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Research Article

MICROBIAL FUEL CELL BASED ON WASTEWATER AS A NUTRIENT SOURCE AND EFFECT OF PERMEABILISER

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ABSTRACT

In Microbial Fuel Cell (MFC) metabolic process within microorganisms helps in the production of voltage due to generation of electrons. When these electrons move across the electrodes they generate a potential difference between electrodes that is the main reason for voltage production. In this study, waste water was used as a nutrient source. A lot of studies are going on the electricity production using organic matter from the wastewater as substrate. Waste biomass is a readily available, cheap source of electrons for microbes that are capable of producing electrical current outside the cell. Waste water was enriched with indigenous microflora of *E.coli* and *E. aerogenes* that was cultured in Nutrient broth. The study was conducted with normal MFC, additional carbon source (D-Glucose (0.5%)) and with additional nitrogen source (Ammonium chloride (0.01%)). The maximum voltages obtained after 11 days of culturing were 528mV from normal MFC, 167.4mV from additional carbon source and 308mV with additional nitrogen source. MFC is a new upcoming research area for generation of energy source from waste water. Microbial fuel cells (MFCs) gives a completely new long lasting, affordable, accessible and environment friendly approach to waste water treatment with production of sustainable energy.

INTRODUCTION

The increasing urbanization and industrialization has resulted in a very high rate of pollution. Water pollution is a serious challenge because of the hazards it causes to the animals and human race (Angenent *et al.* 2004). A Microbial Fuel Cell (MFC) based on wastewater can result in voltage production and simultaneous degradation of the organic wastes (Ghangrekar *et al.* 2006; Zhen He *et al.* 2006; K. Scott *et al.*, 2007; Byung Hong Kim *et al.*, 2007; The Hai Pham *et al.*, 2008; Bernardino Virdis *et al.*, 2008; Sang-Eun Oh *et al.*, 2005). This is a novel wastewater treatment process with energy recovery from the waste (Byung Hong Kim *et al.*, 2007). Electricity generation from domestic wastewater has recently received attention as a potential method to achieve both power generation and wastewater treatment (Booki Min *et al.*, 2004; Hyung Sool Lee *et al.*, 2007; Sonal G. Chonde 2014; Bruce E Logan *et al.*, 2006). Efficiency of treatment was dependent on the conductivity of wastewater with an added benefit of electricity generation rather than consumption of power (Liping Huang *et al.*, 2008). This offers opportunity for the development of economical method to clean wastewater with the added benefit of recovering a proportion of the energy required for wastewater treatment. The working of MFC is similar to the chemical fuel cells; having a steady supply of fuel to the anode and oxidant to the cathode, thereby generating electrical power (K. Scott *et al.*, 2007). Electricity generation in a MFC could be interfered by several reasons such as toxicity due to the high concentrations of ammonia in the wastewater or to volatile acids produced during hydrolysis and fermentation of the substrates or activity of the biocatalysts, electron transfer

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losses both at the anodes and the cathodes, and the internal resistance (B. Min *et al.* 2005; The Hai Pham *et al.*, 2008).Wastewater collected from Yamuna river provided the nutritional requirements.

Thus, this study was aimed to assess the MFC using wastewater as the nutrient source. Also, MFC's efficiency under different working conditions was assessed. The wastewater was enriched with microorganisms by inoculating 5% of indigenous microflora to the wastewater. For preparing the culture of indigenous microflora, the wastewater was cultured on Eosine Methylene Blue agar (EMB agar) and the most prominent colonies of *E.coli* and *E. aerogenes* were cultured in Nutrient broth. The cultured nutrient broth was used to inoculate the wastewater to speed up the degradation process accompanied with voltage development.

MATERIALS AND METHOD

Materials

For the study an anodic chamber, a cathodic chamber and a salt bridge were made, all of which were connected using connecting wires. Growth media such as nutrient media and EMB agar used were of analytical grade. Nutrient media is very suitable for culturing bacteria thus it was used to culture bacterial colonies isolated from the wastewater sample. Whereas, EMB agar is very selective in nature and allow the culturing of only *E.coli* and *E.aerogenes* with these microbes being most prominent in sewage water. Toluene was used as permeabilizer and microorganisms used were *E.coli* and *S.cerevisiae.*

An anodic chamber (containing anode) was prepared by providing anode (Carbon rods) and affixing salt bridge to cover (Korneel Rabaey *et al.* 2003, K. Scott *et al.*, 2007; Sang-Eun Oh *et al.*, 2005; Peter Aelterman *et al.*, 2008). Anode was wrapped around by copper wire to prevent electrical insulation and all possible zones of leaks were covered. Whereas cathodic chamber (containing cathode) was prepared by providing cathode and affixing salt bridge to cover. Cathode was made by spirally coiling copper wire so as to increase the available surface area. Cathode was wrapped around by copper wire to provide electrical continuation and all possible zones of leaks were covered. Salt Bridge was prepared using 1.2%agar and 1.125%KCl. After pouring in the solution, salt bridge was allowed to solidify.

