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Bio-electricity production from industrial effluents using mediator less microbial fuel cell (MFC)

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Abstract

Renewable bio-energy is considered as one of the ways to alleviate the current global energy crisis. It is evident that humankind is increasingly dependent on energy with the advancement of science and technology. Rapidly developing microbial electrochemical technologies, such as Hydrogen Fuel Cells and Microbial Fuel Cells, are part of a diverse platform for future sustainable energy. Microbial fuel cell is a technology that converts the energy stored in chemical bonds in organic/inorganic compounds to electrical energy through catalytic reactions by micro-organisms. The microorganism generally presents in anode chamber of fuel cell act as biocatalyst and generates electrons (e^-) and protons (H^+) by way of anaerobic respiration of organic substrate. The electron transfer through the anode integrated with an external circuit to cathode and protons diffuse through the proton exchange membrane. The potential between the respiratory system and electron acceptor generates the current and voltage needed to make electricity

In the present work, bio electricity is generated using mediator-less Microbial fuel cell utilizing effluents from dairy and sugar industries which are rich in organic substrates as well as microorganisms. The maximum voltage and power densities generated from diary and sugar industry effluents measured using USB data logger with an external resistance across anode and cathode were 450 mV, 143.6 mW/m^2 and 400 mV, 113.4 mW/m^2 respectively for a period of twenty one days. The observed results would form a basis for harnessing energy from industrial effluents and sustainable production of bioelectricity.

Keywords: Bioelectricity, Microbial fuel cell, Dairy and Sugar Industry effluents.

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Introduction

Electricity is one of the most important things needed for day today life. New technology to produce electricity from renewable resources without a net carbon dioxide emission is much desired (Lovley, 2006, Davis and Higson, 2007). The production of electricity by nuclear fuel is radioactive and harmful for the environment. Oil and natural gas method releases greenhouse gases in the air when they burned and they are too expensive to generate electricity. The electricity generation by chemicals also too expensive and they are very harmful for the environment. To overcome these entire problems, research focussed on biological resources to generate electricity without affecting the environment and at low cost is required. A technology using microbial fuel cells (MFCs) that convert the energy stored in chemical bonds in organic compounds to electrical energy achieved through the catalytic reactions by microorganisms has generated considerable interests among academic researchers in recent years (Allen and Bennetto, 1993; Gil et al., 2003; Moon et al., 2006; Zhang et al., 2009; Patil et al., 2011).

In an MFC, microbes in the anode chamber oxidize the organic substrates (as fuel) to produce electrons and protons. Protons migrate through proton exchange membrane /salt bridge to cathode chamber filled with aerated water and the electrons are transferred to cathode through the external circuit connected to a volt meter. Marine sediment, soil, wastewater, fresh water sediment and activated sludge are all rich sources for these microorganisms (Niessen et al., 2006, Zhang et al., 2006). A number of recent publications discussed the screening and identification of microbes and the construction of a chromosome library for microorganisms that are able to generate electricity from degrading organic matters (Back et al., 2004; Holmes et al., 2004; Logan et al, 2005). Several bacteria including many *Geobacter* species, *Shewanella*

putrefaciens, Rhodoferax ferrireducens, Clostridium butyricum and *Aeromonas hydrophila* have been known to oxidize organic substances with the electrode serving as the electron acceptor to produce electrical current (Shijie You et al., 2007).

An attempt has been made in our laboratory to generate electricity using mediator less Microbial Fuel Cell utilizing effluents from dairy and sugar industries which are rich in organic substrates as well as microorganisms.

2. MATERIALS AND METHODS

2.1 Waste water sample

Dairy effluent was collected from Aavin Dairy industy, Sholinganallur, Chennai, and effluent from sugar industry was obtained from Thiruvalangadu, Arakkonam.

All the wastewater samples were kept in a refrigerator at 4ºC before use. The wastewaters were used as the inoculum for all MFC tests without any modification such as pH adjustment or addition of nutrients, mediator or trace elements. Experiments were conducted using full strength wastewater, at 37ºC.

2.2 Construction of MFC

A low cost MFC was constructed as shown in (Fig.1). We used 2 pet bottles of one litre capacity as anode and cathode chambers which are linked by a pvc tube which served as salt bridge (cation exchanger) to transfer protons from anode to cathode chamber. The salt bridge is filled with a mixture of 1M KCl and 2 g agar in 100 ml of distilled water.

Figure 1. Microbial fuel cell with data logger

A graphite electrode with the dimension of 10 cm x 1.5 cm was inserted into each chamber and their terminal wires were taken out through the cap.

2.3 Isolation of microorganisms

Culturable microbial/bacterial colonies were isolated from dairy and sugar industry effluents using Nutrient Agar medium (Beef Extract-3 g; Peptone - 5g; Sodium Chloride - 5g; Agar – 20 g; Distilled Water -1000 ml, pH 7.0). The bacteria were identified based on colony characteristics, Gram staining and by biochemical tests as given by Bergey's (1984) Manual of Determinative Bacteriology and selective media.

