GENERATION OF BIOELECTRICITY AND SIMULTANEOUS TREATMENT OF WASTE WATER USING MICROBIAL FUEL CELLS (MFC)

ABSTRACT

This era is definitely an era of renewable energy generated from resource which are naturally replenished on a human timescale .The human inquisitiveness in search of a sustainable and environment friendly energy source has led to several advancement in this field. The microbial fuel cell (MFC) is a new form of renewable energy technology that can generate electricity from what would otherwise be considered waste. The reasons for this recent interest in using bacteria to generate electricity are a combination of the need for new sources of energy, discoveries about microbial physiology related to electron transport, and advancement of fuel cell technologies. The MFC performance was successfully carried out for about 40 days with two different microbes (*E.coli* and *P.aeruginosa*) which were isolated, inoculated and identified by Gram's staining. Biochemical analysis confirms the purity of the respective microorganisms. The physical parameters estimated and studied showed *E.coli* as an efficient source of degradation for BOD, TDS, COD and DO in all the selected samples. The efficiency of salt degradation like nitrate, chloride, sulphate and phosphates) was shown more by *P.aeruginosa* compared to *E.coli* in all the samples. Further the efficiency of microbes in degrading waste materials and simultaneous production of electricity was statistically proven with ANOVA table showing the best voltage production in two samples by *P. aeruginosa* (419.8mV and 380.7mV) in lake water and apartment samples respectively. Similarly, the third sample collected from sewage treatment plant (STP) the maximum volt efficiency of 344.16mV was shown by *E.coli.*

Keywords : (Renewable energy, Bacteria, E.coli, P.aeruginosa, Biodegradation, Electricity, Microbial fuel cell)

1. INTRODUCTION

Energy calamity in India is rising each year, as there is constant acclivity in the price of fuels and also due to depletion of fossil fuels to a larger level. The demand for an alternative fuel has erupted extensive research in discovering a potential, economical and reusable source for energy manufacturers. For constructing a sustainable world we require to minimize the expenditure of fossil fuels as well as the pollution generated.

These two aims can be accomplished all together by treating the waste water (From disposing waste to using it). Industrial waste, agricultural waste and household waste are ideal substrates for energy productions as they are rich in organic contents. .
Microbial fuel cell (MFCs) have emerged in recent years as a promising yet challenging technology. MFCs are the major type of bio-electrochemical systems (BESs) which convert biomass spontaneously into electricity through the metabolic activity of the microorganisms. MFC is considered to be a promising sustainable technology to meet increasing energy needs, especially using wastewaters as substrates, which can generate electricity and accomplish wastewater treatment simultaneously.

Microbial fuel cells are devices that directly convert chemical energy to electricity through catalytic activities of microorganisms. Electricity has been generated in MFCs from various organic compounds including carbohydrates, proteins and fatty acids. One of the greatest advantages of MFCs over conventional fuel cells like hydrogen and methanol fuel cell is that a diverse range of Organic material can be used as fuels. A microbial fuel cell (MFC) is a device that converts chemical energy to electrical energy with the aid of microorganisms (Pandey *et al*., 2011). The fact that bacteria can oxidize the substrates to produce electricity makes MFCs an ideal solution for wastewater treatment and domestic energy production (Schwartz, 2007).

A MFC consists of anode and cathode separated by a cation specific membrane. Microbes in the anode oxidize fuel here bacteria gain energy for metabolism by transferring electrons from an electron donor, such as glucose or acetate to an electron acceptor such as oxygen and the resulting electrons and protons are transferred to cathode through the circuit and the membrane respectively. Electrons and protons are consumed in the cathode, reducing oxidant usually oxygen. The electrons obtained from this oxidation are transferred into anode chamber where they get departed through an electrical circuit before entering into cathode region (Uma et al., 2016).Since the microbial cells are electrochemically inactive due to nonconductive cell surfaces structure, mediators are employed to facilitate electron transfer from the microbial cells to the anode in MFCs. Production of electrical energy using microorganisms through microbial fuel cells (MFC) is one such renewable and sustainable technology that is considered to be one of the most efficient (HaoYu et al., 2007; Salgado, 2009) and carbon neutral energy sources (Lovley, 2006).
Application of MFCs for wastewater treatment is very attractive due to energy recovery from waste as well as reducing production of excess sludge, disposal of which is very expensive. It is expected that this process would generate much less sludge than a

conventional activated sludge process, since the major part of energy available from the oxidation of the organic contaminants is converted to electricity, and the remaining energy is used for microbial growth.

