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Two Chamber Microbial Fuel Cells for Electricity Generation Using Different Carbon Sources

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Authors' contributions

This work was carried out in collaboration between both authors. The study was planned by author PS and executed by author DA. Manuscript preparation contributed by the both authors. Statistical analysis was carried out by author PS. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Electricity generation from wastes using Microbial Fuel Cells (MFCs).
Materials and Methods: *Bacillus* sp._1, *Bacillus* sp._3 *Pseudomonas* sp., and *Citrobacter* sp., isolated from soil sediment were used for electricity generation in sewage water, glucose, soil sediment and acetate containing medium under different incubation periods.
Results: Of the four isolates, generation of electricity started from 1 hour of incubation and reached maximum at 2h of incubation by *Pseudomonas* sp., and *Bacillus* sp., then declined but *Citrobacter* sp., showed gradual increase till stationary phase of growth. In glucose containing medium, during log phase of growth *Bacillus* sp._1, showed maximum of 336±0.4 mV. In non-supportive acetate medium *Citrobacter* sp., generated 316±0.3 mV during 1 h of incubation. Also in soil sediment medium *Citrobacter* sp., generated maximum of 160±1.2 mV during 5h of incubation.
Conclusion: It is recommended that a longer incubation period required in complex medium for electricity generation.

Keywords:Microbail fuel cell; two chamber method; Citrobacter sp; sewage treatment; soil sediment; electricity generation.

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1. INTRODUCTION

The need for the sustainable fuel energy is increasing worldwide. The use of fossil fuels, especially petroleum, in recent years has accelerated a global energy crisis. The combustion of fossil fuels releases more CO_2 to the atmosphere and causes global climate change. Therefore, a carbon-neutral, sustainable energy sources as an alternative to fossil fuels is needed to alleviate the global energy crisis and climate change.

Large amount of liquid and solid wastes are generated from agriculture, industrial and food processing sectors and are deliberately released into the environment that causes serious environment concern in disposal and health related issues. There are several pathways enabling the conversion of biomass to useful bioenergy. Methanogenic anaerobic digestion based technology, which emerged during the seventies, is now well established. In addition to that, ethanol fermentation and hydrogen fermentation are also approaches for biomass-to-bioenergy conversion.

A technology using microbial fuel cells (MFCs) in which microorganisms mediate direct conversion of chemical energy stored in organic matter or bulk biomass into electrical energy has gained considerable interests to electrical energy [1-3]. The conversion can occur at temperatures below 20℃ and at low substrate concentration levels. So MFCs is considered as a promising technology for sustainable energy generation.

A typical MFC is consisting of two chambers, i.e. the anaerobic anode chamber and the aerobic cathode. The two chambers are separated by a membrane (e.g., proton exchange membrane) where protons are transferred from the anode chamber to the cathode chamber, while electrons from the anode chamber are transferred to the cathode chamber through an external electrical circuit and a resistor for electricity production [4-6].

Various organic compounds and sources of wastes have been successfully utilized for power generation in MFCs [5,7]. Microorganisms play a key role in the anaerobic bioconversion of substrate to energy. In MFCs different types of organisms are involved in generation of electricity among which *Shewanella putrefaciens*, *Pseudomonas aeruginosa, Geobacter* sp., *Rhodoferax ferrireducens* are common [8,9]. MFCs also operated at high temperature using a thermophilic bacterium, such as *Bacillus licheniformis* or *Bacillus thermoglucosidasius* have also been described.

Electricity generation using MFC has certain common limitations, such as high internal resistance, high energy losses, high costs for construction and operation and difficulty of scaling up [3]. However, these technologies are still in their infancy and have not been moved from bench scale operation. So the present study is conducted in order to recycle the waste and reduce the waste discharge using MFCs. The main objective of this project was to improve the performance, reduce the construction cost, and expand the application scopes of two chamber MFC-based systems. Specific objectives are to isolate and produce electricity using different carbon sources using MFC.

