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Bioremediation of Dye effluent waste through an optimised Microbial Fuel Cell.

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Abstract

A microbial fuel cell (MFC) or biological fuel cell is a bio-electrochemical system that generates current by using bacteria and mimicking bacterial interactions found in nature. Sewage wastewater collected from different locations and effluents from Textile industries in Mumbai city were screened for generation of electricity using a "Two chambered H-type MFC unit". The present study demonstrated that maximum electricity was generated from effluent of textile industry using MFC, while at the same time accomplishing biological waste treatment of the same. Highest current output was obtained with 10% KCl and 7% agar concentration in salt bridge after running it for 120 hrs. The effects of different cathodic electron acceptors were tested and optimum catholyte obtained was 40mM potassium ferricyanide which showed maximum current production of 0.64 mA. Effect of various sugars was screened and 1 % glucose and 1% sucrose exhibited optimum growth of indigenous flora present in the waste water. Hence 0.5% of molasses in textile dyeing effluent generated maximum current. Scanning electron Microscopy of anode biofilm showed formation of nanowires. Optimised MFC system with Textile Dye industry effluent generated maximum current of 0.768mA with 76.4% of BOD reduction.

Keywords: MFC, salt bridge, molasses, catholyte, nanowires, BOD.

Introduction

Energy crisis in India is increasing every year, as there is continued hike in the prices of fuels and also due to depletion of fossil fuels to a greater extent (Reddy et al., 2007). The need for an alternate fuel has ignited extensive research in identifying a potential, cheap and renewable source for energy production. The building of a sustainable society will require reduction of dependency on fossil fuels and lowering of the amount of pollution that is generated. Waste treatment is an area in which these two goals can be addressed simultaneously. As a result, there has been a paradigm shift recently, from 'disposing of waste to using it' (Chaudhuri and Lovely, 2003). Waste from household, industries and agriculture are ideal candidates of

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substrates for energy generation since they contain high levels of easily degradable organic material which are metabolised by the viable bacterial species present in the wastewater itself and produces electricity during the course.

Three E's -energy security, energy growth, environmental protection, are the national Energy policy drivers of any country of the world. Energy has become an indispensable part of every human life which is compromising the environmental protection. The global warming situation is worsened by the fact that power generation is continuously increasing through the world using fossil fuel. Low reserves of fossil fuels and the environmental impact of their use to produce energy are leading to a search for novel renewable energy technologies (Singh and Songera, 2012). One of the solutions to the above emerging problems is the development of Microbial fuel Cells (MFC's) where, the conversion of complex and impure organic matter into useful energy sources represents an area of immense potential. It has been known for several years that bacteria can be used to generate electricity that can be harvested in Microbial fuel cells. Thus, Microbial fuel cell research is a rapidly evolving field providing solutions to many future related problems (Kiely et al., 2011; Logan and Regan, 2006).

Microbial fuel cell is rapidly growing environmental technology, where microorganisms are used to convert the chemical energy stored in biodegradable materials to direct electric current. This platform technology has been intensively studied and developed in the past decade and it opens up a new interdisciplinary field for and development research which integrates microbiology, electrochemistry, material science, engineering, and many related areas together. MFCs not only provide a unique environment to understand the largely unexplored microbial electrochemistry, they also offer a flexible platform for many different engineering functions to be developed. Thus Microbial fuel cell would serve as a model to demonstrate how fundamental principles of Biology, Chemistry and Physics could be integrated into an attractive solution to resolve fuel and environment related problems.

A number of scientific publications on MFC have increased exponentially. A number of research articles and papers are published in National as well as International journals. The earliest MFC concept was demonstrated by Potter in 1910 (Ieropoulos et al., 2005). Electrical energy was produced from living cultures of Escherichia coli and Saccharomyces by using platinum electrodes (Potter, 1912). This didn't generate much interest until 1980s when it was discovered that current density and the power output could be greatly enhanced by the addition of electron mediators. Typical synthetic exogenous mediators include dyes and metallorganics such as neutral red (NR), methylene blue (MB), thionine, meldola's blue (MelB), 2-hydroxy-1, 4-naphthoquinone (HNQ), and Fe (III) EDTA (Ieropoulos et al., 2005; Park and Zeikus, 2000; Tokuji and Kenji, 2003; Vega and Fernandez, 1987; Allen and Bennetto, 1993). Unfortunately, the toxicity and instability of synthetic mediators limit their applications in MFCs. Some microbes can use naturally occurring compounds