This study aimed to 1) Isolate ,Identify and Procure of the effective micro-organisms (*Escherichia coli*, *P.aeruginosa*) and 2) evaluation of the effective microbes generating the maximum electricity from the given waste sample.

2. MATERIALS AND METHOD:

2.1 WATER COLLECTION:

Waste water was collected from three different places 1) Apartment waste 2) Lake water 3) Secondary treatment plant waste around 1000ml of each waste water surrounded by Hebbal region of Bangalore.

2.2 CONSTRUCTION OF THE MFC SET UP

PREPARATION OF SALT BRIDGE**:** For the preparation of salt bridge, a water solution containing concentrations of 3% sodium chloride and 1.6% agar was autoclaved and poured into PVC pipe of length 10 cm and diameter 22.2mm covered at one end with polythene. The set up was thereafter allowed to cool for nearly two hours inside High efficiency Particulate Air Filter. The salt bridges were thus ready for use. **(Fig 1).**

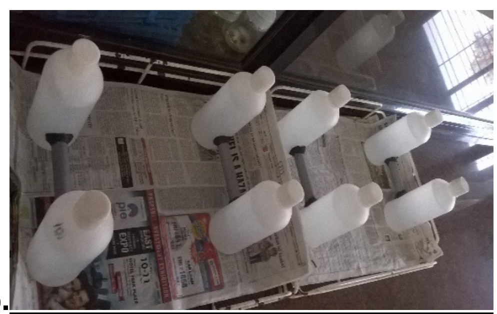


Fig 1: Preparation of salt bridge

DOUBLE CHAMBER SET UP

The set up consisted of two reagent bottles made of plastic. The two were connected by making an opening on one side of each bottle such that the salt bridge could be fitted into them. The corners of the openings were sealed with M-seal to ensure that the apparatus was made completely leak proof (Fig 2).

The 3 distinct anode chambers were filled with 500ml of apartment waste, lake water, STP sample waste water and 5ml of *E. coli* culture was inoculated along with methylene blue as electron mediator respectively. Same way to the other set of samples *P. aeroginosa* was inoculated respectively. The cathode chambers were filled with 500ml of phosphate buffer to those 3 sets of sample respectively of pH-more than 7. Clean graphite electrodes (extracted from 1.5 Volt batteries) were coiled with copper wire and introduced into the chambers. The set up was left 25 days under anaerobic conditions.

Cathode chambers would be equivalent of the oxygen sink at the end of the electron transport chain, only now it would be external to the biological cell. The solution would be an oxidizing agent that would pick up the electrons at the cathode. Potassium ferricyanide can be used an oxidizing agent which can be added to the cathode to accept electrons. It is very reactive with the graphite electrode. Ferricyanide has a fairly positive potential compared to the organic matter in the anode and helps to drive the flow of electrons. The set ups were kept for 30days with intermittent addition of inoculum of 3ml to the respective bottles after every 6 days.

Fig 2: Construction of double chamber set up

2.3 DEVELOPMENT OF PURE MICROBIAL CULTURE:

- Isolation and identification of Escherichia coli and P.aeruginosa by GRAM STAINING.

- Biochemical analysis by Catalase test, Indole test, MR test, Voges Proskauer test and oxidase test.

2.4 PRELIMINARY AND POST TREATMENT WATER ANALYSIS WAS CARRIED OUT BY: Determination of PH, Dissolved Oxygen, Biological Oxygen demand, Chemical Oxygen Demand, Total Dissolved Solids, Calcium estimation, Phosphate estimation, Sulphate estimation, Chloride estimation & Nitrate estimation.

2.5. STATISTICAL ANALYSIS:

The statistical significance was determined by one-way analysis of variance (ANOVA) to determine if the data obtained was significantly varied from one another. Statistical significance between different microorganisms (*E.coli* and *P.aeruginosa*) on 3 different samples (Lake Water, Apartment waste, STP sample) was determined. P value of <0.05 was considered significant.

2.6. ELECTRICAL MEASUREMENT

The voltage readings were taken with the help of multimeter and it is converted into current. Current was measured at a resistance (R) from the microbial fuel cell. I (mA) is the current, and R (Ω) is the external resistance.

4. RESULTS AND DISCUSSION

The present research work entitled **"Generation *of Bioelectricity and Simultaneous Treatment of Waste Water Using Microbial Fuel Cells (MFC)*"** was carried out at Indian Academy Degree College, Centre for Research and PG studies, Bangalore.

For the performance MFC the two different microbes (*E.coli* and *P.aeruginosa*) were isolated and inoculated which were identified by Gram's staining and further biochemical analysis confirms the purity of the respective microorganisms.

