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ISSN: 2394-1081

Generation of Bioelectricity and Simultaneous Treatment of Waste Water Using Microbial Fuel Cells (MFC)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SUM and GP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GP managed the analyses of the study. Authors Pooja and GJ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/30927 Editor(s): (1) Anil Kumar, Professor & Head (Chair), School of Biotechnology, Devi Ahilya University, Madhya Pradesh, India. Reviewers: (1) Sarfraz Hashim, Bahauddin Zakariya University, Pakistan. (2) Sanjay Mishra, IFTM University, India. (3) Nvatwere D. Mganga, Mkwawa University College of Education, Tanzania. Complete Peer review History: http://www.sciencedomain.org/review-history/17174

Original Research Article

Received 29th October 2016 Accepted 28th November 2016 Published 8th December 2016

ABSTRACT

This era is definitely an era of renewable energy generated from resources which are naturally replenished on a human time scale. This study provides information that initiates to reveal correct mechanisms involved with electron transfer to microbial fuel cell (MFC) electrodes using two bacterial isolates. The MFC performance was successfully carried out in P. aeruginosa and E. coli microbial inoculum for 30 days. Biochemical analysis confirms the purity of the respective microorganisms. The comparative physical parameters showed E. coli as an efficient source of degradation than P. aeruginosa in Lake water for Biological oxygen demand (1.6 mg/lt), Total dissolved solids (920 mg/lt), Chemical oxygen demand (64 mg/lt) and Dissolved oxygen (0.8 mg/lt) with respect to apartment & STP waste. In contrast, the efficiency of salt degradation like nitrate (570 mg/lt), chloride (92.49 mg/lt), sulphate (1000 mg/lt) and phosphates (3200 mg/lt) calcium (0.56 mg/lt) was shown more by P. aeruginosa compared to E. coli in STP than Lake and apartment waste. Further the efficiency of microbes in degradation of waste materials and production of electricity was statistically proven with ANOVA showing the best voltage production in two samples by *P. aeruginosa* (419.8 mV and 380.7 mV) in lake water and apartment samples respectively. Similarly, the third sample collected from sewage treatment plant (STP) showed the maximum volt efficiency of 344.16 mV 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. Industrial waste, agricultural waste, and household waste are ideal substrates for energy productions as they are rich in organic contents.

Microbial fuel cells (MFCs) have emerged, in recent years, as a promising yet challenging technology. MFCs are the major type of bioelectrochemical 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 waste water as substrates, which can generate electricity and accomplish waste water treatment simultaneously. In general, an MFC functions same as a fuel cell acts for, and is developed on its fuel cell based principle, the difference here is, that the reactions are catalyzed by the microorganism and the fuel is, the growth medium and the substrate [1]. The choice of substrate can be made by the process being involved (waste water utilization, type of microorganism, etc.)

Electricity has been generated in MFCs from various organic compounds including carbohydrates, proteins and fatty acids [2], [3]. 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 [4]. An MFC is a device that converts chemical energy into electrical energy with the aid of

microorganisms [5]. The fact that bacteria can oxidize the substrates to produce electricity makes MFCs an ideal solution for wastewater treatment and domestic energy production [6].

An MFC consists of anode and cathode separated by a cation-specific membrane. Microbes in the anode oxidize fuel where 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 [7]. Since the microbial cells are electrochemically inactive due to the nonconductive cell surface structure, mediators are employed to facilitate electron transfer from the microbial cells to the anode in MFCs. Production of electrical energy using microorganisms through MFC's is one such renewable and sustainable technology that is considered to be one of the most efficient [8], [9] and carbon neutral energy sources [10].

MFC can be best defined as a fuel cell where microbes act as catalyst in degrading the organic content to produce electricity. It is a device that straight away converts microbial metabolic or enzyme catalytic energy into electricity by using usual electrochemical technology [11]. All the microbes are not equally efficient in generating Hence, the current potential difference. investigation was focused to compare two electro chemically active micro organisms for their electrogenic properties and to study their degradation capacity in sewage samples. This inturn will combat the excess environmental burdens and helps in developing environmentally safe eco-friendly method.

2. MATERIALS AND METHODS

2.1 Water Collection

Wastewater was collected from three different places in and around Hebbal region of Bangalore. (1) apartment waste; (2) lake water; and (3) secondary treatment plant waste.

2.2 Construction of the MFC Setup

2.2.1 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 22.2 mm covered at one end with polythene [12]. The setup was thereafter allowed to cool for nearly 2 hrs inside high efficiency particulate air filter. The salt bridges were thus ready for use (Fig. 1).



Fig. 1. Preparation of salt bridge

2.2.2 Double chamber setup

The setup 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 Mseal to ensure that the apparatus was made completely leak proof (Fig. 2).

The three distinct anode chambers were filled with 500 ml of apartment waste, lake water, STP sample waste water, and 5 ml of *E. coli* culture was inoculated along with methylene blue as electron mediator respectively. Similarly, with the other set of samples *P. aeroginosa* was inoculated. The cathode chambers were filled with 500 ml of phosphate buffer to the three sets of samples with pH \geq 7. Clean graphite electrodes (extracted from 1.5 V batteries) were coiled with copper wire and introduced into the chambers. The setup was left for 25 days under anaerobic conditions [13].