2. MATERIALS AND METHODS

2.1 Isolation of Organisms and Culture Conditions

The sewage sediment collected in a sterile container and transported immediately to the laboratory. Collected sample was serially diluted, plated on nutrient agar plates and incubated at 37ºC for 48 h. Isolated colonies were subcultured on nutrient agar plates and cultures plates were stored at 4ºC. Isolated organisms were identified using standard cultural, microscopic, and biochemical tests. Flasks containing 100 ml of sterile nutrient broth were inoculated with 1ml of each culture (1 OD at 600 nm), incubated at room temperature. Intensity of growth of each organism was recorded by taking OD at 540 nm at 30 minutes of time interval (Fig. 1).

2.2 Measurement of Electricity Generation by Isolates in two Chamber Method

The microbial fuel cell is divided into aerobic and anaerobic chambers. The aerobic half is cathode or positively charged (iron rod) electrode and is filled with 500ml of saturated sodium chloride solution. The anaerobic half was maintained in anaerobic condition allowing a negative or anode electrode (zinc rod) and this filled with 500ml of sewage water collected from waste discharged from a house. The chambers were separated by a plastic connector filled with 1% of semisolid agar which keeps oxygen out of the anaerobic chamber while still allowing hydrogen ions to

pass through. The chambers were continuously connected and incubated at 25ºC for 48h. Generation of electricity was measured and recorded using digital multimeter at 30 min of time interval (Fig. 2). All experiments were conducted in triplicates and results were recorded by calculating the mean and standard deviations manually.

2.3 Influence of Carbon Sources on Electricity Generation

The microbial fuel cell is divided into two chambers aerobic and anaerobic. The aerobic half was filled with sodium chloride solution. The anaerobic half was filled with 2% of glucose solution or 2% acetate solution or soil sediment. Soil sediment was collected from near drainage pipe, consists of half weathered food wastes, leaf litters and liquid. Half of the chamber was filled with semisolid organic matter rich sediment and remaining area filled with liquid waste. By above mentioned procedure electricity was measured for each experimental design. All experiments were conducted in triplicates and results were recorded by calculating the mean and standard deviations manually.

Fig. 1. Measurement of electricity in two chamber containing sewage

Fig. 2. Two chamber method showing electricity generation using different carbon sources

3. RESULTS

3.1 Isolation and Identification of Bacteria

On nutrient agar plates morphologically distinguishable four bacterial colonies were identified and sub-cultured on nutrient agar plates and tubes.

The isolated organisms were named serially as isolate - 1, 2, 3, & 4. The isolate-1 was Gram positive, spore forming, non-fermentative rod shaped organism, and identified as *Bacillus* sp._1, isolate–2 was Gram negative, citrate, oxidase and catalase positive, non-fermentative organisms and was confirmed as *Pseudomonas* sp., the isolate-3 was Gram positive rod shaped, spore forming bacteria confirmed by non-fermentative metabolism as *Bacillus* sp._3, and the isolate – 4 showed positive for indole and citrate utilization and negative for gelatine hydrolysis so it was confirmed as *Citrobacter* sp.

3.2 Growth Profile of Isolates

During 10 h of incubation period of growth profile showed that all isolates showed a short lag phase between 30 and 60 min of incubation period and entered into log phase of growth. Recorded values were given in Table 1 and Graph 1. Log phase extended till 150 min by *Bacillus* sp._1, and *Citrobacter* sp., then entered into stationary phase of growth. *Pseudomonas* sp., remained in log phase till 180 min and *Bacillus* sp._3, retained its log phase till 240 min and then both entered into stationary phase of growth till the time of incubation. Of the four bacteria highest density was reached by *Bacillus* sp., and a steady growth profile was observed for *Citrobacter* sp. The recorded results confirms that isolates enters to log phase from 60 min of incubation period and maintained in stationary phase of growth for long period. So it is essential to study optimum phase of growth for electricity generation by these organisms.