4.1. ISOLATION AND IDENTIFICATION OF MICRO-ORGANISMS: *Escherichia coli* was isolated from the sewage sample using Eosin Methylene Blue Agar mediumand *P.aeruginosa* were isolated using *P.aeruginosa* isolation agar medium. The given micro-organisms were identified by performing gram staining. Further the conformity of the identified micro-organisms was tested at the biochemical level.On the basis of morphological, cultural and bio-chemical characteristics, the isolated *E.coli* bacteria was Gram negative in nature and showed positive response to Catalase, indole, MR test and showed negative results for oxidase, VP test, Urease Test while the *P.aeruginosa* bacteria showed positive response to Catalase, oxidase, tests and showed negative response for indole, MR test, VP test.. These results are in consent with the work reported by Priya Iyer1*et al.,* 2013;

4.2. IMPACT OF MFC ON WASTE WATER QUALITY

To check the efficiency of microbes certain physical analysis of three different samples (Lake Water, Apartment waste, STP sample) were carried out before and after treatment in MFC (with microbial inoculum).

The results analysed indicates that *E.coli* was well performed in treating all 3 samples by the reduction in the level of BOD, TDS, COD, DO and CALCIUM. The lake water, apartment waste and STP samples are all rich in great biomass sources (organic matters) for MFCs, (Suzuki et al 1978; Oh and Logan 2005; Min et al 2005 and Zuo et al 2006). Liu and Logan (2004) suggested the vital role of the chamber setup (double) which greatly influences the COD level of waste water. This setup removes up to 80% of COD present in water sample. The present study showed that the role of microorganisms *(E.coli)* is another added feature to convert rich organic matter into generation of electricity by reducing concentration of COD BOD and DO. This is in consent with the work done by Mercy et al 2012. Further there is an important role of formation of biofilm in the MFC setup in degradation of waste. It was found that the reduction of dissolved solids increased with the increase in biofilm concentration (Hampannavar et al 2011). The study revealed the formation of increased biofilm in *E.coli* cultures compared to *P.aeruginosa*. The calcium removal capacity with respect to *E.coli* and *P.aeruginosa* in MFCs is not reported in the literature and further investigations are necessary to confirm the mechanism behind the nitrogen removal and its effect on overall power production in the MFC. But in case of other parameters like NITRATE, CHLORIDE, SULPHATE, PHOSPHATE estimations *P.aeruginosa* performed efficiently in reducing these amounts in samples. Since *P.aeruginosa* is a sulphide oxidizing microorganism it has been reported that they are efficient anaerobic microorganisms which involve in reducing the concentration of sulphate in the samples (Dambe, T; Quentmeier et al 2005). Sulfate reduction in a MFC fed with carbohydrates has been described previously (Rabaey,K et al 2004). According to Vanita Roshan Nimje et al 2012, as a result of substrate oxidation, liberated electrons which gradually decrease the level of nitrate but the role of microbes in reducing nitrates were not described earlier. Even the phosphates and chlorides removal capacity with respect to *E.coli* and *P.aeruginosa* in MFCs were also not reported in the literature and further investigations are necessary to confirm the mechanism behind the nitrogen removal and its effect on overall power production in the MFC.

Table 1: Comparative analysis of physical parameters of 3 samples before and after treatment by isolated micro organisms

Physical parameters	Lake water			Apartment waste			STP waste		
	Before treatment	After treatment		Before treatment	After treatment		Before treatment	After treatment	
		E.coli	*P.aeruginosa*		*E.coli*	*P.aeruginosa*		*E.coli*	*P.aeruginosa*
PH	8.6	8.3	7.4	7.3	8.6	8.0	7.4	8.3	7.5
BOD	3.2mg/Lt	1.6mg/Lt	2.4mg/Lt	4.5mg/Lt	1.2mg/Lt	1.6mg/Lt	4.4mg/Lt	2mg/Lt	3.2mg/Lt
TDS	20.4g/Lt	0.92mg/Lt	1.08g/Lt	12.4g/Lt	1.84g/Lt	2.08g/Lt	10.8g/Lt	1.76g/Lt	1.88g/Lt
COD	136mg/Lt	64mg/Lt	92mg/Lt	596mg/Lt	244mg/Lt	316mg/Lt	364mg/Lt	152mg/Lt	184mg/Lt
DO	2mg/Lt	0.8mg/Lt	1.2mg/Lt	5.7mg/Lt	3.6mg/Lt	4.1mg/Lt	4.8mg/Lt	2.5mg/Lt	3.4mg/Lt
NITRATE	0.39mg/ml	0.15mg/ml	0.09mg/ml	0.36mg/ml	0.24 mg/ml	0.20mg/ml	0.90mg/ml	0.60 mg/ml	0.57mg/ml
CHLORIDE	192.4mg/Lt	72.4mg/Lt	49.9mg/Lt	417.3mg/Lt	139.9 mg/Lt	124.9mg/Lt	342.3mg/Lt	112.4 mg/Lt	92.49mg/Lt
SULPHATE	1.8mg/ml	0.5mg/ml	0.3mg/ml	1.9mg/ml	0.2mg/ml	0.0mg/ml	2.6mg/ml	0.4mg/ml	0.1mg/ml
PHOSPHATES	6.4mg/ml	4.2mg/ml	4.omg/ml	7.4mg/ml	3.6mg/ml	2.6mg/ml	15mg/ml	6.8mg/ml	3.2mg/ml
CALCIUM	1.52mg/Lt	0.32mg/Lt	0.72mg/Lt	1.68mg/Lt	0.48 mg/Lt	0.80mg/Lt	0.72mg/Lt	0.32 mg/Lt	0.56mg/Lt