Cathode chambers were made equivalent of the oxygen sink. The solution used was an oxidizing agent that would pick up the electrons at the cathode. Potassium ferricyanide was used as an oxidizing agent which was added to the cathode to accept electrons. Ferricyanide has a fairly positive potential compared to the organic matter in the anode and helps to drive the flow of electrons. The setup was kept for 30 days with intermittent addition of 3 ml of inoculum to the respective bottles after every 6 days.

2.3 Development of Pure Microbial Culture

- Isolation and identification of *E. coli* and *P. aeruginosa* was performed by standard Gram staining method.
- Biochemical analysis by Catalase test, Indole test, MR test, Voges Proskauer test, and Oxidase test [14].

2.4 Pre and Post Water Analysis Treatment

Pre and post treatment of 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, and nitrate estimation [15].

2.5 Electrical Measurement

The voltage readings were taken in a multimeter and then converted into current. Current I, mA was measured at an external resistance R, Ω from the microbial fuel cell.

2.6 Statistical Analysis

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

Maheswari et al.; JABB, 10(4): 1-9, 2016; Article no.JABB.30927



Fig. 2. Construction of double chamber setup

3. 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.

3.1 Isolation and Identification of Microorganisms

Escherichia coli was isolated from the sewage sample (collected near Indian academy college Bangalore) using dearee Eosin Methylene Blue Agar medium and P. aeruginosa were isolated using P. aeruginosa isolation medium. The given micro-organisms adar 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 biochemical 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, and urease test. However, P. aeruginosa bacteria showed positive response to catalase test, oxidase, test and showed negative response for indole, MR test, and VP test. These results are in consent with the work reported by [14].

3.2 Impact of MFC on Wastewater Quality

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

The results analysed indicate that E. coli was well performed in treating all three samples by the reduction in the BOD, TDS, COD, DO and CALCIUM levels. The lake water, apartment waste, and STP samples are all rich in great biomass sources (organic matters) for MFCs [16], [17], [18], [19]. It's been seen that the vital role of the chamber setup (double) greatly influences the COD level of wastewater [20]. 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 [21]. 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 [22]. 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 are involved in reducing the concentration of sulphate in the samples [23]. Sulphate reduction in an MFC fed with carbohydrates has been described [24]. The result of substrate previously liberated oxidation. electrons gradually decrease the level of nitrate but the role of microbes in reducing nitrates was not described earlier [25].

3.3 Performance of MFC

The apparatus was set up with the combination of three different samples with respective microorganisms 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 mV 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 (10.00 AM and 4.00 PM) under room temperature.

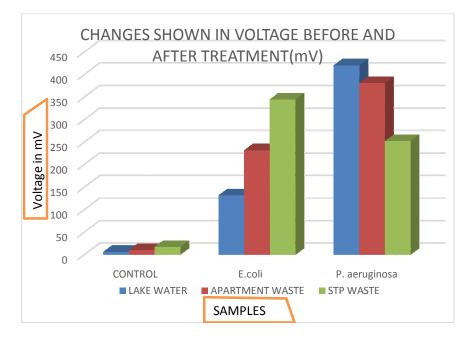
The voltage reading in the control samples decreased gradually as the day's move on approximately till the day 12 the voltage was obtained, later the values were found to be as 0.00mV. Simultaneously the voltage (mV) obtained in the samples treated with the respective microorganisms (*E. coli* and *P.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

The voltage obtained on intermittent addition of inoculum was carried out for efficient voltage result. 3 ml of inoculum was added for every 6 days into the respective samples in the MFC setup 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 30 days.

The voltage (mV) was converted into current (amperes) and was calculated as follows:

V= IR

where, I is Current (mA) and R the resistance (Ω) .



