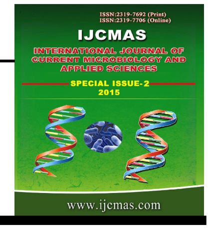

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Original Research Article

Isolation and Characterization of Facultative Anaerobic Bacteria for Generation of Bioelectricity Using Microbial Fuel Cell Setup

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A B S T R A C T

The use of oil and gas, especially fuels, in last decade has accelerated and this triggers energy crisis. But requirement of this basic need is fulfilled by paying high capital price. Taking a glimpse of India we observe many villages which have not seen this basic commodity while many cities suffer power cut. Microbial Fuel Cells (MFC) are devices that use bacterial metabolism to produce an electrical potential from a wide range of organic substrates. The objective of this study was to generate electricity with the help of facultative anaerobic bacteria using Microbial Fuel cell setup. We constructed a two chamber MFC where salt bridge was used to connect the chambers. Samples of cowdung, soil and industrial effluent were obtained from Shivajinagar gavthan, Kas pathar, Satara, and Anuraj sugar industry, Theur respectively. Samples were processed for isolation of facultative anaerobic organisms. Sodium thioglycholate medium was used for this purpose. Isolates were further analyzed for their morphological characters and biochemical tests such as catalase, oxidase, nitrate reduction and oxidative fermentative test. Isolates were tested for generation of bioelectricity, the bacterial isolate IE3 was found best among the organisms which produced 0.23 volts. In consortium all isolates reduce the performance of MFC. MFC was optimized for different parameters such as carbon source, nitrogen source, salt-bridge concentration, pH in anode chamber and buffer concentration in cathode chamber. During optimization, glucose as a carbon source was replaced by 1% sucrose, starch, mannitol and lactose while peptone as a nitrogen source was replaced by 1% potassium nitrate, ammonium sulphate and urea of which lactose and urea showed 0.13V and 0.30V electricity generation respectively. 1M KCl with 3% agar when used in salt bridge and pH 8 showed optimum electricity generation.

Keywords

Bioelectricity, Microbial Fuel Cell, facultative anaerobes

Introduction

Microbial fuel cells are devices which convert the chemical energy to electrical energy by means of electrochemically active bacterial decomposition. Bacteria require energy to survive and the consumption of energy occurs in two steps; oxidation which requires the removal of electrons from source of organic matter and reduction which gives electrons to the final electron acceptor i.e. oxygen. Certain types of bacteria respire by releasing the electrons into the surrounding medium. These released electrons pass through the semi-permeable membrane or agar salt-bridge that connects the two chambers of the Microbial Fuel Cell (MFC). The transferred electrons generate a current and can be used to power devices (Zuwei Du et al., 2007).

An electrochemically active bacterium develops biofilm on the MFC electrode. A biofilm is an extracellular polymeric substance which immobilizes the bacteria by providing greater opportunity for cell to cell contact and communication. The biofilm producing bacteria include; Gram negative bacteria such as *Pseudomonas aeruginosa, Geobacters ulphurreducens* and Gram positive bacteria like *Clostridium acetobutulicum* and *Enterococcus faecium* (Gregory *et al.,* 2004) and many others.

Biofilms have capacity for Extracellular Electron Transfer (EET). For extracellular electron transport, the bio electrochemical systems were taken into account. In this process, electrons are pumped towards the electron acceptor outside the cell using direct or indirect mechanisms. Direct EET occurs via the flow of electrons through the outer membrane proteins by making substantial contact with the anode with the help of pili. In Indirect EET, it involves the exogenous or endogenous soluble molecules called as mediators or redox shuttles, which act to shuttle the electrons through the extracellular aqueous matrix from the cells to the anode (Suzanne *et al.,* 2010).

Various environmental factors affecting microbial growth are temperature, pH, and oxygen.The effect of temperature on MFC performance mainly include 4 aspects; first, within certain range of temperature, the activity of anode microorganisms will increase, second, community structure and distribution proportion of nutrients varies with temperature, third, when temperature is increased by 10° C, the chemical reaction rate would be increased twice and lastly, conductivity of electrolytes differs with temperature.