4.3 PERFORMANCE OF MFC

Once the apparatus was set up on combination of 3 different samples with respective micro-organisms i.e., Lake Water with *E.coli* and Lake water with *P.aeruginosa* followed by Apartment waste with *E.coli* and Apartment waste with *P.aeruginosa* finally, STP sample with *E.coli* and STP sample with *P.aeruginosa*. The voltage in mili volts was checked with the help of multimeter in both control as well as in the samples with respective inoculum for about 30 days at a time point per day the readings were taken for two times (10.0AM and 4.0PM) under room temperature.

The voltage reading in the control samples was gradually found to decrease as the day's move on approximately till the day 12 the voltage was obtained, later the values were found to be as 0.00mV. Simultaneously the voltage values (mV) obtained in the samples treated with the respective micro-organisms (*E.coli* and *P.aeruginosa)* initially shown high values in the *E.coli* containing samples than *P.aeruginosa.*

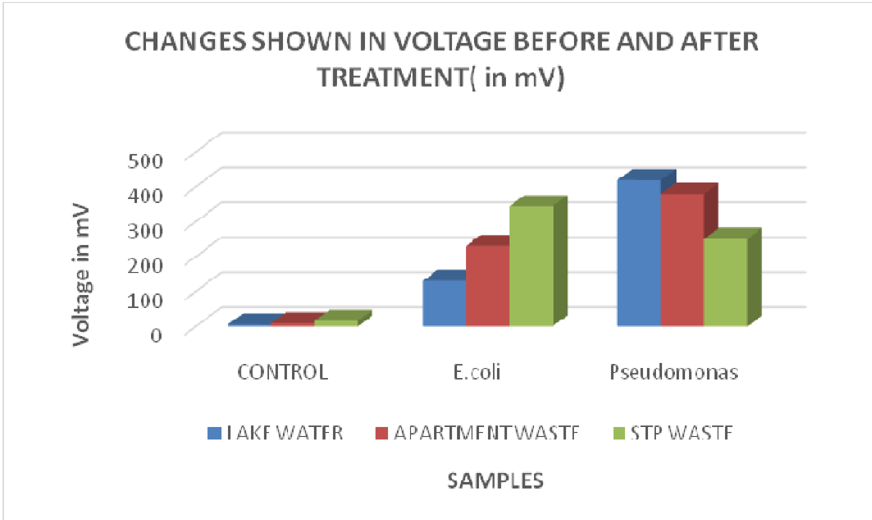
The voltage values were obtained on intermittent addition of inoculum was carried out for efficient voltage result. 3ml of inoculum was added for every 6 days into the respective samples in the MFC set up.as per the values the voltage was found to decrease gradually but when the inoculum was reloaded into the medium the value was found to be increasing. The cycle was carried out for 30days as tabulated in **TABLE 1**.
The voltage values were converted into amps with the help of formula. The current produced is calculated as follows:

$$I = V/R$$

Where, I= Current in mA
V=Voltage in mV
R= Resistance in Ω
Ω-100Ω

Table 2: Changes shown in voltage (mV) before and after treatment.

SAMPLES	***CONTROL(mV)***	***E.coli(mV)***	***P.aeruginosa(mV)***
LAKE WATER	5.88333333	131.18	419.80
APARTMENT WASTE	9.96666667	231.18	380.72
STP WASTE	16.5833333	344.17	251.82



Graph 1: Effect of microbial inoculum in generation of voltage (mV) against samples compared with control.