2.4 Generation of electricity from MFC

The anode chamber was filled with dairy (pH= 6.1) and sugar industry (pH= 5.5) effluents separately. The cathode chambers of the MFC were filled with aerated tap water (pH 7.2) and a graphite electrode was inserted through the cap of anode and cathode chambers. The cap of the anode chamber was sealed air tight to maintain strict anaerobic environment. The entire set up was kept on magnetic stirrer at room temperature (37ºC). The voltage was measured with an external resistance of 470 Ω across the anode and cathode electrodes using a data logger (EL-USB-3 Voltage data logger) at an interval of 24 hrs over a period of 20 days. The power densities were calculated from $P=E^2_{cell}/A_{An}R_{ext}$, where E is voltage, A is surface area of anode and R is the external resistance (Logan et al., 2006).

2.5 Statistical analysis

All experiments were conducted using 2 separate microbial fuel cells. When a single microbial fuel cell was used, the experiments were repeated at least 3 times and results were presented as average values. The presented data were statistically significant.

3. RESULTS AND DISCUSSION

3.1 Bacterial species isolated from effluents

The dominant bacteria isolated in our laboratory from sugar industry effluent were *E. Coli* (EMB Agar medium), *Staphylococcus* sp. (Mannitol salt agar medium), and *Saccharomyces cerevisiae* (Oxytetracycline, glucose yeast extract (OGYE) medium) and some other unidentified bacteria. The dairy industry effluent contained *Pseudomonas* sp (King's B medium), *Lactobacillus* sp. (Lactobacillus selective agar) and several other unidentified cocci. Gram staining of the bacterial colonies also revealed the presence of both gram negative and gram positive bacteria in all samples.

3.2. Generation of electricity

The performance of Microbial fuel cell was analyzed from the data obtained from the data logger. Fig.2 presents the voltage variation with days. In our experiments, Dairy and Sugar industry effluents produced a maximum of 450 milli volts (mV) on 12^{th} day and 400 mV on 10^{th} day respectively and beyond which the voltage dropped, suggesting that the viability of the microbial community is lost due to depletion of nutrients in this closed system. It indicates that microorganisms in the effluents were able to utilize the carbohydrate (mainly lactose in dairy effluent and sugar in sugar industry effluent) existing in the effluents for generation of bioelectricity as long as the microorganisms are viable.

Fatemi et al. (2012) reported that MFCs using *S. cerevisiae* in pure culture produced a maximum of 290 mV while a mixed culture in

dairy effluent generated 500 mV without mediators. Our result of dairy effluent is in conformation with Fatemi et al. (2012). However, it is interesting to note that Dalvi et al. (2011) reported the generation of 453 mV by a single culture of *Klebsiella pneumoniae* inoculated into autoclaved dairy effluent and Prasad et al. (2007) found that *Hansenula anomola* belonging to yeast family produced a maximum voltage of 520 mV without mediators. The above reports suggest that it is the capacity of the individual organism to effectively transfer the electrons to anode to generate more voltage irrespective of the presence or absence of other factors.

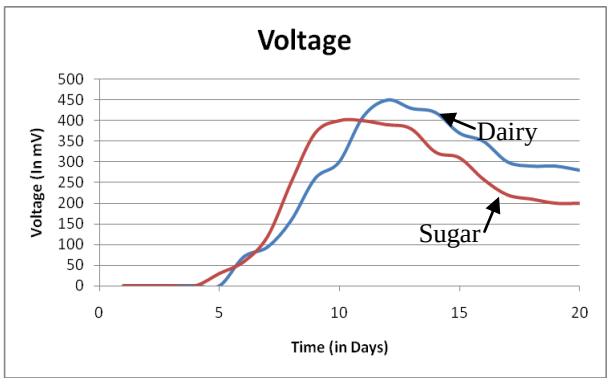

Figure 2. Voltage generated from Dairy and sugar industry effluents on different days.

Mathuriya and Sharma (2009) have used Sugar Industry waste water and Dairy waste water in their MFC without mediators and found high current output (11.39 mA) was obtained with sugar industry waste water compared to dairy waste water (8.39 mA) on 5th day. In our study, we found a maximum current output of 0.957 mA and 0.851 mA respectively for Dairy and sugar industry waste water. The high current output reported by Mathuriya and Sharma (2009) might be due their use of Nafion 117 Proton exchange membrane, which is reported to be the best PEM in microbial fuel cell experiments. In our case, we have used salt bridge instead of Nafion membrane. Another reason could be external resistance of 10 ohms used by them, where as we used 470 ohms as external resistance, the higher ohmic resistance could have reduced the current output in our study.