After setting the apparatus, surface sterilization was provided by a 15 minutes exposure to UV-radiation (Booki Min *et al.*, 2005). Then autoclaved media was poured and inoculation of microorganisms was done. Thereafter, vessels were immediately sealed by using para film in order to maintain anaerobic conditions. $CuSO_4$ solution (0.1M) was added to the cathodic vessel to complete the set-up. $CuSo_4.5H_2O$ was used as oxidizing solution.

Development of MFC's

Normal/Control MFC

Normal MFC refers to the MFC operating under normal conditions. This MFC contains wastewater obtained directly from source and utilizes it as the nutrient source.

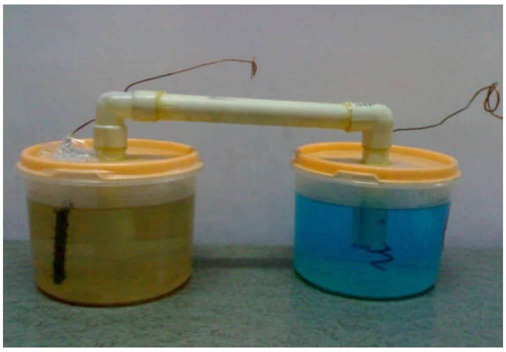


Figure 1 Normal MFC with wastewater as the nutrient source

Extra-C MFC

Extra-C MFC contains additional carbon source in the form of D-Glucose. In this MFC, D-Glucose was added at the concentration of 0.5% to the original wastewater (Korneel Rabaey *et al.* 2003). This was done to assess how the microorganisms isolated from the wastewater behave in presence of higher sugar content.

Figure 2 Extra-C MFC with wastewater as the nutrient source

Extra-N MFC

Extra-N MFC contains additional nitrogen source in the form of Ammonium chloride (Peter Aelterman *et al.*, 2008). In this type of MFC, Ammonium chloride was added at the concentration of 0.01% to the original wastewater. This was done to assess behavior of the microorganisms isolated from the wastewater in the presence of higher nitrogen content.

Figure 3 Extra-N MFC with wastewater as the nutrient source

Effect of permeabilizer on electron transfer

Permeabilizer creates pores in the cell's outer covering therefore used to increase the free electron availability by enabling the release of those electrons which were present inside the cell and could not move out. Effect of Permeabilizer was studied on both microbes. In case of *E.coli*, effect of higher inoculation and extra aeration at cathodic chamber was also studied.

Luria Bertani broth was used to culture *E.coli* and *S.cerevisiae* was cultured using Sabourand Dextrose broth. 21 hours old cultures of E.coli and S.cerevisiae were used for inoculating 600ml vessels. The inoculum size was kept at 5% for *E.coli* grown under normal and extra aeration conditions whereas inoculation density of 10% was used for *E.coli* (higher inoculation) and *S.cerevisiae*. All inoculations were done at an interval of 3 minutes. For control MFC with *E. coli.* 0.001% of permeabilizer was used, 0.005% was used for *E.coli* MFC with higher inoculation and *S. cerevisiae* MFC whereas 0.002% permeabilizer was used for *E.coli* MFC with extra aeration. Permeabilizer was added to respective Microbial Fuel Cells on day8. The MFCs were placed in incubator. The vessels with *E.coli* were incubated at 37°C and *S.cerevisiae* at 25°C.

Figure 4 MFCs for permeabilizer effect testing placed in incubator

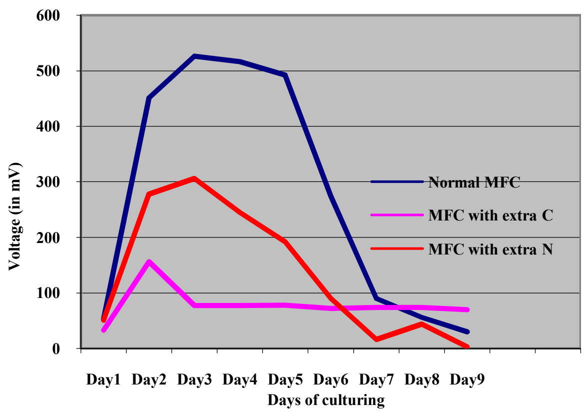
Statistical analysis

All readings were taken in duplicate using multimeter. The data was analyzed using EXCEL and differences were considered significant at a level of $p<0.05$.

RESULTS AND DISCUSSION

MFC based on wastewater

All the MFCs were placed in incubators (37°C) just after inoculation to provide appropriate environmental conditions.



