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MICROBIAL FUEL CELL - AN EFFECTIVE STRATEGY FOR EXPLOITING THE CATABOLIC POTENTIAL OF BACTERIAL ISOLATES

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ABSTRACT
Microbial fuel cells are devices that use microbes as the catalyst for oxidizing organic matter to generate electricity. The present work focuses on the construction of a novel microbial fuel cell (MFC) with stainless steel as electrodes, 0.20 µm syringe filter as microbial filter and polyacrylic sheet as the material of construction. Phenol degrading strain *Alcaligenes sp. d2* was used to degrade phenol in the MFC. Working of the MFC was standardized and current generation at various intervals of phenol degradation was studied. Maximum voltage output was obtained in the 32 hours of incubation of the microbe in the medium where phenol served as the only source of carbon. The power density was found to be 0.7875 mW/cm^2. Phenol degradation studies were carried out in MFC and also under optimal conditions in the flask for comparison. Comparative study of degradation rate and pattern were made on biodegradation of phenol inside the MFC and also in the optimized conditions in flask. FTIR analysis of the ether extract biodegraded phenol and spectrophotometric estimation of phenol were done and it was found that degradation rate in the MFC is little lower than that of flask system. However the MFC systems generate sufficient current during the degradation of phenol, which might be trapped for other applications. Further characterization of MFC based on operating conditions may improve the power generation from the system with the same substrate.

KEYWORDS: Microbial fuel cell, phenol biodegradation, *Alcaligenes sp. d2*, current generation.

INTRODUCTION
Microbial fuel cell (MFC) technologies represent the newest approach for generating bio-electricity generation from biomass using bacteria. A MFCs or biological fuel cells are devices that use bacteria as the catalyst to oxidize organic and inorganic matter and generate current.[1] In an MFC, microorganisms degrade (oxidize) organic matter producing electrons that travels through a series of respiratory enzymes in the cell and make energy for the cell in the form of ATP. The electrons are then released to a terminal electron acceptor (TEA) and becomes reduced. Many TEAs such as oxygen, nitrate, sulfate and others readily diffuse into the cell where they accept electrons forming products that can diffuse out of the cell. However some bacteria can transfer electrons exogenously to a TEA such as a metal oxide like iron oxide. These bacteria can exogenously transfer electrons are called exoelctrogens. That can be used to produce power in a MFC.

MFCs can be utilized for various applications, besides as an alternative source of electricity (may be in future) it is used for processing of waste water, bioremediation and also in biological hydrogen production. A modification of MFC assembly is done for the production of bio-hydrogen (another source of energy). This requires an energy input and that’s why the system is called Microbial Electrolysis cell (MEC). MFC can be used in bioremediation of various toxic compounds like phenol.

Many different configurations are possible for MFCs. A widely used and inexpensive design is a two chamber MFC built in a traditional “H” shape, consisting usually of two chambers connected by a tube containing a separator which is usually a proton exchange membrane such as nafion or ultrex or a plain salt bridge.[1] “H” shape systems are acceptable for basic parameter research, such as examining power production using new material or type of microbial communities that arise during biodegradation of specific compound, but they typically produce low power densities.

In addition to conventional instruments used for chemical measurements in microbial systems (e.g., for determining substrate concentrations and degradation products), MFC experiments can require specialized electrochemical instrumentation. In most cases, cell voltages and electrode potentials are adequately measured with commonly available voltage meters, multimeters, and data acquisition systems connected in parallel with the circuit. Current is calculated using Ohm's law (I) =E cell/R) using the measured voltage.

Polarization curves represent a powerful tool for the analysis and characterization of fuel cells.[2] A polarization curve represents the voltage as function of current. Using a periodic decrease of the load, the voltage is measured and current is calculated using Ohm's law. In MFCs, linear polarization curves are mostly obtained and for a linear polarization curve, the value of internal resistance of MFC is easily obtained from polarizing curve as it is equal to the slope of the curve.