including microbial metabolites (Endogenous mediators) as mediators. Humic acids, anthraquinone, the oxyanions of sulphur (sulphate and thiosulphate) all have the ability to transfer electrons from inside the cell membrane to the anode (Lovley, 1993). In recent years, rapid advances have been made in MFC research and the number of journal publications has increased sharply in the past three years with more researchers joining the research field. Logan et al. (2006) reviewed MFC designs, characterizations and performances. The microbial metabolism in MFCs was reported by many reviewers (Rabaey and Verstraete, 2005; Lovley, 2006) who mainly focused on the promising MFC systems such as Benthic Unattended Generators (BUGs) for powering remotesensing or monitoring devices from the angle of physiologies. Pham et al. microbial (2006)summarized the advantages and disadvantages of MFCs compared to the conventional anaerobic digestion technology for the production of biogas as renewable energy. Chang et al. (2006) discussed both the properties of electrochemically active bacteria used in mediator less MFC and the rate limiting steps in electron transport. Bullen et al. (2006) compiled many experimental results on MFCs reported recently in their review on biofuel cells. A real breakthrough was made when some microbes were found to transfer electrons directly to the anode (Chaudhuri and Lovely, 2003). Cathodes can serve as electron donors as studied in Thiobacillus ferrooxidans suspended in a catholyte for an MFC system that contained microbes in both anodic and cathodic chambers (Prasad et al., 2006).

In this study, a two chambered H - type mediator less microbial fuel cell was set up to maximize current production from Textile Dyeing industry effluent by optimizing various physicochemical parameters, enriching with crude nutrient source and simultaneously facilitated bioremediation.

Materials and Methods

Materials

Customised borosil glass bottles of 1000 mL capacity used as reservoir in the preparation of MFC were acquired from Scientific Apparatus Manufacturing company (SCAM India), Mumbai, India. Graphite rods extracted from exhausted EverReady AA batteries were used as electrodes. Electrical hardware like external resistance, copper wires and Digital multi-meter were brought from a local vendor.

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Voltages for all experiments were recorded on Digital Multi-meter (Haoyue-DT830D). All other chemicals of Analytical grade were obtained from Hi-Media Pvt. Ltd., Mumbai, India.

Substrate collection- Wastewaters

Waste waters which served as the substrate for the MFC were collected from different locations in Mumbai like Tilak Nagar, Bandra creek, Textile Dyeing Industry, Mahim creek and Worli. The natural microbial consortium present in the waste water was used in the study.

After the waste water was collected, it was left undisturbed under static conditions so as to settle the solid particulate contents; 1000 mL supernatant was taken for setting up a batch of MFC.

MFC construction

Anode (Anaerobic)

The anode chamber was a glass bottle of 24 cm in height and 9 cm in diameter having capacity of 1000 mL. It was fitted with a screw cap which had one inlet of 2 mm diameter for holding the graphite electrode. An opening of 2.0 cm in diameter at the right side of the chamber for attachment of the salt bridge.

Cathode (Aerobic)

The cathode chamber was a glass bottle 24 cm in height and 9 cm in diameter having capacity of 1000 mL. It was fitted with a screw cap having two inlets, one of which was 7 mm diameter while the other 2mm diameter-one for holding the graphite electrode and the other for supply of oxygen through air pump in the chamber. A few trails required the use of air pumps. This inlet was sealed off when not in use. An opening of 2.0 cm in diameter at the right side of the chamber was for the attachment of salt bridge.

Salt bridge

The salt bridge glass tube measured 12 cm in length and 2.0 cm in diameter was inserted into side arms of both the bottles.

MFC inoculation and operation

The two glass bottles were autoclaved at 15 psi at 121°C for 20 mins and used for set up. Graphite electrodes were soaked in distilled water for 24 hrs and rinsed with 0.2M Phosphate buffer solution at the time of setting up the fuel cell. The two chambered MFC was constructed appropriately for the batch operation as shown in fig. 1.