3.3 Generation of Electricity Using Sewage

The generation of electricity was measured with multimeter in millivolts (mV). In two chamber system, isolates were inoculated along with the native organism in sewage water and generation of electricity under different incubation periods were measured and tabulated in Table 2. *Bacillus* sp._1, produced maximum of 335±0.5 mV at 5 h of incubation and remained stable till incubation period, similarly *Pseudomonas* sp., showed maximum 426±0.7 mV of electricity generation at 1 h of incubation and decreased drastically to168±0.4 mV at 5 h of growth and maintained at the same till 12h of study. Another *Bacillus* sp._3, produced maximum electricity of 366±0.4 mV at 2h of incubation and decreased to 249±0.3 mV at 5 h and remained stable till 12 h of incubation. *Citrobacter* sp., produced maximum of 356±1.0 mV at 4h of incubation and remained stable till 12 h of incubation. Generally all the isolates produced electricity, though the facultative anaerobic organism *Citrobacter* sp., produced 356±1.0 mV of electricity at 4 h of incubation which was comparatively lower than electricity generated by *Pseudomonas* sp., (maximum 426 mV) but was stable till stationary phase of growth.

3.4 Generation of Electricity using Different Carbon Sources

In two chamber system isolates were inoculated in media having different carbon sources such as glucose, acetate and soil sediment and generation of electricity during the incubation periods were measured and tabulated in Table 3 and Graph 2.

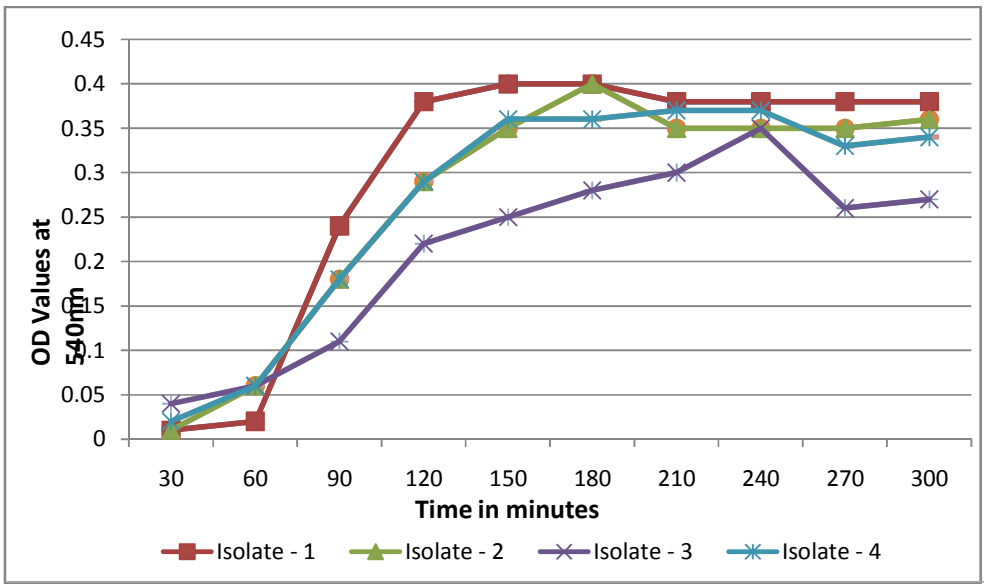
In glucose containing medium *Bacillus* sp._1, produced 125±0.4 mV at 1 h of incubation, serially increased to 336±0.2 mV at 4 h of incubation, after that started decreasing; in acetate medium electricity production was found to be very poor, maximum recorded as 25±0.4 mV at 5 h, in soil sediment overall production was affected, production was 46±0.6 mV at 1 h and was remained as 124±1.4 mV till 5 h of incubation.