Phenolic compounds are hazardous pollutants that are toxic at relatively low concentration. Accumulation of phenol creates toxicity both for flora and fauna. Since phenol is toxic and cause pollution, it must be removed from the environment.[3] Phenol has been detected in effluents from industries, including coal gasification, pharmacy, and productions of pesticides, fertilizers, dyes and other chemicals. Although phenol is biodegradable both aerobically and anaerobically, it can be growth inhibitory to microorganisms at elevated concentrations, particularly above a concentration of 400mg/L.[4] Biodegradation is the effective tool for the detoxification of phenolic compounds. Several phenol degrading strains like *Bacillus sp*, *Pseudomonas sp*, *Alcaligenes sp.* were reported to be capable of utilizing phenol as carbon source. Phenol is usually degraded by two pathways- ortho and metapathways.

This works focuses on the current generation by MFC using phenol as substrate. A*lcaligenes sp. d2* strain is used to degrade phenol and the electrogenic activity of the species during this process is evaluated. *Alcaligenes sp. d2*, a potent strain for degrading recalcitrants usually follows meta pathway of phenol degradation. The aim of this work is to detect power density generated by *Alcaligens sp.d2* while degrading phenol in MFC and comparison of degradation of phenol in MFC is made with that at ideal degrading situation.

MATERIALS AND METHODS

Construction and working of Microbial Fuel Cell

Two chambered Microbial fuel cell was built with polyacrylic sheets in a traditional H shape. Compartments were made of polyacrylic sheets, 0.20 µm syringe filter was fitted in between the PVC pipes connecting the two chambers, stainless steel electrodes were fixed on the lid of the compartments using nuts and bolts. A 1.5 V battery was connected to the assembly, to define cathode and anode (+ ve to anode and –ve to cathode). The stirrer of the agitator was fitted to the anode compartment through a hole made on the lid of the anode chamber[5]

Phenol Biodegradation study in MFC

Mineral salt phenol medium with the following composition Ammonium sulphate- 1 g, Potassium dihydrogen phosphate- 1 g, Magnesium sulphate-0 .9 g, Calcium chloride- 0.005 g, 5% phenol - 200 microlitre was prepared in 1 L of distilled water. *Alcaligens sp. d2*[6] and *Bacillus sp.* strains were obtained from the culture collections SBS, M.G University and were sub cultured at regular intervals of two weeks. Both species were grown in nutrient broth separately and 1 O.D culture in Physiological saline was inoculated in mineral salt phenol medium.At regular interval of 4 hours upto 32 hours, 20ml of sample were collected and centrifuged at 10000 rpm for 10 minutes, then pellet was removed and the supernatant is stored.

Estimation of Phenol

Estimation of phenol was done by 4-amino anti pyrine method as per modified method of *Mordocco*[7] spectrophotometrically at 510 nm. The conductance of the supernatant is measured using a portable multiparameter conductivity meter (PSC Testr 35).

Voltage, Current, Power and Polarisation Curve

Voltage drop across 1000 ohm were noted. Current generated can be find out by using ohm's law (V=IR). Usually current generated is low (µA) so they can't be easily detected. In this work the battery were turned off before taking the reading and allowed the multimeter to reach a stable reading and the same was .The following calculation was then attempted.

Current generated can be find out using ohm's law $I = V/R$

Then power generated, $P = VI$ or $P = V^2/R$

Power density = P/A of anode

To test the current producing capability of the cell, they were allowed to discharge through different range of resistances and the polarizing curves were[8] constructed.

FT/IR analysis

70 ml of analyte is taken out through inflow of the compartment in the optimized working condition of the MFC and centrifuged at 10000 rpm for 10 minutes and the supernatant obtained is extracted with diethyl ether and concentrated for FT/IR analysis.

RESULTS AND DISCUSSION

Construction of the Microbial Fuel Cell

Microbial fuel cell technology is a recently evolved technology which can be used to produce electricity from biomass. In this work, a double chambered fuel cell was designed using inexpensive materials and working of the MFC was monitored using phenol as the substrate.