Figure 1. Laboratory scale two chambered working Microbial Fuel Cell. Experimental set up- 1: Anode 2: cathode 3: salt bridge 4: copper wires 5: multi-meter

The anodic and cathodic chambers of MFCs were joined by a salt bridge (15 mL) containing 10% KCl and 7% Agar. The two identical cylindrical graphite rods were used as electrodes. The anode chamber was filled with 1000 mL of waster waters collected from various locations in Mumbai while cathodic chamber was filled with 20mM potassium ferricyanide in 0.2M phosphate buffer solution as catholyte mediator. The anode chamber was covered with a layer of paraffin oil to provide anaerobic conditions. The anode and cathode were connected with an external resistance of 500 and the current produced was calculated by measuring the voltage across a resistor every 24 hrs using a digital multi-meter (Zhang et al., 2009).

Effect of varying resistances on MFC performance (Validation of Ohms Law)

The validation of utilisation of Ohms Law as a parameter to estimate current output was studied, here multiple resistors of 10, 120, 220, 330, 470, 820, 1000, 2200, 3300, 8200, 10000 ohm were used and corresponding voltage was recorded (mV) and expressed in current (mA). Anode chamber was filled with 1000 mL of Textile Dyeing industry effluent and cathode with potassium ferricyanide (20mM).

Measurement of Output

The MFC setup was run for duration of 120 hrs and voltage generated measured at an interval of 24hrs. After each of the waste water sample is run in the MFC for 120 hrs, the result of the open circuit voltage recorded are studied and compared. The output of the MFC was recorded in terms of Voltage (mV) and expressed by means of current (mA). For this purpose multi-meters were used and were calibrated each time before use. The current was calculated using Ohms Law (V=IxR) where V is the Voltage across resistance, I is the current generated and R is the external resistance. Resistance of 500 was employed in all experiments and hence calculations were based on it, readings from the multi-meter were noted only after a steady and constant value which took approximately 1 - 2 hrs. (Deval et al., 2014; Muralidharan et al., 2011). Results are expressed as mean values from a triplicate set

Effect of varying concentration of Agar in salt bridge.

Salt bridges were prepared with various concentrations 5-10% with an interval of 1% Agar. The six fuel cells with dye effluent as substrate were set up with above

mentioned varying Agar concentrations in salt bridge. The fuel cells were run for 120 hrs and readings were noted at regular intervals of 24 hrs.

Effect of varying concentration of salt (KCl) in salt bridge.

Salt bridges were prepared with various concentrations 4–14% with an interval of 2% KCl and with agar concentration of 10%. The six fuel cells with dye effluent as substrate were set up with above mentioned varying Salt concentrations in salt bridge. The fuel cells were run for 120 hrs and readings were noted at regular intervals of 24hrs.

Effect of cathodic electron acceptors and varying concentration of potassium ferricyanide on the performance of microbial fuel cell

To explore the effect of different cathodic electron acceptors on MFC performance, diverse catholytes (20mM) like potassium permanganate, potassium dichromate, potassium ferricyanide and potassium chromate. To study the effect of potassium ferricyanide concentration, performance of microbial fuel cell was checked in presence of 10 to 50 mM potassium ferricyanide (Guerrero-Rangel et al., 2010).

Screening of different sugars for the optimum growth of indigenous flora

The suitability of various sugars as growth supportive nutrient for the indigenous flora was determined. To study this effect, different sugars (1%) like lactose, glucose, sucrose, fructose and mannitol were added to M9 medium. The Textile Dyeing industry effluent sample was centrifuged at 3000 rpm for 15 mins. The supernatant discarded and pellet resuspended in sterile phosphate buffered saline and 1 mL of this was added to a side arm flask containing 50 mL of M9 medium containing 1% of respective sugar. Increase in cell number was measured using optical density (O.D_{530nm}) as a parameter at an interval of 24 hrs for 96 hrs to determine the optimum growth supportive sugar.

Effect of varying concentrations of molasses on MFC performance.

Molasses (46.3% sucrose) obtained from cane sugar manufacturing unit from Solapur was procured and used as a co-substrate to enhance MFC performance. To study this effect varying concentration of molasses ranging from 0.01% -1% was used in the anode which constituted of 1000 mL of effluent containing respective concentrations of molasses.

BOD measurement of waste water before and after current generation in MFC.

