the fuel.

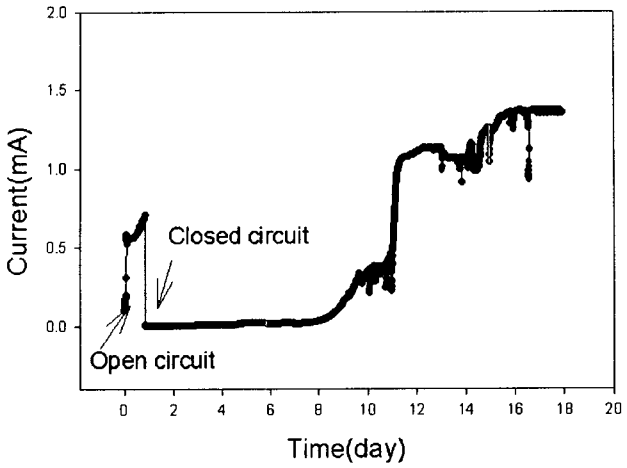

Fig. 1. Typical current generation from a microbial fuel cell during the enrichment process using acetate as the fuel. MFCs were inoculated with activated sludge and fed continuously with artificial wastewater containing 5 mM acetate as the fuel at the hydraulic retention time of 2.5 h and the current was monitored through a resistance of 500 ohm after open circuit operation for 10 h. Initially 6 MFCs were used, and they gave similar results.

Denaturing gradient gel electrophoresis (DGGE)

DNA was extracted from the anode of the MFCs and the inocula used, and amplified using a forward primer with GC clamp (GC-341f; 5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CCC TAC GGG AGG CGA CAG) and a reverse primer (534r; 5'-ATT ACC GCG GCT GCT GG) for DGGE (Fig. 2). The DGGE patterns are different from each other showing that bacterial populations in MFCs are different from the inocula and determined by the fuel used.

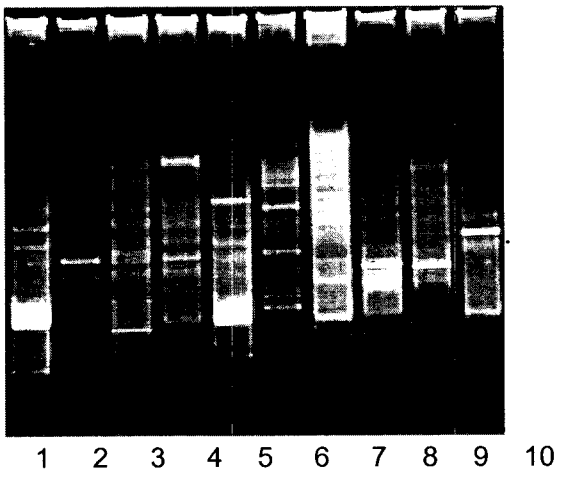

Fig. 2. DGGE patterns of 16SrDNA amplified from the microbial fuel cells and sludge used to inoculate them. Denaturing gradient used was from 30 to 60%. Lane 1; sludge (inoculum), 2; acetate enriched, 3; glucose+glutamate enriched, 4; starch processing wastewater enriched, 5; starch processing wastewater sludge, 6; acetate enriched (open circuit), 7; oligotrophic MFC, 8 and 9; bacterial isolates from acetate enriched MFC, 10; *Escherichia coli.*

Electron micrographs

Electrodes retrieved from MFCs were observed under an electron microscope (Fig. 3). The MFC enriched with acetate show biofilm developed onto the electrode whilst microbial clumps were observed in addition to biofilm in the MFCs enriched with wastewater. Confocal scanning laser micrograph showed that the biofilm and the bacterial clump consist of micro-colonies of gram positive and negative bacteria.

It is hypothesized that the bacterial clumps are inhabited by fermentative bacteria that ferment the fermentable substrate into fermentation products, and that the biofilm is formed by electrochemically active bacteria that oxidize the fermentation products to transfer the resulting electrons to the electrode.

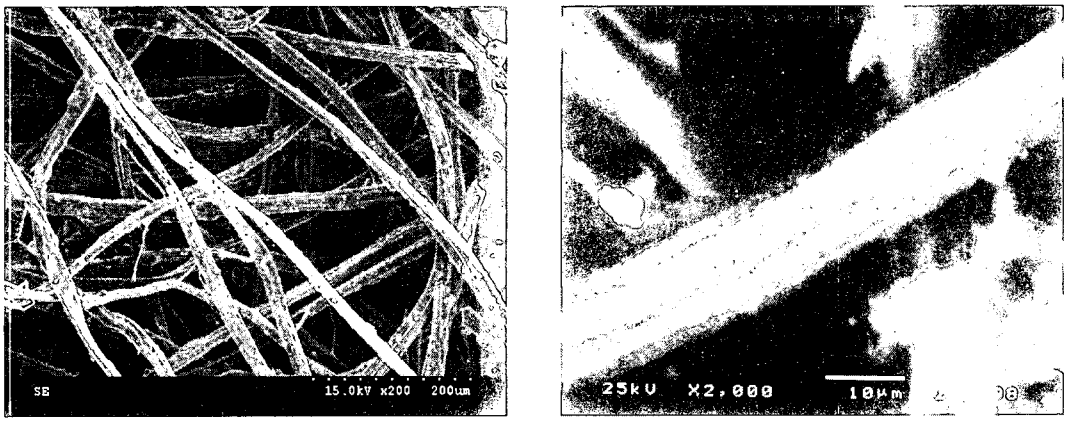

Fig. 3. Low vacuum electron micrographs of the anode retrieved from acetate (left) and wastewater (right) enriched microbial fuel cells.