Pseudomonas sp., showed maximum of 28±0.1 mV of electricity at 2 h of incubation in glucose containing medium, then gradually decreased to 165±0.9 mV at 5 h, a decreased production of 75±0.3 mV at 4 h in acetate medium then production was decreased, and in soil sediment electricity generation was slowly increasing from 45±0.1 mV to 125±1.5 mV at1 h to 5 h of incubation.

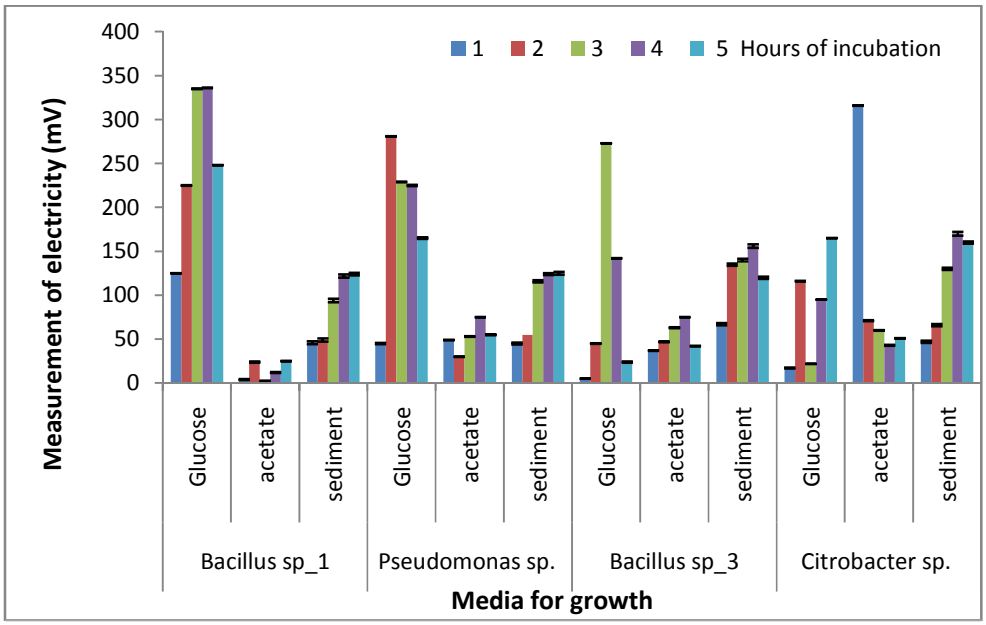
Bacillus sp._3, which showed minimum electricity generation among the isolates, maximum of 273±0.3 mV at 3 h of incubation was recorded in glucose medium, an average of 53 mV during different incubation period in acetate medium then production was gradually decreased and in

soil sediment an average of 123 mV of electricity was generated, maximum of 156±1.9 mV recorded at 4 h of incubation then decreased.

Citrobacter sp., started generating electricity from 17±0.6 mV at 1 h of incubation in glucose medium; production steadily increased till 5 h of incubation, recorded maximum was 165±0.3 mV of electricity at 5 h in glucose medium, maximum of 316±0.3 mV of electricity generated at 1 h of incubation and drastically production was reduced to 51±0.3 mV at 5 h of incubation in acetate medium, and in soil sediment electricity production started from 47±1.2 mV at 1 h and gradually increased to 160±1.2 mV at 5 h of incubation. Though *Citrobacter* sp., was not a maximum producer but showed steady and gradual increase in electricity generation in complex organic sources.