Plate. 1. Microbial Fuel Cell (MFC) designed for phenol biodegradation by *Alcaligens sp. d2.*

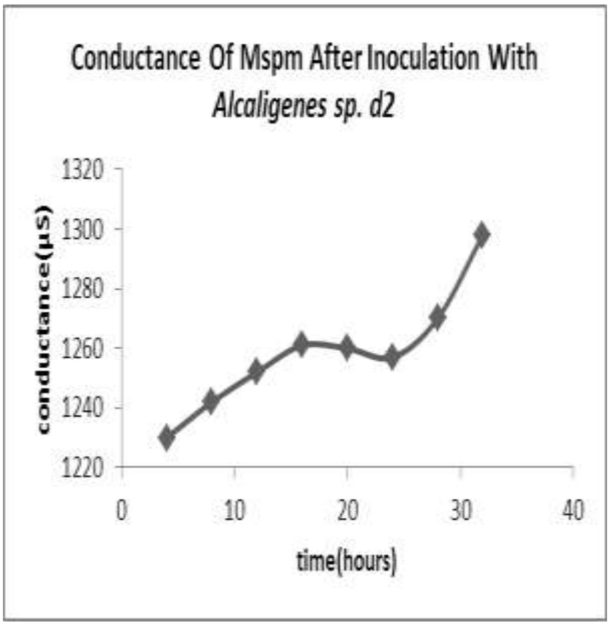


Fig.2.Conductance of Mineral Salt Phenol Medium after incubation with *Alcaligenes sp.d2.*

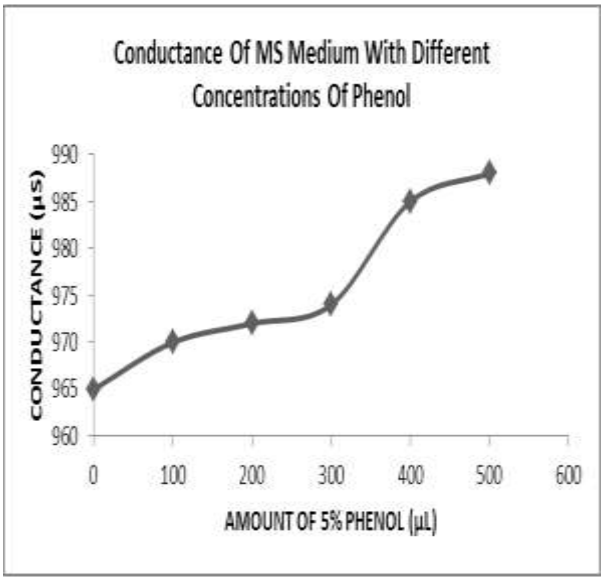


Fig. 1. Conductance of mineral salt media under different concentration of phenol in MFC.

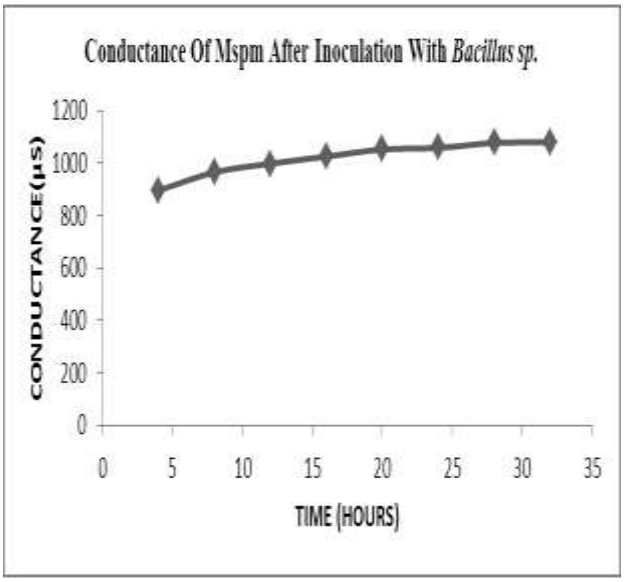


Fig. 3. Conductance Of Mineral Salt Phenol Medium After Incubation With *Bacillus Sp.*

The conductance of MS medium was found to be increases with increase of concentration of phenol in the medium (Fig.1). It was found that the conductivity of mineral salt phenol medium used for *Alcaligens sp. d2* have conductance of 972 µS.