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

Physical	Lake water			Apartment waste			STP waste		
parameters	Concentration before	Concentration after treatment (mg/lt)		Concentration before	Concentration after treatment(mg/lt)		Concentration before	Concentration after treatment(mg/lt)	
	treatment (mg/lt)	E. coli	P. aeruginosa	treatment(mg/lt)	E. coli	P. aeruginosa	treatment(mg/lt)	E. coli	P. aeruginosa
PH	8.6	8.3	7.4	7.3	8.6	8.0	7.4	8.3	7.5
BOD	3.2 mg/Lt	1.6 mg/Lt	2.4 mg/Lt	4.5 mg/Lt	1.2 mg/Lt	1.6 mg/Lt	4.4 mg/Lt	2 mg/Lt	3.2 mg/Lt
TDS	20400 mg/Lt	920 mg/Lt	1080 mg/Lt	12400 mg/Lt	1840 mg/Lt	2080 mg/Lt	1080 mg/Lt	1760 mg/Lt	1880 mg/Lt
COD	136 mg/Lt	64 mg/Lt	92 mg/Lt	596 mg/Lt	244 mg/Lt	316 mg/Lt	364 mg/Lt	152 mg/Lt	184 mg/Lt
DO	2 mg/Lt	0.8 mg/Lt	1.2 mg/Lt	5.7 mg/Lt	3.6 mg/Lt	4.1 mg/Lt	4.8 mg/Lt	2.5 mg/Lt	3.4 mg/Lt
NITRATE	390 mg/Lt	150 mg/Lt	90 mg/ Lt	360 mg/ Lt	240 mg/ Lt	200 mg/ Lt	900 mg/ Lt	600 mg/ Lt	570 mg/ Lt
CHLORIDE	192.4 mg/Lt	72.4 mg/Lt	49.9 mg/Lt	417.3 mg/ Lt	139.9 mg/Lt	124.9 mg/Lt	342.3 mg/Lt	112.4 mg/Lt	92.49 mg/Lt
SULPHATE	1800 mg/ Lt	500 mg/ Lt	300 mg/ Lt	1900 mg/ Lt	200 mg/ Lt	0.0 mg/ Ľt	2600 mg/ Lt	400 mg/ Lt	100 mg/ Lt
PHOSPHATES	6400 mg/ Lt	4200 mg/ Lt	4000 mg/ Lt	7400 mg/ Lt	3600 mg/ Lt	2600 mg/ Lt	15000 mg/ Lt	6800 mg/ Lt	3200 mg/ Lt
CALCIUM	1.52 mg/Lt	0.32 mg/Lt	0.72 mg/Lt	1.68 mg/Lt	0.48 mg/Lt	0.80 mg/Lt	0.72 mg/Lt	0.32 mg/Lt	0.56 mg/Lt

Table 1. Comparative analysis of physical parameters of 3 samples before and after treatment by isolated microorganisms

Table 2. Cumulative statistical analysis by One way ANOVA for voltage generated (mV) between treatment vs microbial inoculum (P≤0.05)

Samples	<i>E. coli</i> (mV)	<i>P. aeruginosa</i> (mV)	Fold change	P value
Lake water	131.1833	419.8	3.200102	2.58E-23 ≤0.05
Apartment waste	231.1833	380.7167	1.646817	1.89E-07 ≤ 0.05
STP sample	344.1667	251.8167	1.366735	0.005454 ≤0.05

The comparative results of microbial efficiency to generate the best voltage compared to control (GRAPH 1) was seen best in lake water by *P. aeruginosa* (419.8 mV) compared to (131.18 mV) in *E. coli*. The apartment waste sample generated 380.7 mV and 231.18 mV in *P. aeruginosa and E. coli* respectively. Which again supports *P. Aeruginosa* to be an efficient micro organism.

On the other hand in STP sample, *E. coli* has shown a better voltage (344.16 mV) than that of *P. aeroginosa* (251.81 mV). Graphical representation was well established to compare the activity of inoculum in different samples (GRAPH 1).

The control and the treated voltage values for 30 days showed fluctuation in readings upto the maximum level followed by a decline phase. This result supports the generation of high potential during the exponential phase when microbial inoculum was added intermittently for every 6 days. In *Pseudomonas aeruginosa* and *Escherichia coli* used together as a biocatalyst in substrate degradation [26].

Further, a high voltage of 419.8 mV and 380.7 mV was observed in lake water and apartment waste samples. These samples were treated by the pure cultures of *P. aeruginosa*. Contrary to this *E. coli* inoculum gave a very low voltage output of 131.18 mV and 231.18 mV inlake water and apartment waste samples respectively. This can be attributed to the fact that *P. aeruginosa* is electrochemically active in nature and *E. coli* electrochemically inactive when added as a pure culture, while mixed consortia comprises both of them. Hence, electrochemically active nature supports the pumping out of redox powers (H+ and e-) from the outer membrane of biocatalyst.

Higher bio potential was observed with *Pseudomonas aeruginosa* which might be because of its electrochemical nature and the involvement of soluble shuttlers for the redox powers [26].

In case of STP the voltage was seen high in the *E. coli* treated sample (344.16 mV) as compared to that of *P. aeruginosa* (251.81 mV). The reason for better efficiency of *E. coli* in STP can be suggested due to the fact that the substrate level for releasing the electrons and oxidizing the organic compound was higher in *E. coli* inoculum than in *Pseudomonas*.

4. 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 three samples. The efficiency of salt degradation (NITRATE, CHLORIDE, SULPHATE AND PHOSPHATES) resulted better in *P. aeruginosa* inoculum.

The electricity generation in lake water was facilitated more by the organism *P. aeruginosa* compared to *E. coli* which was proven statistically. Similarly in apartment waste *P. aeruginosa* has given a best voltage and current but there was slight difference seen in case of STP sample where *E. coli* gave the maximum voltage.

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 production. The for electricity 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. The method adopted was cost effective and efficient at the industry and residential level. Application of microbial fuel cell (MFC) for wastewater treatment could be an attractive alternative to reduce the cost of treatment and generate electricity.

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: http://sciencedomain.org/review-history/17174