Graph 1. Line diagram showing growth profile of isolated bacteria



Graph 2. Electricity generation in different carbon sources

Table 1. Growth profile of isolates

Time in minutes	Growth of organism in OD values at 540nm			
	***Bacillus* sp._1**	***Pseudomonas* sp.**	***Bacillus* sp._3**	***Citrobacter* sp.**
30	0.01	0.01	0.04	0.02
60	0.02	0.06	0.06	0.06
90	0.24	0.18	0.11	0.18
120	0.38	0.29	0.22	0.29
150	0.40	0.35	0.25	0.36
180	0.40	0.40	0.28	0.36
210	0.38	0.35	0.30	0.37
240	0.38	0.35	0.35	0.37
270	0.38	0.35	0.26	0.33
300	0.38	0.36	0.27	0.34

Table 2. Generation of electricity using sewage

Incubation time (hours)	Measurement of voltage in millivolts			
	***Bacillus* sp._1**	***Pseudomonas* sp.**	***Bacillus* sp._3**	***Citrobacter* sp.**
1	280 ±1.1	426±0.7	345±0.9	342±0.6
2	278±0.8	415±0.8	366±0.4	336±0.7
3	295±0.7	385±0.6	352±0.8	337±0.4
4	300±0.3	365±0.7	335±0.4	356±1
5	335 ±0.5	168±0.4	249±0.3	345±0.8
6	280±0.8	169±0.3	271±0.9	323±0.9
7	280±1	172±0.5	254±0.8	354±0.2
8	289±0.6	170±0.3	285±0.7	346±0.3
9	287±0.9	165±0.5	291±0.4	331±0.7
10	286±0.8	167±0.2	297±0.5	323±0.8
11	289±0.7	170±0.4	256±0.6	312±0.6
12	280±0.3	169±0.9	274±0.3	320±0.8

Table 3. Generation of electricity in different medium

Incubation time (hours)	Measurement of voltage in millivolts			
	***Bacillus* sp_1**	***Pseudomonas* sp**	***Bacillus* sp._3**	***Citrobacter* sp.**
Glucose as carbon source				
1	125±0.2	045±0.7	005±0.1	017±0.6
2	225±0.5	281±0.1	045±0.4	116±0.5
3	335±0.3	229±0.3	273±0.3	022±0.2
4	336±0.4	225±0.6	142±0.2	095±0.1
5	248±0.2	165±0.9	024±0.7	165±0.3
Acetate as carbon source				
1	004±0.6	049± 0.1	037±0.4	316±0.3
2	024±0.7	030± 0.5	047±0.62	071±0.6
3	002±0.8	053± 0.4	063±0.5	060± 0.3
4	012±0.5	075± 0.3	075±0.3	043± 0.4
5	025±0.4	055± 0.64	042±0.4	051± 0.3
In soil sediment				
1	046±1.6	045±1.1	067±1.4	047±1.2
2	049±1.8	055±1.4	135±1.3	066±1.4
3	094±1.9	116±1.3	140±1.5	130±1.3
4	122±1.8	124±1.2	156±1.9	170±2.1
5	124±1.4	125±1.5	120±1.3	160±1.2

4. DISCUSSION

Microorganisms are exploited for production of various economically important products at industrial scale. Compared to eukaryotes, their abundant numbers, rapid growth and the ability to utilize wide variety of carbon sources thereby releases energy at rapid rate enabled them to use for various purposes. Major advantage of using microorganisms is, under harsh anaerobic conditions carries out fermentation or anaerobic respiration yielding electrical energy. During respiration, organic matter or biomass are oxidized at the anode producing carbon dioxide, protons and electrons [10]. In ordinary chemical battery (chemical fuel cell) chemicals are used to produce electrons and movements of electrons towards anode produce electricity and power a device. Similarly in microbial cells, by microbial respiration electrons are generated and moves towards cathode hence called as microbial fuel cells (MFCs).

An alternative source for the energy generation is the major focus of research using various strategies. The present study exploits bacterial respiration to generate electricity from different carbon sources. The objective of the study is to isolate a high and stable electricity producing organism from household waste and exploit it for energy generation.

