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CHARACTERIZATION OF ELECTRICITY PRODUCING BACTERIA FROM PADDY FIELD SOIL.

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ABSTRACT

In this study soil samples collected from paddy fields of Lonavala. Were screened for bacteria having electricity producing ability. Electron transfer from electrodes to cells has many rewards over indirect electrical stimulation of microbial metabolism through electron shuttles or hydrogen production. From which two isolates (D1&D2) were selected for electricity production. These isolates were identified by morphological and biochemical studies. The identified isolates were *Pseudomonas fluorescens*, and *Klebsiella ozaenae.* These two culture were separately grow on two different media i.e. nutrient medium and Succinate medium and their electricity producing potential were studied in MFC. *Pseudomonas fluorescens* showed more electricity generation in nutrient medium.

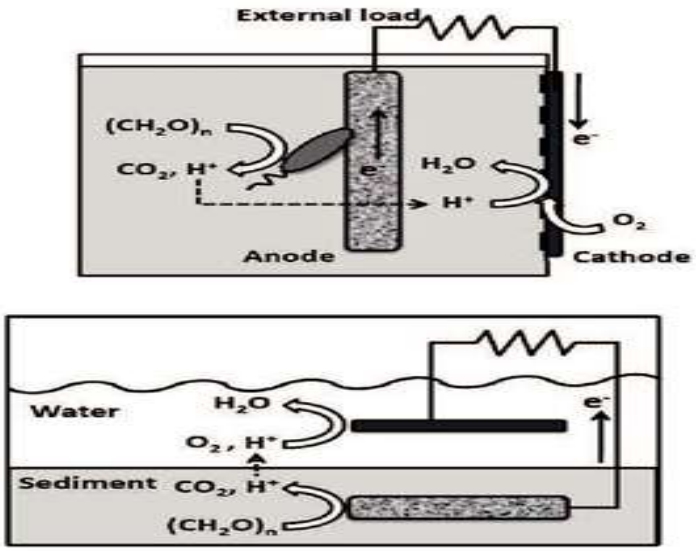
KEYWORDS: Nutrient medium, MFC, bioelectrical production.

INTRODUTION

Bacteria able to transfer electrons to metals are key agents in biogeochemical metal cycling, subsurface bioremediation, and corrosion processes. More recently, these bacteria have gained attention as the transfer of electrons from the cell surface to conductive materials can be used in multiple applications [Enrico Marsili 2008]. The integration of biomolecules with electronic elements to yield functional devices attracts substantial research efforts because of the basic fundamental scientific questions and the potential practical applications of the systems. The research field gained the buzzword ‘‘bioelectronics’’ aimed at highlighting that the world of electronics could be combined with biology and biotechnology [Willner & Hoffmann, 2002]. MFCs are devices that exploit microbial catabolic activities to generate

electricity from a variety of materials, including complex organic waste and renewable biomass. In MFCs, microbes utilize organic compounds as energy and carbon sources. In order to generate energy for growth, organics are decomposed, and chemical energy is released (i.e., fermentation). In addition, high-energy electrons released from organics are transferred to oxidized chemicals (i.e., electron acceptors, such as molecular oxygen) to conserve electrochemical energy (i.e., respiration). In microbial cells, electrons released from organics are initially accepted by intercellular electron-shuttling compounds (e.g., nicotinamide adenine dinucleotide [NAD]), and subsequently transferred to electron acceptors via respiratory electron-transport chains. If a mechanism is present by which electrons released from organics can be transferred from any step in the intercellular electron-transfer pathway to an extracellular electrode (i.e., anode), then microbial oxidation of organics can be coupled to electricity It has been suggested that MFCs have many possible future applications; these include water treatments coupled to energy recoveries, portable fuel cells, biosensors, and in-situ energy sources. Among them, the first one, waste treatments, is considered to be the most promising, and MFCs may be able to work as energy- and cost-saving options for waste treatments (Watanabe 2008).

The MFC technology has however not yet been applied to practical waste treatments. This is primarily because it is an emerging technology and more time is required for technical maturation generation (i.e., an MFC). Recent studies have identified that there exist bacteria that can use self-sustaining extracellular electron transfer mechanisms to respire MFC anodes (Lovley 2008; Watanabe et al. 2009). Some bacteria excrete water-soluble electron-shuttling compounds that are reduced by bacterial cells and oxidized by transferring electrons to MFC anodes (Watanabe et al. 2009). Other bacteria use secreted and/or cell-surface electron-transporting proteins (e.g., cytochromes) for the electron transfer toward MFC anodes (Lovley 2008). An important point is that they have self-sustaining anode-respiring mechanisms without the need of artificial assistance, e.g., the supplementation of MFC with artificial electron.



Schematic diagrams for a single-chamber MFC (A) and sediment MFC (B).

Shuttles. This finding is really important, when one consider the application of MFC to waste treatment. Furthermore, with such bacterial self-sustaining electron-transfer mechanisms, sediment MFC (this includes RPF electricity generation) can also be constructed (Fig. 1B). We consider that deeper understanding of bacterial self-sustaining electron-transfer mechanisms will facilitate more efficient MFCs. (Kazuya Watanabe & Koichi Nishio).

Operated MFCs with bio cathodes at which bacteria catalyze the electron transfer from the cathode to electro positive terminal electron acceptors, such as oxygen or nitrate. This resulted in a complete biological MFC with both bacteria at the anode and cathode and therefore self-replenishing biological catalysts on just electrode materials, such as carbon or graphite the circumvention Besides of expensive metal catalysts, exciting recent work has shown that bio cathodes in photosynthetic MFCs also reduce carbon dioxide (Co2). The aim of the present project is to study bioelectricity generation in paddy plant microbial fuel cells. & objective To evaluate the factors which induce bioelectricity generation by using paddy plant & ground soil of paddy plant microbial fuel cell.