Construction of MFC

A typical MFC consists of an anode chamber (anaerobic) and a cathode chamber (aerobic) connected by Proton Exchange Membrane (PEM) or agar salt bridge. The two chambers are built in an "H" shape configuration (Figure 1 and 3). In the H-configuration the two chambers are connected by PVC pipes and filled with agar and salt. Power generation can be affected by the surface area of the anode and the surface of the membrane. Using ferric-cyanide as the electron acceptor in the cathode chamber power density can be increased. In the development of MFC identification of an electrode material is vital because it should be durable, conductive and bio-compatible. Carbon electrode, carbon cloth or graphite belt can be the material of choice for electrodes. Gold has been identified as a potential material for the MFC anode development. It is highly conductive and can be vapor deposited. MFC using gold as the anode material gave more reproducible results (Bruce Logan *et al.,* 2006).

Electron Transfer in MFC

The anodic electron transfer mechanism in MFC is a fundamental issue in understanding the theory of how MFCs work. Microbes transfer electrons to the electrode through an electron transport system that either consists of series of components in the bacterial extracellular matrix or together with electron shuttles dissolved in the bulk solution. Electrons generated during microbial oxidation of organic compounds are delivered to the anode via microbial membrane-associated components, soluble electron shuttles or nanowires (Lovely *et al.,* 2004; Vargas *et al.,* 1998). Electrons flow from anode to the cathode trough the external electrical circuit. This electron transfer events completes the circuit.

MFC discovers metabolic potential of microbes for conversion of organic substrate into electricity by transferring electron from cell to circuit. In anodic chamber, oxidation of substrate by respiratory bacteria in the absence of oxygen produces electron and proton that are passed onto terminal electron acceptor. In absence of electron acceptor, some microorganism pass electron onto anode either by electron shuttle or by means of electron shuttling mediators. Direct electron transfer to anode by bacteria requires some physical contact with electrode for current generation. Plunge line-up between bacterium and anode surface involves outer membrane bound cytochromes or putative conductive pili called nanowire. Electron mediators like Mn^{4+} or neutral red incorporated into the anode enhance the performance of MFCs. (Lovely *et al.,* 2004; Vargas *et al.,* 1998; Holmes *et al.,* 2004)

Microbes used in MFC

Many microorganisms possess the ability to transfer the electron derived from the metabolism of organic matter to anode. Marine sediments, soil, wastewater, freshwater sediments and activated sludge are all rich sources for these microorganisms (Table 1).

Types and applications of MFC

MFC can be of four different types, MFC with membrane, MFC without Membrane, Mediator MFC and Mediator less MFC. MFC can be used in various fields such as wastewater treatment, power supply to remote sensor, in BOD sensor and in hydrogen production. These important applications signify research in this area and need of detailed investigations.

Materials and Methods

Sample collection

Samples of cowdung, soil and industrial effluent were collected in sterile polythene bag and sterile glass bottle from Shivajinagar gavthan, Kas pathar (Satara) and Anuraj sugar industry (Theur) respectively.

Isolation and screening of facultative anaerobic bacteria for electricity production

Samples were streaked and sub-cultured on sterile Sodium thioglychollate agar plates (Peptone 15, yeast extract 5, glucose 5.5, NaCl 2.5, sodium acetate 3.0, cystein-HCl 0.5, and sodium thioglychollate 0.5, sodium resazurin 0.001 g/L) for isolation of facultative anaerobic bacteria. Plates were incubated at 37˚C in anaerobic conditions.

Inoculum development for MFC

Inoculum was developed by growing the highest electricity producing isolate in sterile sodium thioglycollatebroth (10 ml) which was incubated at 37˚C. After 24 h the inoculums was adjusted to 0.10 OD with sterile sodium thioglycholate medium and then inoculated in anode chamber.

MFC construction and operation

Anode and cathode chambers with dimensions 15cm × 13cm × 13cm and volume 2.5 L were connected by salt bridge (PVC pipe: 7cm).The H- shape Microbial Fuel Cell was constructed from acrylic sheets (Figure 3). Graphite electrodes were fitted into the lid of anode and cathode chamber.

This entire assembly was surface sterilized with 70% ethanol and was exposed to UV rays for 30 minutes in laminar air flow before inoculation. Multimeter was connected to the anode and cathode electrodes with crocodile clamps to record the readings in volts.