Microorganisms produce electric current by the bacterial decomposition of organic compounds in water and soil. Soil sediment consists of diverse group organisms with stress tolerable ability. So soil sediment was collected near liquid waste discharge area of a house, and four different bacteria were isolated by serially dilution-agar plate technique. By the standard biochemical test procedures, the isolates were identified as *Bacillus* sp._1, *Pseudomonas* sp., *Bacillus* sp._3 and *Citrobacter* sp. According to the report of Anurag Vjay et al., in 2011 [11], bacteria like *Escherichia coli, Pseudomonas methanica, Pseudomonas fluorescence, Pseudomonas putida, Proteus vulgaris, Bacillus subtilis* and many more that lives at anode are able to consume sugars under anaerobic conditions, so the selected organisms were used for electricity generation. The exogenous electron transfer in MFC and presence of related genes were identified in *Geobacter sulfurreducens* and *Shewanella oneidensis*. Produced electrons and protons combine with an oxidant at cathode by which electricity is generated with carbon dioxide and other useful by products.

The Hai Pham et al., in 2010 [12] reported that the abundant presence of organism in the biofilm of MFC. *Pseudomonas* sp., is the predominant member of biofilm, metabolite produced by *Pseudomonas* sp., enabled the establishment of other bacteria. These bacteria can transfer electrons to the electrode via self-produced phenazine-based mediators. A MFC fed with acetate where several *Pseudomonas* sp., enabled the establishment of a Gram-positive bacterium *Brevibacillus* sp. PTH1. These literatures substantiated that the isolates were capable to generate electricity.

Growth profile of these organisms showed that among the four isolates, during 10 hours of growth analysis, all isolates remained in lag phase for 1 h, and then entered into log phase of growth. *Bacillus* sp._1, and *Citrobacter* sp., from 1h to 5h remained in log phase and reached stationary phase of growth. *Pseudomonas* sp., reached its log phase by 3h and showed slow growth, whereas *Bacillus* sp._3, showed fast growth and log phase between 1h to 8 h, found to be fast growing organism. According to the results within 5h of incubation period isolates have reached log phase of growth, so 5h of study planned for energy generation.

Microbial fuel cell works on the capacity of microorganisms to convert carbohydrate substrate into microbe specific by-products and simultaneously producing electrons which help in production of electricity. MFC consists of two different chambers namely anodic chamber and cathodic chamber called as two chamber system. Both the chambers here are partitioned by a salt bridge consisting of salt. From the anodic half consisting of bacteria and their substrates (in anaerobic conditions) electrons flow to cathodic chamber (aerobic conditions) consisting of the electrolytic solution through an external electrical connection maintained by the wires. Microbes present in the anodic chamber oxidize the substrates present in the same to generate electrons through their metabolism consuming the carbohydrates.

Sewage consists of liquid waste with suspended solid having high BOD level with varied group of microbial population. Disposal of sewage is major concern, microorganisms while utilizing wastes present in the sewage could generate electricity and other end products. Under the

study condition all isolates produced maximum electricity between 1 h to 4 h of incubation. Though the facultative anaerobic organism *Citrobacter* sp., produced 356 mV of electricity which was comparatively lower than electricity generated by *Pseudomonas* sp., (maximum 426 mV) but was stable and increasing till stationary phase of growth. The optimum phase of electricity production varies among the species.

In two chamber system, isolates were inoculated along with the native organism in sewage water and generation of electricity under different incubation periods was measured. Of the four isolates, generation of electricity started immediately from 1st hour of incubation and reached its maximum at 2h of incubation by *Pseudomonas* sp., and *Bacillus* sp., then declined but isolate - 4, *Citrobacter* sp., showed continuous production of electricity. A strain *Citrobacter* sp. SX-1 showing high similarity with *Citrobacter* sp. sdy-48 produced electricity from citrate, acetate, glucose, sucrose, glycerol, and lactose in MFCs with the highest current density of 205mA/m generated from citrate. Study also reported by cyclic voltammetry analysis that membrane associated proteins plays an important role in facilitating electron transfer from the bacteria to the electrode. This is the first study that demonstrates the *Citrobacter* species can transfer electrons to extracellular electron acceptors. Also reported that *Citrobacter* strain SX-1 capable of generating electricity from a wide range of substrates in MFCs. While utilizing wide range of substrate transfers extracellular electron electrode, thereby selected as potential organism of MFCs in renewable energy generation and waste treatment [13].