Followed by the voltage comparative results to see the efficiency of microbes between them in generating best voltage and as per the **TABLE 2** it was seen that in lake water *P.aeroginosa* was resulted in better voltage (**419.8mV**) when compared to that of *E.coli* (**131.18mV**) and same in case of apartment waste (*E.coli*-**231.18mV**, *P.aeruginosa*-**380.7mV**) but there was little difference was appeared in case of STP sample, *E.coli* were shown better voltage (**344.16mV**) than that of *P.aeroginosa* (**251.81mV**). Graphical representation was well established to compare the activity of inoculum in different sample (**GRAPH 1**). Similarly the control and treated values were obtained by the OHM's law thereby converting mV into mA. One way ANOVA was performed for average voltage (mV) readings to show the level of significance separately for three different samples and it was found that the Lake water, Apartment waste, STP sample there was a significant as obtained *P* value is less than 0.05.

Cumulative statistical analysis was performed to calculate the fold change and compare the *P* value obtained for voltage generated (mV) between treatment vs microbial inoculum (**TABLE 3**).

Table 3: Cumulative statistical analysis for voltage generated (mV) between treatment vs microbial inoculum.

SAMPLES	*E.coli*(mV)	*P.aeruginosa*(mV)	Fold change	*P* value
Lake water	131.1833	419.8	3.200102	2.58E-23
Apartment waste	231.1833	380.7167	1.646817	1.89E-07
STP sample	344.1667	251.8167	1.366735	0.005454

The control voltage values and treated voltage values were taken for 30 days respectively during that period of time intermittent (for every 6 days) addition of inoculum facilitated to show fluctuation in voltage readings up to maximum level and decline phase this is because when the microbial inoculum at exponential phase it shows high potential and it gradually decreases due to stationary and death phase S. Veer Raghavulu et al 2011.

Upon addition of *E.coli* and *P.aeruginosa* in three different samples showed change in voltage reading (mV) as well as current reading (mA) among which in lake water and apartment waste, *P.aeruginosa* resulted in high voltage of **419.8mV and 380.7m V** respectively whereas *E.coli* resulted voltage in lake water and apartment waste was found to be **131.18mV and 231.18mV.** Among the pure cultures studied *P.aeruginosa* is electrochemically active in nature (Logan 2009) and *E. coli* is electrochemically inactive, while mixed consortia are comprised of both of them. Electrochemically active nature supports the pumping out of redox powers (H+ and e-) from the outer membrane of biocatalyst. According to S. Veer Raghavulu et al 2011, higher bio potential observed with *Pseudomonas aeruginosa* might be because of its electrochemical nature and the involvement of soluble shuttlers for the redox powers. In case of STP sample the voltage was seen high in the *E.coli* treated sample (**344.16mV)** than *P.aeruginosa* (**251.81mV).** The efficiency of *E.coli* in STP is due to the substrate level where the micro-organism oxidize organic compounds present in waste water, electrons are released yielding a steady source of electrical current.

The relation between volume of waste water, its characteristics, organic content, microbial load in anode chamber, the potential of electrode etc need to be standardised for improving the efficiency of the MFC.

SUMMARY AND CONCLUSION

Under present investigation, bioelectricity was successfully generated from Lake Water, apartment and Secondary Treatment Plant waste water using Microbial Fuel Cell Technology (MFC).

The physical parameters estimated and studied showed *E.coli* as an efficient source of degradation for BOD, TDS, COD and DO in all the 3 samples. The efficiency of salt degradation (NITRATE, CHLORIDE, SULPHATE AND PHOSPHATES) was shown more by *P.aeruginosa* compared to *E.coli* in all the 3 samples.

According to the results obtained from this study, the electricity generation in Lake water was facilitated more by the organism *P.aeruginosa* compared to *E.coli* which was proven statistically by the ANOVA table, similarly in case of apartment waste *P.aeruginosa* was giving best voltage and current but there was slight difference seen in case of STP sample where *E.coli* gave the maximum voltage when compared with that of *P.aeruginosa*. Since the ANOVA values obtained in the all three samples was less than *P* value (0.05) hence the results were found to be significant.

MFC is a promising technology for bioelectricity generation and waste water treatment. Recent research and development and analysis of literature review showed that higher power densities can be obtained from improved MFC designs with the use of cost effective materials. Hence, large scale MFC's can solve the future energy crisis undoubtedly. More research and development is required for assessing suitability of microorganisms for better efficiency for electricity production. The ultimate achievement in MFCs will be when they can be used solely as a method of renewable energy production.

So the present investigation led to a conclusion that the two suitable microorganisms *E.coli* and *P.aeruginosa* proved to be equally efficient in degrading the waste depending on the source with simultaneous production of electricity.

Further, a detail study for this upcoming technology for power generation need to be studied and developed.

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