Performance of the MFC inoculated with Cells

The conductivity of mineral salt phenol media after incubation with *Alcaligenes sp.d2* was found to be increases with the incubation with increase in incubation time. (Fig.2) It suggested that the products formed after phenol degradation were more conducting than that of phenol in the MS medium.

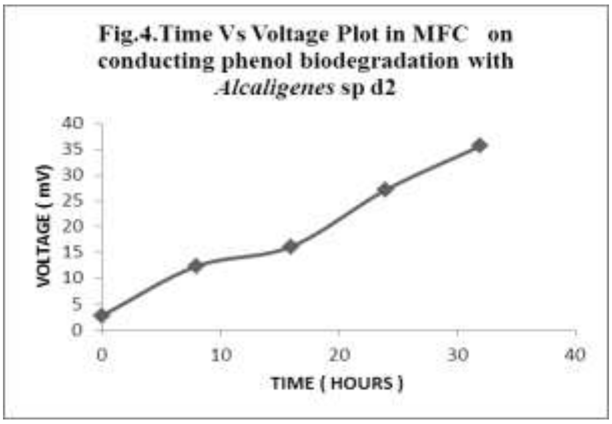
It was found that the conductance of the mineral salt medium increases after incubation with *Bacillus sp* (Fig.3) also. When conductance measurements of medium inoculated with *Bacillus sp.* was compared with that of *Alcaligenes sp. d2*, it was found that medium inoculated with *Alcaligenes* have more conductance values. It followed that it was better to conduct experiment with *Alcaligenes* - a gram negative bacteria and fast phenol degrading strain than with *Bacillus sp.* eventhough both can act on phenol. Earlier studies have already established the efficiency of *Alcaligenes* sp d2 in performing the degradation of phenol.

Several procedures were followed to get the correct working condition of the MFC. When 1% inoculum where given to the MSPM in the anode chamber, the catholyte used was uninoculated MSPM. It was unable to take reading because of the uninoculated MSPM in the cathode chamber got contaminated. Hence the catholyte was changed and the MS medium without carbon source was used as the catholyte. 1% inoculums were given to the MSPM in the MFC and the readings were noted in every 8 hours upto 32 hours. From the experiment we could find that microbes did not cross the membrane to the cathode chamber but there was no voltage generation .As far as concerned, the microbial filter functions rightly and we can conclude that use of syringe filter (.22 μM) in the assembly was a right option.

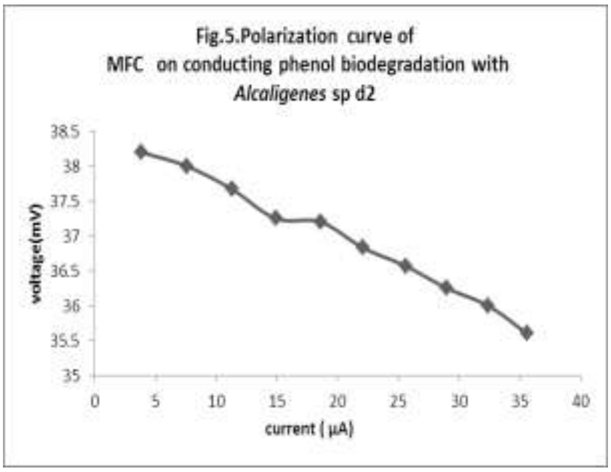
Then percentage of inoculums to the MSPM is increased to 3% without external voltage supply (it is based on the concept that, the chamber where microbial oxidation is taking place was the anode). The procedure was repeated. During 16th hour of incubation a voltage output of 2mV was shown in the multimeter after which no further voltage output could be observed. After that, to the assembly an external voltage supply was provided to define the electrodes into anode and cathode. 3% inoculum were given to the MSPM in the anode chamber and observed for voltage output. Maximum output was observed at 32 hours.