BOD of the Textile Dyeing industry effluent was measured following Standard methods by APHA (2005).

SEM Analysis of Anode Biofilm

Field emission Gun – Scanning electron microscopy (FEG-SEM) was executed using JSM 7600 F of sample 1 (Test) which was obtained from the anode of MFC run for 120 hrs and sample 2 (control) which was obtained from the anode of a disconnected MFC by coating with platinum using JFC 1600 fine auto coater, at Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of Technology (IIT), Mumbai.

Results and Discussion

Comparative current production using different wastewaters in MFC.

Large volumes of high-strength wastewaters are produced annually from industrial and agriculture operations. Treatment requires lots of energy and resources. Waste water treatment system based on a MFC provides a great opportunity to develop sewage treatment technology without energy input. Sewage water, serving as a rich nutrient medium is a natural source of wide range of microbial communities having a great possibility that it may contain electrigens. (Rezaei et al., 2009; Oh et al., 2009; Velasquez-Orta et al., 2009). Wastewater collected from different locations in Mumbai like Tilak Nagar, Bandra creek, Textile Dyeing Industry, Mahim creek and Worli were screened for current generation by inoculating it in a "Two chambered H-type MFC unit". Based on the

graph pattern for the comparison between the results of the current generated by the 5 samples as shown in fig. 2, Textile effluent produced maximum current of 0.258 mA compared to other sewage samples collected from different locations in Mumbai, which may be due to presence of readily oxidisable substrates in Textile Dyeing Industry Effluent. Sudarsan et al. (2014) has reported a current output of 0.64 mA, 0.54 mA and 0.34 mA from hostel sewage, sugar wastewater and dairy wastewater respectively after 37 days and our study showed a current output of 0.258 mA within a period of 3 days which is more significant. The difference in the current production by different waste waters may be due to the presence of different types of microorganisms that exist in the sample and the rate of electron transfer. The current output given by the mixed microbial consortia present in different waste waters varies due to differences in their nutrient adaptability to handle a broad substrate range and its concentrations present in waste water and their ability to resist stress .This is supported by many previous studies (Holmes et al., 2004; Jung and Regan, 2007; Rabaey et al., 2004; Ki et al., 2008). Waste water sources that have been used in MFCs include domestic wastewater (Lui et al., 2004), swine wastewater (Min et al., 2005), food processing wastewater (Kim et al., 2004), hydrogen fermentation reactor effluent (Oh and Logan, 2005), Paper Industry wastewater (Mathuriya and Sharma, 2009) and corn stover hydrolysates, liquefied corn stover (Zuo et al., 2006) chocolate industry wastewater (Patil et al., 2009). Further studies on the fundamental understanding of types of nutrients required by the microorganisms and physicochemical other parameters like optimum salt bridge conditions, potential catholyte were studied in order to optimize the MFC process for higher current production.





Effect of varying resistances on MFC performance (Validation of Ohms Law)

According to Ohms law, current production is directly affected by the value of external resistance employed, varying the resistance and plotting graph with respect to voltage produced gives an almost linear relationship between both parameters as predicted by ohms law (graph not shown). However considering for over potential losses and internal resistance a nonlinear graph is observed. (Rabaey and Verstraete, 2005) this may be due to the variation seen in bacterial populations, and other physicochemical parameters.

Effect of varying concentration of agar on MFC performance.

Salt bridge is the economic alternative to highly priced proton-exchange membrane in the construction of MFC. By altering the concentration of agar in the fabrication of salt bridge, the performance of doublechambered MFC was observed using Textile Dyeing as the substrate. Agar concentration ranging from 5%-10% was used for the study and optimum concentration was observed to be 7% as it showed

maximum current production of 0.096 mA at voltage of 47.9 mV after 72 hours (fig.3). The current developed shows a comparative hike as the concentration of agar increases from 5% to 7%. This is due to effective proton transfer and the gel being highly polymerized, it prevents the diffusion of oxygen from the cathode chamber to the anode chamber through the salt bridge thus maintaining a better anaerobic environment in the anode chamber encouraging the growth of anaerobic bacteria for increased electron release. (Nair et al., 2013; Kim et al., 2008). There is a decrease in current production from 8% to 10% agar as the extremely polymerised gel prevents the effective movement of protons, increasing the concentration of protons in the anode chamber, reducing the pH, making the anodic environment highly acidic for the microbes to survive. The increased polymerization of agar gel also results in elevating the internal resistance of the set up thus minimizing current production (Kim et al., 2008; APHA, 2005). The observed results are in accordance with Nair et.al. (2013) who reported increase in current with increase in agarose concentration from 7% to 10% while decrease current production at 11 and 12% of agarose.