MATERIALS AND METHODS

Collection of soil sample

Samples were collected in sterile polythene bags from paddy fields of Lonavala.

Isolation and identification of Bacteria

Firstly soil sample were serially diluted and streaked on nutrient agar plates and these plates were incubated at 37^0C for 24 hrs. After incubation colonies having different colony characters .and studies on colony morphological and bacterial colony characters.

Maintenance of culture

Bacterial cultures were maintained on sterile nutrient agar slants and stored in a refrigerator.

Detection of current formation

Nutrient broth were prepared to check the efficiency current formation medium was inoculated separately and then incubated. After 24 hrs. incubation time current formation was measured. Using digital Multimeter.

RESULT AND DISSCUSSION

Isolation of bacteria

The soil samples were collected from paddy fields of Lonavala. This soil sample was use as a source of microorganisms the bacteria were isolated from it and preserved for further studies.

Screening of current forming bacteria

After isolation of bacteria screened current forming bacteria from which isolates. During screening of current forming bacteria, it was noted that out of 6 isolates, only 2 isolates were current forming capacity.

In the present study 6 different isolates were obtained from paddy fields of Lonavala. Out of which 2 isolates were found to generate electricity. Research conducted by Sample et.al show bacteria isolates from different sources soil sample collected from agricultural land.

Identification of current forming bacteria

Those organism which showed current producing capacity were used for the identification purpose. With the help of morphological and biochemical characters 2 isolates were identified.

Table no. 1: Colony Characters of Electricity production isolates from paddy soil.

Sr. No	Bacterial Isolates	Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
1	**D1**	1mm	Circular	Pigmented Green	Regular	Flat	Opaque	Moist
2	**D2**	1mm	Circular	Colourless	Regular	Flat	Opaque	Moist

Table no. 2: Gram Nature & Motility.

Sr. No	Bacterial Isolates	Gram Nature	Motility
1	**D1**	Gram negative	Motile
2	**D2**	Gram negative	Motile

Table no. 3 Biochemical Test.

Bacterial Isolates	TEST									
	Glucose	Maltose	Manitols	Lactose	Indole	MR	VP	Citrate	Urease	Lysine
D1	A/G	A/G	-	A/G	-	+	-	+	+	-
D2	A/G	A/G	-	A/G	-	+	-	-	-	-

Table no. 4 Identified Bacteria.

Sr. No	Isolate Code	Identified up to species level
1	**D1**	Pseudomonas fluorescens
2	**D2**	Klebisella ozaenae

With the help of biochemical characterization it has confirmed that the current forming organism are Pseudomonas fluorescens, Klebisella ozaenae.

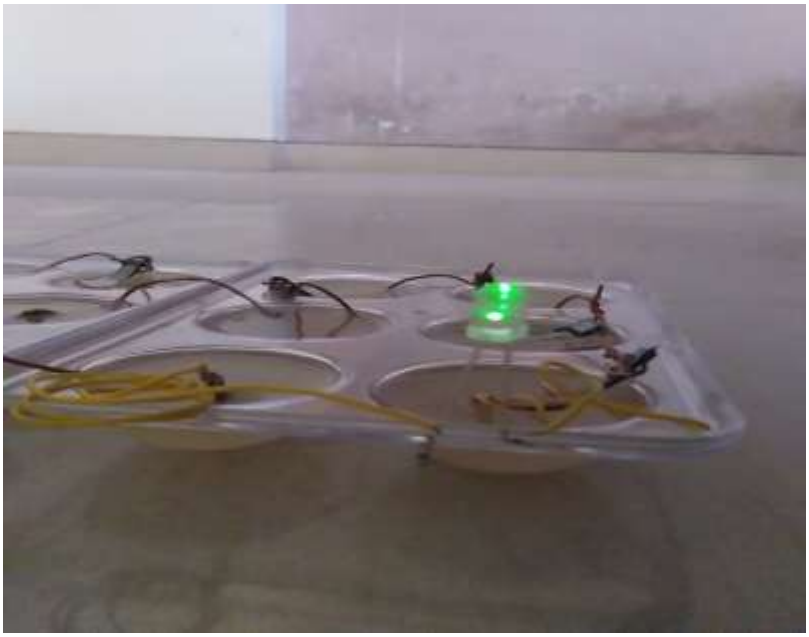
Detection and development of MFC

The isolates organisms where separately incubated in nutrient broth and kept for incubation for 37^0C. During incubation electricity production was maintained every 24 hrs. Electricity is measured by digital multi meter in the units of millivolts. using anode copper (Cu) rods & cathodes aluminum (Al) & LED was burned.

Table no. 5: Detection of Current formation with the bacterial isolates.

Hours	Pseudomonas fluorescens		Klebisella ozaenae	
	Current in nutrient medium (mV)	Current in Succinatemedium (mV)	Current in nutrient medium (mV)	Current in Succinate medium (mV)
0	300mV	290mV	300 mV	290mV
24	666 mV	600mV	650 mV	610mV
48	710 mV	690mV	700 mV	666mV
72	823 mV	800mV	800 mV	750mV
96	764 mV	760mV	760 mV	700mV

It was observed that there was an current formation after every 24 hrs. upto 96 hrs. with both the isolate cultures.



Production of Electricity Showing By LED bulb.

Out of The six isolates D1, D2 were found to produce electricity. The identified isolates were Pseudomonas fluorescens, Klebsiella ozaenae. D1 isolate generates more electricity than the D2 isolates.

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