Graph 1 Effect of waste water based MFC on voltage production

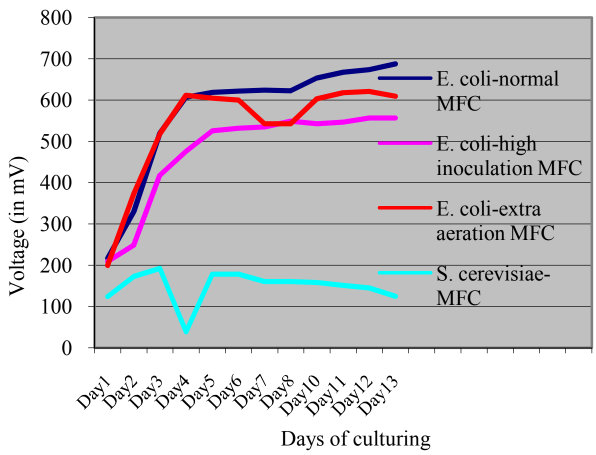
From the graph it can be seen that the best response was shown by the MFC-Normal. However, the poorest results were shown by MFC-ExtraC. The response of MFC-ExtraN was intermediate to the responses shown by the other two MFCs.

All the 3 types of MFCs had shown an increase in the voltages initially and the pattern was continued for a few days followed by the drop of potential difference. The drop in the voltages can be related to the exhaustion of the substrate contained in the wastewater. It can be significantly noticed that the voltage drop recorded was highest for the Normal MFC and then by the MFC with extra nitrogenous content. MFC with higher sugar content showed a stationary phase with no significant voltage dip as in case of other 2 types. Results showed that voltages obtained after 11 days of culturing were 528mV for normal MFC, 167.4mV for MFC with extra C and 308mV for MFC with extra N.

It is imperative from the results obtained that the best results were obtained with MFC operating under normal conditions and least effective was the MFC with extra carbon content. Thus it can be said that the micro flora isolated from the sewage wastewater was compatible with wastewater with no addition and hence very good results were obtained in case of MFC-Normal.

Presence of extra carbon and nitrogen had negative influence on the microbial culturing and hence the results obtained were not fruitful. Towards the end, MFC-Normal and MFC-Extra N showed drastic decrease in voltage owing to substrate exhaustion. However stationary phase towards end of MFC-Extra C signifies that microbial growth was highly restricted in presence of additional carbon source.

Effect of permeabilizer on MFC operation



Graph 2 Effect of permeabilizer on MFC

It was observed that desired results were obtained from the Microbial Fuel Cells based on *E.coli* but not from the one based on *S.cerevisiae*. All the MFCs using *E.coli* as the microorganism showed a linear growth initially but thereafter reported almost stationary phase untill permeabilizer was added. The growth pattern of *S.cerevisiae* was the same as before with no significant voltage development. However, addition of permeabilizer has shown somewhat positive response in case of MFCs with *E.coli* under normal and extra aeration conditions but nothing was clearly obtained in case of MFCs with higher inoculation (*E.coli*) and *S.cerevisiae*.

Following were the maximum voltages obtained under different cases after 13s days of culturing:

- *E.coli* (normal) : 688mV

- *E.coli* (higher inoculation) : 558mV
- *E.coli* (extra aeration) : 622mV
- *S.cerevisiae* : 193mV

Voltages obtained in case of *E.coli* (Normal) and *E.coli* (Extra aeration) were superior.

Permeabilizer was added with an intention of releasing cellular electrons which were thought to be entrapped inside the cell. Permeabilizer concentration used in case of *E.coli* (normal) and *E.coli* (extra aeration) was better compared to *E.coli* (higher inoculation). This might be the reason for no good results obtained in that case. However, MFC based on *S.cerevisiae* has shown no positive results thus it cannot be effectively used for the purpose of MFC.

CONCLUSION

Wastewater contains various nutrients and could thus be considered as a potential media for MFC. Such water is a threat to environment and thus, its use in MFC would mean that it is used judiciously. Wastewater use in MFC also results in decrease of BOD, COD etc. of the wastewater employed. Thus, this study was based on developing MFC based on waste water. Also effect of different nutrient conditions of waste water on MFC was studied i.e. normal conditions, wastewater with extra carbon content and wastewater with extra nitrogen content. Results have shown that best outcomes were obtained on culturing microorganisms under normal conditions. Under provided nitrogen content growth was better compared to extra-C case. This clearly implies that the bacterial growth is considerably hindered under high sugar concentrations.

Anaerobic culturing of *S.cerevisiae* didn't produce significant voltage indicating that for this microbe the electron transport chain doesn't run in the membrane thus hindering the electron transfer to the surrounding media. So, the numbers of electrons available in free state were very less, thereby, resulting in lower potential difference development across the two electrodes.

Also, the use of permeabilizer such as toluene (a chemical which permeabilizes the cell) can make electrons accessible. Use of permeabilizer after culturing provided positive results with *E.coli* but didn't affect *S.cerevisiae*. Thus, it can be concluded that *E.coli* can be beneficially exploited for MFC production while use of *S.cerevisiae* required several other aspects to be covered.

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