Two more structural improvements i.e. provision for aeration and agitation was added to the MFC assembly. 3% inoculum was given to the MSPM and an agitator was provided at the anode chamber. Also a sparger was provided to the cathode chamber. Both components were turned on and readings were taken. But during 16th hour a black colouration was developed to the medium in the anode chamber and no further readings were recorded. This may be due to the aerial contamination at increased agitation provided in the activated biodegradation medium for longer time. Hence we decided to give occasional agitation to the system. 30 minutes agitation was provided to the system in every interval of 8 hours. However continuous sparging was provided in the cathode chamber. 3% inoculum was given to the MSPM and voltage output was observed in every 8 hours upto 32 hours.

It is found that during 32 hours of incubation maximum voltage is generated at a constant load (1000 ohm) (Fig.4). The current generated was calculated and the polarizing curves were made on the basis of the observation at 32nd hour of incubation.



Further, the experiments were repeated at varying load and the voltage drop across different resistances at 32 hours were calculated as follows and polarization curve was constructed (Fig.5)



In MFCs linear polarization curves are mostly encountered. In the present study also the experimental values could generate almost a linear representation satisfying y=mx+c with M as the slope representing internal resistance offered by the MFC. On regression analysis the obtained data can be interpreted to a linear relationship.(Fig.6)

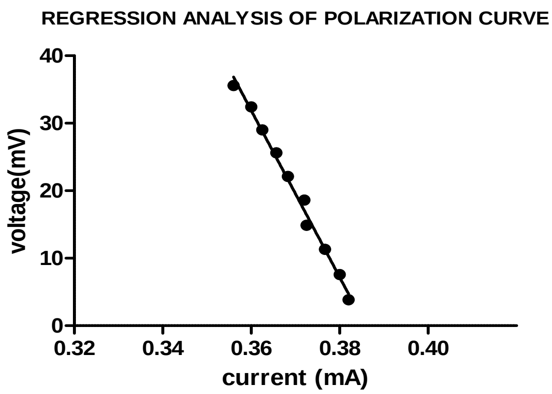


Fig.6: Regression analysis of Polarization curve in MFC on conducting phenol biodegradation with *Alcaligenes* sp d2.

From the regression analysis of the polarization curve it was found that the slope of the graph was 1235±39.27.Hence the internal resistance of the MFC assembly is 1235±39.27 mOhms.

Polarization curve is generally used as criteria in evaluating the performance of fuel cell. A linearity in the polarization curve obtained is indicative of the consistency in the performance shown by the MFC in the given set of conditions.

CALCULATION OF POWER AND POWER DENSITY

After 32 hours of incubation the maximum is generated and power generated can be calculated.

V = 35.6mV, R = 1000 ohm Then the current generated = V/R =0.0356 mA

POWER P= V*I = 35.6*0.0356 mW=**1.26 mW**

Power density P_D = P/ Area of the anode = 1.26mW/16cm^2 = **0.07875 mW /cm^2**

The power density obtained in the proposed MFC is indicative of satisfactory performance as far as the energy output is concerned.

Phenol Biodegradation in The MFC

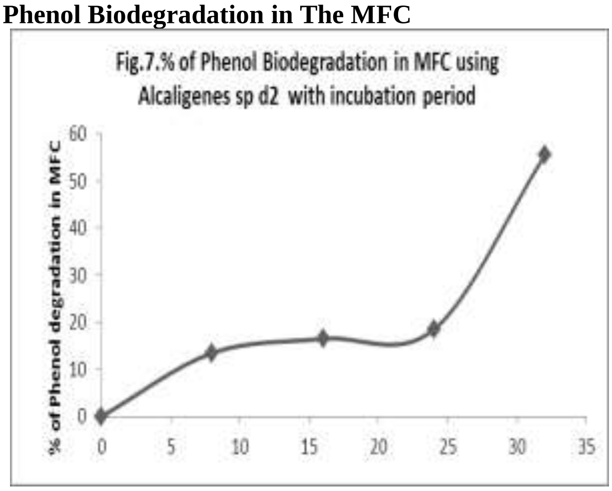


Fig.7.% of Phenol Biodegradation in MFC using Alcaligenes sp d2 with incubation period

The percentage of phenol content after biodegradation of phenol by *Alcaligens sp. d2* in the MFC after 32 hours is found to be 55.5%. Percentage of biodegradation of phenol in optimized condition in the flask is found to be 88.5%. (Fig.7).