Instead of electricity generation by self, metabolites like biosurfactant and phenazine-1-carboxamide produced by *Pseudomonas* sp., enable other Gram-positive bacteria such as *Brevibacillus* sp. PTH1 to achieve extracellular electron transfer in MFCs. Vanita et al., in 2009 [14] reported from the study using *B. subtilis* that in biofilm these organisms are electrochemically active to produce a maximum of 115 mV of power and the electron transfer mechanism is mainly due to the excreted redox compounds (mediator) in the broth solution and not to the membrane-bound proteins. *Pseudomonas* sp., and *Bacillus* sp., generates energy by secondary process. These bacteria have been grown on simple soluble substrates, such as glucose or acetate that can be directly taken into the cell and used for energy production [15].

Microbial fuel cells have been used to produce electricity from different compounds, including acetate, lactose and glucose. In the present study a simple medium containing glucose, easily consumable medium containing acetate, and complex medium with soil sediment were used. In glucose containing medium, during log phase of growth more power generation was observed. During 3 h and 4 h of incubation *Bacillus* sp._1, showed maximum of 335mV. Acetate poorly supported electricity production in all isolates among which *Citrobacter* sp., generated 316±0.3 mV during 1 h of incubation. Soil sediment was found to be non-supportive for growth of isolates initially, but electricity production was gradually increased and *Citrobacter* sp., generated 160±1.2 mV during 5 h of incubation. It is recommended that a longer incubation period required in complex medium for electricity generation. Yong Yuan et al., in 2008 [15] evaluated the MFC performance in sediment bioremediation and electricity generation under closed-circuit (30, 100, 1,000 Ω) and open-circuit conditions. The study clearly demonstrated that the MFC could be a valid means for in situ sediment bioremediation. Removal of black colour and odour of sediments caused by the rich level of organic matter in sediments was achieved by MFC while stimulating biodegradation of aromatic hydrocarbons with one or two aromatic rings (such as toluene, benzene, and naphthalene) by providing anode as the electron acceptors. As sewage supported growth of isolates and more electricity generation, during treatment of sewage it can be considered for electricity generation to meet the energy demand using *Citrobacter* sp., which showed gradual and stable electricity production in sewage. Liu et al., in 2005 [5] also reported that MFC can be used as a method for simultaneous wastewater treatment and electricity production. Pure cultures have been found to produce both more and less power than the mixed cultures from which they were isolated, depending on the strain and the specific MFCs used [16]. More understanding on its effect with other organisms needs to be analysed.

5. CONCLUSION

Microbial fuel cell technologies are gaining more attention in waste disposal and bioenergy generation sectors. Four different bacteria were

isolated and analysed in different carbon sources for electricity generation. Maximum electricity of 426±0.7 mV at 1 h of incubation was produced by *Pseudomonas* sp., in sewage, *Citrobacter* sp., produced 356±1.0 mV at 4 h of incubation. In soil sediment also similar results were observed. Though *Pseudomonas* sp., and *Bacillus* sp., showed more generation in glucose medium, in complex media *Citrobacter* sp., showed better result and when incubation period was increased production also increased. *Pseudomonas* sp., and *Bacillus* sp., forms biofilm and generates energy as secondary process. It may be beneficial to combine sewage treatment with microbial fuel cells to generate electricity. Use of organism like *Citrobacter* sp., in pure culture produce continuously increasing level of electricity and *Pseudomonas* sp., *Bacillus* sp., like organisms in mixed culture by the formation of biofilm assists in energy generation. Further research on this aspect of large scale treatment opens a way to wealth from waste.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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