Figure 3. Effect of varying concentration of agar on MFC performance.

Effect of varying concentration of Salt on MFC performance.

An appropriate concentration of salt is critical since the transfer of protons through the salt bridge is facilitated by the dissociated ions present in it (Rozendal et al., 2006). In the experiment conducted by employing KCl based salt bridge, it is observed that there is an increase in current production from 4% to 10% KCl and optimum results were observed for salt bridge fabricated using 10% KCL resulting in maximum current production of 0.197 mA (fig.4). This further corroborated the fact that the ionic strength of the salt agar bridge affects the performance of MFC.



Figure 4. Effect of varying concentration of Salt on MFC performance.

Increase in salt concentration probably increases the conductivity of the salt agar bridge system by decreasing the internal resistance. Increased salt concentration might have probably assisted in efficient transfer of the protons from the anode chamber to the cathode chamber, thus allowing the circuit to be completed at a faster rate, with maximum current generated at 10% of salt in the salt bridge (Sevda and Sreekrishnan, 2012). The use of salt bridge is advantageous over a membrane as the membrane based MFC needs replacement due to fouling which decreases the period of its use.





Effect of cathodic electron acceptors and varying concentration of potassium ferricyanide on the performance of microbial fuel cell.

Electricity generation in a two-chamber MFC using 20mM of various electron acceptors (potassium permanganate, potassium dichromate, potassium ferricyanide and potassium chromate) was studied and maximum resultant current output was produced using potassium ferricyanide. Further effect of varying

concentrations of potassium ferricyanide was checked and 40mM resulted in maximum current production of 0.64 mA as represented in fig.6. It is necessary to choose an appropriate concentration to improve current generation because changes in concentration of electron acceptor affect the performance of MFC from Nernst equation and the kinetics of redox reaction (Wei et al., 2012). There are three advantages for potassium ferricyanide serving as cathodic electron acceptor in a two chambered microbial fuel cell. Firstly, for the system with potassium ferricyanide, there is no need to use air pumps for continuous sparging with air, which reduces energy consumption. In addition, due to the low price of potassium ferricyanide, it is possible to generate electricity at low cost. Thirdly, reduction products of potassium ferricyanide are environment friendly and have no effect on continuous cathode reaction. Therefore, potassium ferricyanide is a promising cathodic electron acceptor for waste water treatment in MFC (Wei et al., 2012).



Figure 6. Effect of varying concentrations of potassium ferricyanide on the performance of MFC.

Screening of different sugars for the optimum growth of indigenous flora

The suitability of various sugars as growth supportive nutrient for the indigenous flora was determined. To study this effect, different sugars like Glucose, Lactose, Sucrose, Mannitol, Fructose (1% concentration) were screened to determine potential carbon source. Increase in cell number was measured using optical density as a parameter which was checked at an interval of 24 hrs for 96 hrs to determine Potential growth supportive sugars. The results revealed that lactose, glucose and sucrose were the best growth supportive nutrients (fig.7). Based on the sugar requirement of the indigenous flora as concluded from the above experiment, facilitated the selection of a crude substrate as a feed to the electrigen bearing anode to make the MFC more economically viable. Therefore molasses which is a by-product of cane sugar manufacturing units was selected as a crude substrate as it majorly contains glucose and sucrose.





Effect of varying concentrations of molasses on MFC performance

Molasses was used as carbon source for electricity production in the fabricated MFC. Initially there was an increase in the current production with increase in molasses concentration from 0.01% to 0.5% with maximum bioelectricity of 0.768 mA obtained at 0.5% molasses (fig. 8) which is 20% more than the current obtained in MFC run without molasses. Further increase in molasses concentration resulted in decrease in current production. This is attributed to the toxic effects shown by higher concentrations of crude molasses on bacteria. Crude molasses, a by-product of sugar industry, is thus a promising substrate for the bacterial community in a microbial fuel cell, making the microbial fuel cell process cost effective, and providing an alternative method of utilizing waste product as a feed to achieve higher energy yields. Sirinutsomboon et al. (2013) has reported use of 2% crude glycerine as substrate in MFC resulting 0.02 mA. Different kinds of substrate, simple, organic and inorganic waste water in combination with crude substrates can be used in MFCs as electron donors (Habermann and Pommer, 1991; Mokhtaria et al., 2013).