FTIR analysis of Phenol Biodegradation in MFC

In the FT/IR analysis control (Fig.8) the region between 1600cm^{-1} -1750cm^{-1} there were no significant peak representations and in the case of sample in flask (Fig.9) and MFC sample (Fig,10) there were considerable differences in the peak representations. There was considerable peak representation in the region 1650 cm^{-1} – 1850 cm^{-1} in MFC sample and in control there were no significant peak representation. The Carbonyl group peak representation is more prominent in MFC than in the flask. In the range 700-1000 $^{cm-1}$ C-H bending in aromatic compounds.

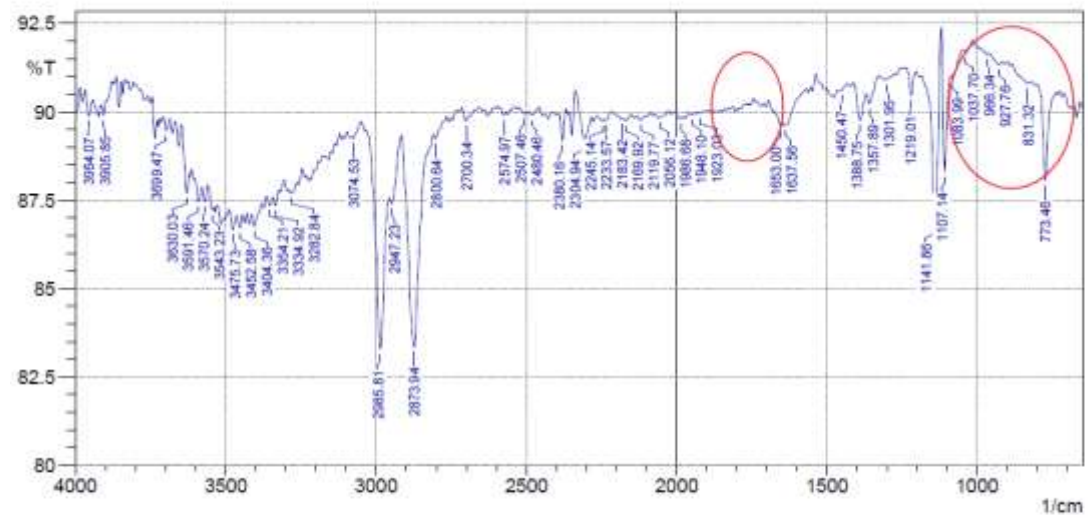


Fig. 8: FTIR analysis of ether extract of uninoculated MSPM after 32 hours (control).