Electron transport in MFCs

The acetate enriched MFC was fed with fuel containing various inhibitors and the current generation was monitored (Fig. 4). The current increased slightly with the additions of terminal oxidase inhibitors such as cyanide and azide, and antimycin A did not affect the current generation. The current generation was inhibited by rotenone, HQNO, *p*-CMPS, DCCD and DNP. These results show that the electron transport in an MFC shares early part of the electron transport chain with aerobic bacteria, but the terminal oxidase is not involved in MFCs. The inhibition of current generation by ATPase inhibitor and uncoupler might be due to the fact that a reverse electron transport step is required in the MFC.

Bacterial diversity in MFCs with differemt fuel

DNA was extracted from MFCs enriched and maintained using different fuel, and used to amplify nearly complete 16S rDNA. The analyses of the small rDNA sequences showed that dominant bacterial classes are different from each other (Table 1). In all cases gram negative bacteria were the dominant. It is interesting to note that *Deltaproteobacteria* are the major class in MFC enriched with acetate but absent in those

enriched with starch processing wastewater and with artificial wastewater containing 10 mg l^{-1} glucose and glutamate as BOD. Firmicutes were absent in the oligptrophic MFCs and low in the copiotrophic MFCs except MFC enriched with artificial wastewater containing glucose and glutamate. Over 60% of the clones found in MFCs showed 16S rDNA homology less than 97% with those in database, suggesting that majority of the bacteria in MFCs are novel.

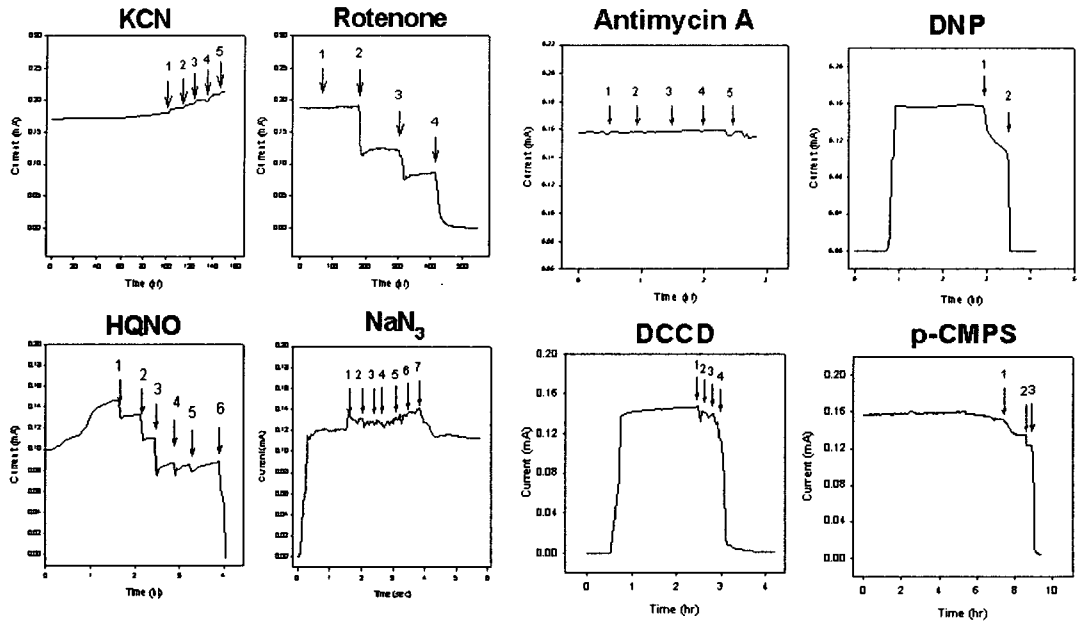

Fig. 4. Effects of various inhibitors on the current generation from acetate-fed MFC

Table 1. Bacterial classes (%) of MFCs fed with various fuels.

fuel (value as COD[a])	Class[b]						Ref
	Alpha Proteo-bacteria	*Beta-Proteo-bacteria*	*Gamma-Proteo-bacteria*	*Delta-Proteo-bacteria*	*Firmi-cutes*	others	
Acetate (300[a])	7.0	1.7	17.3	**68.8**	1.0	3.8	[5]
Starch processing wastewater (400)	27.2	**40.9**	0	0	4.5	27.1	[4]
Copiotrophic AWW (200)	1.4	6.8	**36.5**	14.9	27.0	13.4	[8]
Oligotrophic AWW (10)	**64.4**	21.1	3.3	0	0	11.1	[6]
River water (≈ 5)	10.8	**46.2**	12.9	12.9	0	17.2	[6]

Fuel concentration mg l^{-1} was shown as COD (chemical oxygen demand)[a],
Class composition (%) of bacterial consortium by 16S ribosomal DNA clones[b]
Numbers in bold indicate the most abundant class.

Conclusion

Electrochemically active bacterial consortia can be enriched using a fuel cell-type electrochemical

device. The enriched bacterial population is different from the inocula. Different bacterial population was enriched depending on the nutritional characteristics of the fuel. Biofilm and bacterial clump were observed in MFCs fed with fermentable fuel, whilst only biofilm was present in MFC fed with acetate. Electron transfer to the electrode involves NADH dehydrogenase and coenzyme Q, but not the terminal oxidase. Reverse electron transport might be needed for the anaerobic electron transfer to the electrode. Different classes of bacteria were dominant depending on the fuel supplied.

References

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