The salt bridge was prepared by using 3% agar and 1M KCl, autoclaved at 15 psi for 15 minutes. Sterile sodium thioglychollate medium was added into anode chamber. Paraffin film was used to seal anode chamber and maintain anaerobic conditions in anode chamber. All operations were carried out at room temperature (23^0C - 37^0C) and at pH 7 for isolates. Readings were recorded after specific time interval. Media was discarded when potential difference observed was below 0.5V.

Optimization of MFC parameters

MFC was optimized by using following parameters to increase potential difference between anode and cathode.

Effect of salt bridge concentration

1M, 2M, 3M, 4M, KCl and NaCl and 3% agar concentration in salt bridge were tested for efficient transfer of ions through salt bridge for high electricity generation. The concentration which shows high conductivity was taken further for optimization.

Effect of carbon sources

Different sugar sources were tested for maximum potential difference by replacing glucose in sodium thioglychollate medium with 1% sucrose, lactose, starch and mannitol.

Effect of nitrogen sources

Three different nitrogen sources namely potassium nitrate, ammonium sulphate, urea were added separately at different concentrations (0.5%, 1%, 1.5%, 2%) by replacing peptone in sodium thioglychollate medium.

Effect of pH

pH of sodium thioglychollate medium was adjusted at different values (5, 6, **7**, and 8) to check the efficacy of microbial fuel cell.

Results and Discussion

In this study we emphasized on bioelectricity generation by facultative anaerobic microorganisms isolated from cowdung, soil and industrial effluent. The isolates positive for oxidative as well as fermentative test were selected for further studies. Selected isolates were then screened for bioelectricity generation. Isolates obtained from soil sample showed generation of highest potential difference. Consortia (mixed culture of all isolates) were also studied for electricity generation using isolates obtained from three samples. The isolate giving highest potential difference in screening was further optimized with different parameters essential for the enhancement of bioelectricity generation. Salt bridge with 3% agar and different molar concentration of NaCl and KCl (1M, 2M, 3M, and 4M) was checked for bioelectricity generation.

Table.1 Microbes used in MFC (Zuwei Du et.al.2007)

Microbes	Substrate
Acetinobacillus succinogens	Glucose
Aeromonas hydrophila	Acetate
Clostridium beijerinckii	Starch, glucose, lactose, maltose
Clostridium butyricium	Starch, glucose, maltose
Desulfovibrio desulfuricans	Sucrose
Erwinia dissolven	Glucose
Escherichia coli	Glucose, sucrose
Klebsiella pneumoniae	Glucose
Proteus mirabilis	Glucose
Pseudomonas aeruginosa	Glucose
Streptococcus lactis	Glucose

Figure.1 Microbial fuel cell with membrane (Bruce Logan *et al.*, 2006)

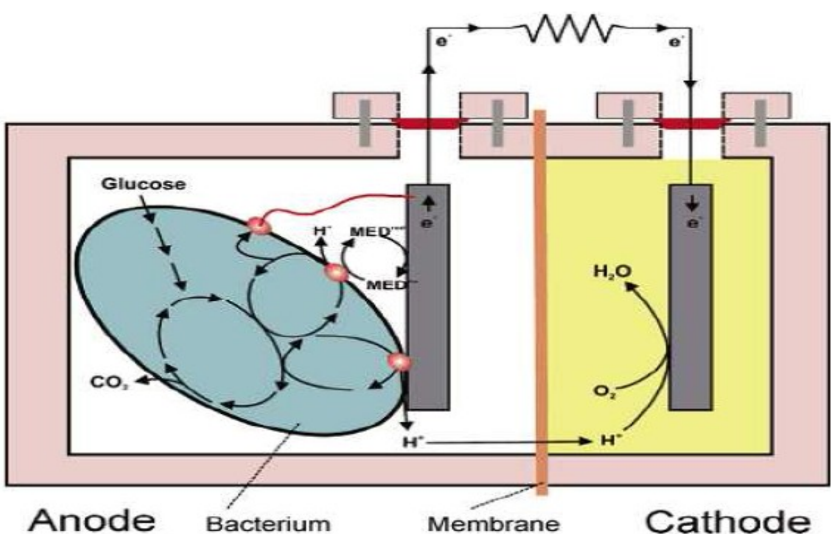


Figure.2 The components which involved in electron transport from cells to the anode in MFC (Lovely *et al.*, 2004)