Min et al. (2005) reported that *Geobacter metallireducens* in nutrient medium produced a power output of 2.2 mW/m^2 in their MFC using a salt bridge between anode and cathode chambers. In contrast, we obtained a maximum power density of 143 mW/m^2 and 113 mW/m^2 (Fig. 3) while using a salt bridge for dairy and sugar industry effluents respectively suggesting that a consortium of microorganisms are able to produce more power synergistically than a single pure culture. It also proves the fact that performance of microbial fuel cells with respect to electricity generation dependent on availability of various types of microbes found in biological waste/ effluents.

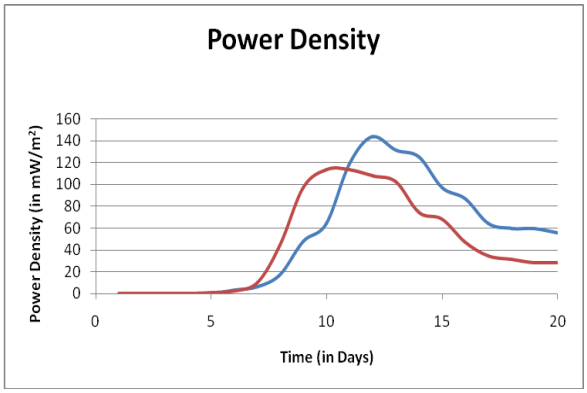

Figure 3. Power density for Dairy and sugar industry effluents on different days (Blue –Dairy effluent, Red – Sugar industry effluent)

Usually mixed cultures in the effluents have good performances (Du et al. 2007). Our data with mixed cultures is in conformity with the above fact. Availability of complex mixed cultures allows much wider substrate utilization and thus release of more electrons. This may be the reason for higher voltage generation in our MFCs using dairy and sugar industry effluents without using any mediators. Some species of *Pseudomonas* enhance their electron transfer potential through excretion of self made redox mediator pyocyanin (Rabaey et al. 2004). The presence of *Pseudomonas* species in our dairy effluent may also have influenced the increase in power output through their self made mediator. However, this needs to be validated.

The mediators in MFCs play a role in enhancing power output as a chemical agent in shuttling electrons between microbes and anode. Many microbes are electrochemically active to transfer the electrons directly to anode by conductance through cell membrane. In these cases the electroactive enzymes present in the outer membrane is responsible for direct electron transfer between microorganisms and electrode. Cytochromes localized to the outer membrane are believed to facilitate the direct electron transfer to the anode from the intact bacterial cells (Kim et al. 2002). However, there are microbes which cannot transfer electrons directly to the anode. In such cases, mediators such as Neutral red, thionin (Park and Zeikus, 2000), methylene blue (Schroder et al. 2003; Grzebyk and Pozniak, 2005), Pyocyanin and Phenazine-1-carboxamide (Rabaey et al. 2004, 2005), Anthraquinone-2,6, disulfonate (Ringeisen et al. 2006) and thionin (Choi et al. 2003) etc. play an important role in electron transfer. Sometimes, mediators have some effect on mediator-less MFCs also. For example, incorporation of neutral red into the anode chamber enhanced the performance of mediator-less MFCs using *Shewanella putrefaciens* (Park and Zeikus, 2002). Ieropoulos et al. (2005) found that *Escherichia coli* in single culture produced highest power output in the MFC in which methylene blue was used as a mediator in anode chamber. This fact has been confirmed by the works of Kim et al., (2002) in which the microbial fuel cell using *E. coli* in the absence of any mediator produced negligible amount of current. Apart from this, finding a suitable microorganism/mediator combination also plays an important part in the development and efficiency of microbial fuel cells. Ferric

chelates have proven to be suitable mediators for *Escherichi coli,* as reported in the literature (Tanaka et al .1983 a, b).

Vega and Fernandez (1987) have made a study with *Lactobacillus plantarum* and *Streptococcus lactis* as they grow rather easily in dairy products, and *Erwinina dissolvens* which grows in residues such as coffee wastes for finding a suitable microorganism/mediator combination with five ferric chelates used as mediators. *Lactobacillus and Streptococcus* did not produce any significant electric output with any of the five ferric chelates used as mediators. The fuel cells with these two microorganisms and the ferric chelates gave maximum potentials of 0.2 V where as result with *Erwinia dissolvens* was high with 0.5 V.

The fact that under very similar experimental conditions, different microorganisms using the same mediators and the same amount of fuel produce different electric yields is an interesting scientific problem still to be clarified. According to Liu et al., (2005) the output power depends on the rate of substrate degradation, the rate of electron transfer from the bacteria to the electrode, microbial synergistic effect, the circuit resistance and proton mass transfer rate in the liquid.

The results obtained with our experiments can be further optimized with various parameters including use of different mediators, different types of electrodes, various microorganisms in pure and as consortium which would increase the power output from our MFC. Further optimization of various parameters is under progress.

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