Figure 8. Effect of varying concentration of molasses on performance of microbial fuel cell

BOD measurement of waste water before and after current generation in MFC

All Waste waters used in Microbial Fuel cell were evaluated for reduction in BOD to enumerate the potential of fuel cell to act as wastewater treatment unit. All wastewater samples displayed substantial BOD reduction as shown in table 1 indicating the function of microbes present in wastewaters in metabolizing the carbon source as electron donors. However the BOD of textile effluent showed a reduction of 76.4% when MFC unit was operated for 120 hours. This BOD reduction performance is more significant than the BOD reduction of 74.7% obtained after 28 days of textile waste water reported earlier by Mise and Saware (2016). The decrease in BOD indicates the potential of the microbial fuel cell as a candidature for bioremediation units and also supporting the concept of microbes utilizing the organic waste through microbial oxidation. Thus the combination of waste water treatment along with electricity generation may help in saving money as far as cost of waste water treatment is concerned.

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Sample	BOD before MFC run (mg/L)	BOD after MFC run (mg/L)	BOD Reduction
Dye effluent	6.9	1.4	76.4%
Bandra Creek	4.0	2.1	52.2%
Tilak Nagar	24.5	11.0	55.1%
Worli	2.56	1.28	49.6%
Mahim creek	1.8	1.0	44.4%

Table 1. BOD of various waste water samples before and after MFC run.

SEM Analysis of Anode Biofilm

The surface microstructure and biofilm adhesion on the electrode were studied using SEM and representative micrographs are shown in fig.9. Biofilm formed in the anode of MFC run for 120 hrs using Textile Dyeing industry effluent showed presence of pilus like surface appendages (nano thread like) associated with rod shaped bacteria as well as cocci shaped bacteria, while nano wires were not observed on the surface of the bacteria present in the biofilm formed in the anode of disconnected MFC. The unsuitable culture condition like stress imposed on bacterial community due to applied potential difference may had an important role in the formation of appendages. However further research such as the

formation mechanism and electrical properties of the appendages needs to be investigated. Thus the SEM Analysis provide evidence for development of biofilm on the electrode surface, which is known to initiate substrate oxidation for the release of electric current. Similar phenomenon was observed when Pelotomaculum thermopropionicum was grown in mono cultures on furmarate and in co cultures with Methanothermobacter thernoautotropicus on propionate (Wu et al., 2014). The earlier studies have also reported Geobacter sulfurreducens to be producing highly conductive nanowires and have a potential for long range exo cellular electron transfer across biofilm via intertwined nano wires (Offei et al., 2016).





Figure 9. SEM Micrographs of Anode biofilm, (A) & (B) biofilm formed in the anode of a disconnected MFC using dye effluent, (C) & (D), biofilm formed in the anode of a MFC run for 120 hours using dye effluent.

Conclusion

Microbial fuel cell operated under optimum conditions, using textile effluent resulted in maximum current of 0.64 mA and when supplemented with molasses as substrate, 0.768mA current generation was achieved, which is a 20% increase. The optimal concentration of Agar and Salt for the fabrication of salt bridge was found to be 7% and 10% respectively. Potassium ferricyanide (40mM) was found to be an excellent cathodic electron acceptor for MFC. The study also demonstrated 76.4% BOD reduction of Textile dyeing industry effluent after its complete MFC run for 120 hours. Thus, the present study holds the potential in bioremediation to clean up the environment and add wastewater to the list of new renewable resources of bioenergy. SEM showed biofilm formation initiating oxidation of organic substrate for generation of electricity aided by nanowires and facilitating mediator less functioning of MFC.

Currently limited applications are possible because of low MFC current output. An understanding of the microbiology of the current producing processes is required to enhance the current production. In MFCs where current production is not the major advantage, wastewater treatment or bioremediation using a cathode or anode maybe much more promising application.

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