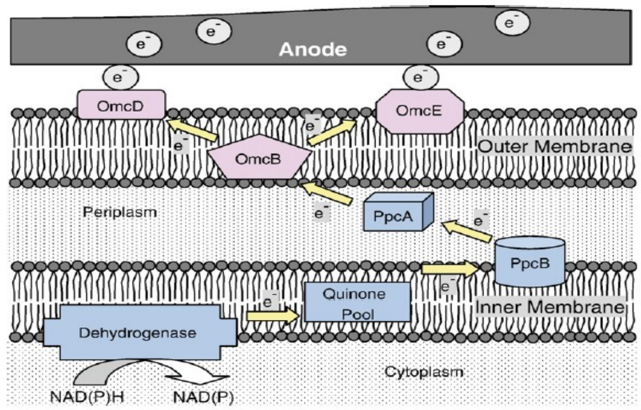


Figure.3 Schematic of microbial fuel cell setup

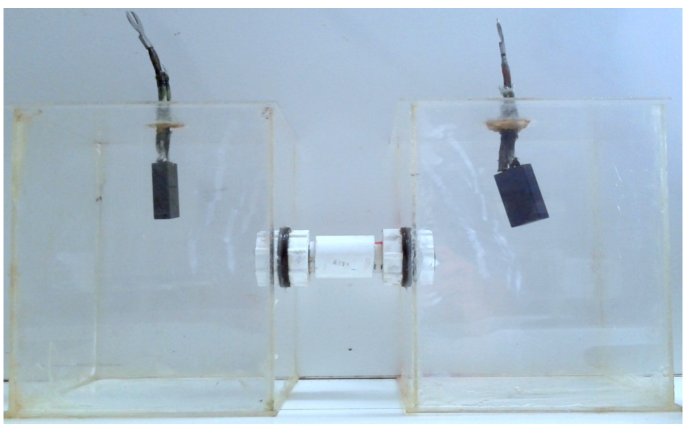


Figure.4 Effect of salt bridge concentration on production of electricity

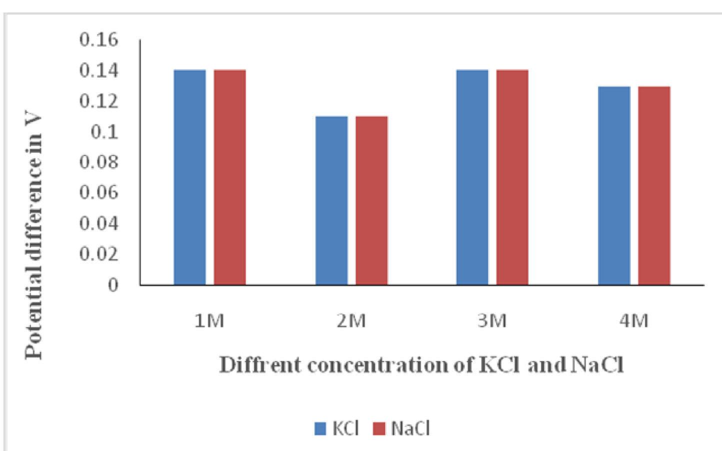


Figure.5 Effect of different carbon sources on production of electricity

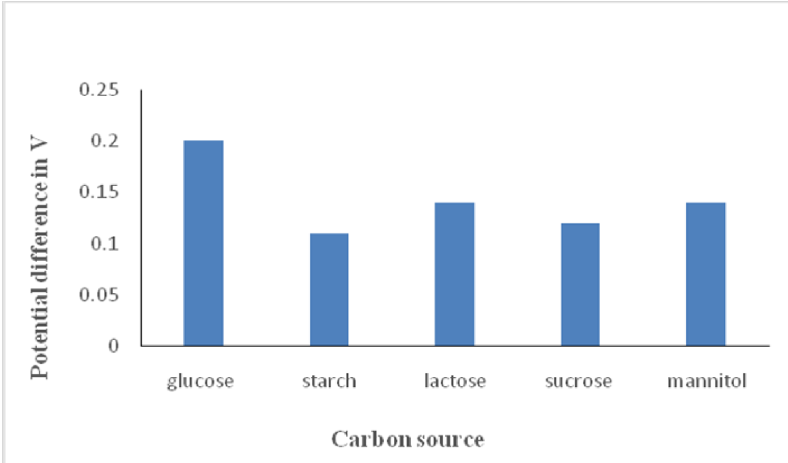


Figure.6 Effect of different nitrogen sources on production of electricity

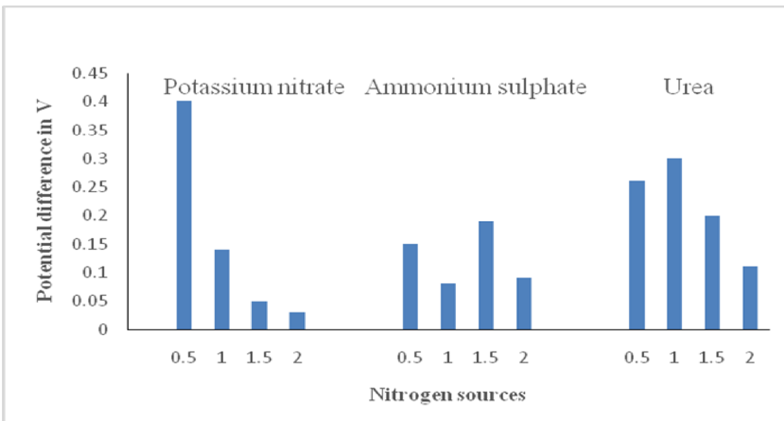
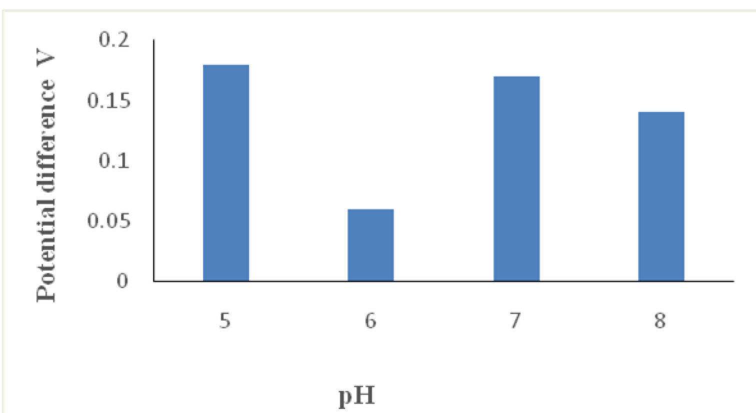


Figure.7 Effect of pH on production of electricity



Among the both 1M KCl gave highest yield of 0.17V while 2M, 3M and 4M KCl and NaCl showed 0.11, 0.14 and 0.13V respectively (Figure 4). Sodium thioglychollate medium was provided with five different carbon sources individually. It was noticed that among the different carbon sources amended into the anolyte, the bioelectricity generation of lactose, starch, sucrose and mannitol was 0.14, 0.11, 0.12 and 0.11 respectively (Figure 5). Glucose produced maximum potential difference whereas starch produced minimum potential difference. Potential difference production was measured for 24h. Similarly for the five different nitrogen sources with different concentration (0.5%, 1%, 1.5%, and 2% Figure 6) amended into the media it was observed that urea yielded maximum bioelectricity of 0.30V at 1% concentration. MFC inoculated with different concentration of potassium nitrate showed lowest potential difference 0.03V at 2% concentration. The MFCs were operated for pH 5, 6, 7 and 8. After 24h, pH 7 showed highest potential difference of 0.18V (Figure 7). The result reported by Shiv et al for lactose as a carbon source and urea as nitrogen source gave maximum yield of 0.626V and 1.575V respectively. In present optimization glucose and urea also showned maximum bioelectricity generation of 0.20V and 0.30V respectively. The reading obtained thus recommend the use of glucose as carbon and urea as nitrogen supplement for isolates in sodium thioglycollate medium.

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