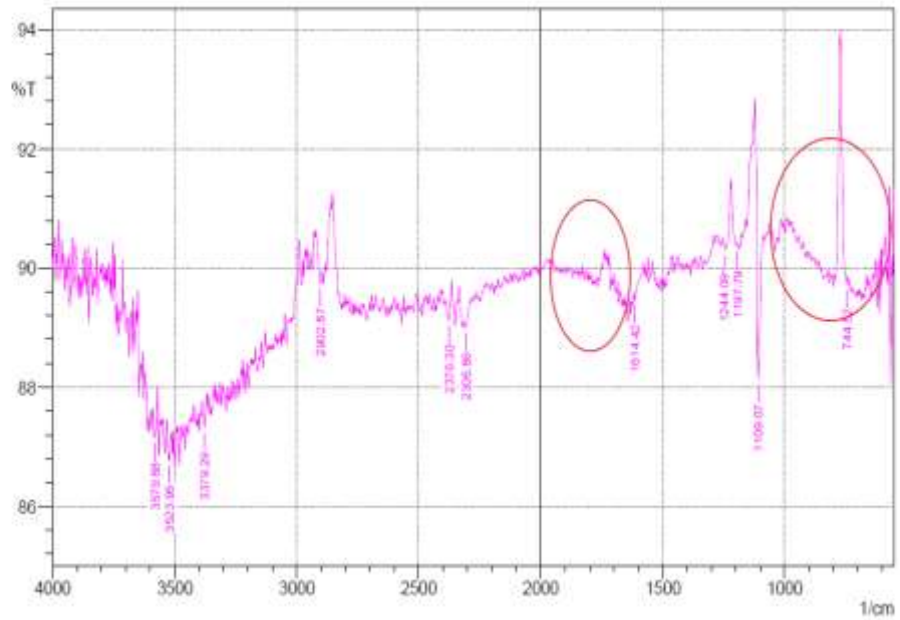


Fig.9. FTIR analysis of ether extract of Alcaligenes sp d2 inoculated MSPM after 32 hours (Flask).

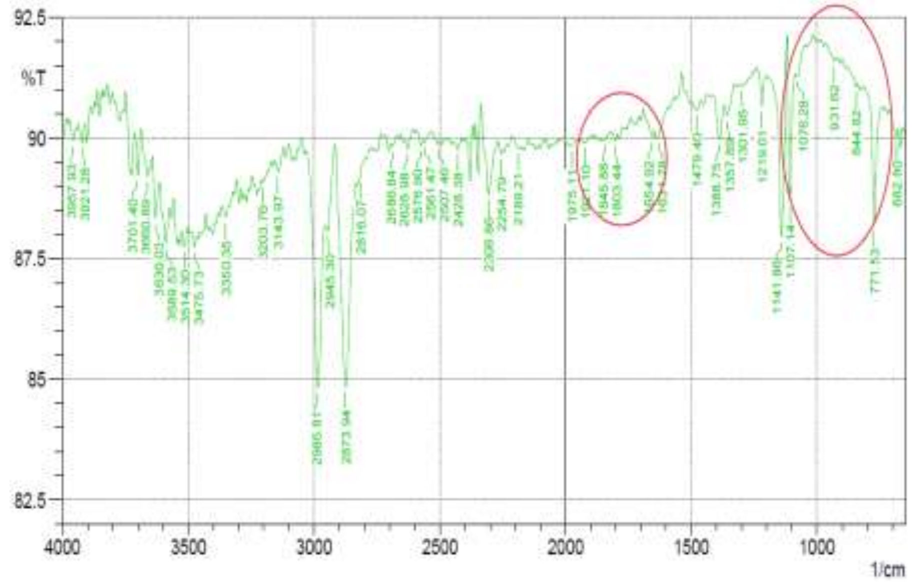


Fig.10: FTIR analysis of ether extract of *Alcaligenes* sp d2 inoculated MSPM after 32 hours (MFC).

Hence the evaluation of the process of biodegradation achieved through MFC using analytical approaches confirmed the structural changes that have occurred during the power generation in MFC. However it was also noticed that the extent of structural changes effected by MFC reactions was comparatively less than that in flask studies.

CONCLUSION

The global energy demand increases day by day. And we depend on unsustainable fossil fuels as the main source of energy. Fossil fuels are non-renewable and their exhaust cause environmental pollution. And that's why we are searching for alternate source of energy. Microbial Fuel Cell technology is one kind alternate source. It is a promising technology for simultaneous treatment of organic wastes and bio-energy recovery in form of electricity. In MFC, microorganisms were used for catalyzing the oxidation of organic compounds.

In this work, a microbial fuel cell assembly was made and the working of the assembly was monitored using phenol as the substrate. *Alcaligens sp. d2* strain was used in the study to degrade phenol in the MFC. The working conditions of the MFC were standardized. It was found that the maximum power density was observed at 32 hours of incubation of bacteria with minimal media containing phenol as the sole carbon source.

A comparative study was made on the phenol biodegradation rate in the MFC and in optimized condition in flask. FTIR analysis of ether extract of biodegraded phenol and spectrophotometric estimation of the phenol in both conditions were done. Degradation rate of phenol in the MFC is found to be slower than that of in the flask. However MFCs generate current during the biodegradation of phenol. This might be trapped for the other applications. MFCs offer potential for in situ bioremediation of recalcitrant compounds and might be going to an alternate source of energy in future.

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