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Research Paper

Comparative Study of Various Substrates and Microorganisms in a Laboratory Designed Microbial Fuel Cell

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Abstract: *Bio-electrochemical systems have recently emerged as an exciting technology to generate the electricity that is considered as the ladder for the development. The most desirable type of bio-electrochemical system is the microbial fuel cell (MFC), in which power could be generated from electron donors that are present in the form of organic matter, substrates and effluents from the industry. A microbial fuel cell consists of an anode, a cathode and proton exchange membrane (PEM, or more precisely a cation exchange membrane (CEM)). The electrons available through the metabolism of the substrates i. e. electron donors, by microorganisms are transferred to the anode of the fuel cell and then to the cathode through the external circuit, where they reduce the oxidant, consuming protons available from the anode through the membrane. A simple microbial fuel cell was designed in laboratory, using four different substrates (glucose, sucrose, starch and sodium acetate) along with the addition of microbial cultures of bacteria, (Escherichia coli, Bacillus subtilis) and fungi (Saccharomyces cerevisiae), at the bottom of the anode chamber. Electron transport system started in the anode chamber due to microbial activity which was accelerated and availed by the use of the mediator, methylene blue and the transport of protons started from anode to cathode through the membrane that was agar salt bridge in this study. The electricity generated was measured by multimeter device and recorded in milliamperes (mA), which was higher in case of bacteria and E.coli being the most potent generator. Glucose as the substrate generated higher voltage and current in comparison to the other three organic substrates viz. sucrose, starch and acetate.*

Keywords: Agar salt bridge, *Bacillus subtilis, Escherichia coli,* Microbial Fuel Cell, *Saccharomyces cerevisiae.*

Introduction

Continued use of petroleum fuels is widely being recognized as unsustainable because of their depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment, which is a major green house gas. Therefore, renewable, carbon neutral transport fuels are necessary for environmental and economic sustainability and are being considered the need of the hour [1]. A microbial fuel cell (MFC), envisaging a novel form of microbial respiration has recently been discovered, it provides new opportunities for the sustainable production of energy from biodegradable compounds. In other words, it is a bioreactor that converts chemical energy present in the organic compounds (in the form of chemical bonds) to electrical energy through catalytic reactions of microorganisms under controlled conditions. One of the greatest advantages of MFCs over conventional fuel cells like hydrogen and methanol fuel cell is that a diverse range of organic materials can be used as substrates or fuels. In this the microbes, specifically the bacteria, need to establish an electrical link with these insoluble electron donors or acceptors [2,3]. A simplest MFC consist of an anode and a cathode separated by a cation specific membrane. In the anode compartment microorganisms oxidize fuel (substrate) generating electrons and protons, these protons and electrons are transferred to the cathode compartment, the protons pass through the cationic membrane, whereas, the electrons move through the external circuit, thereby generating electric current. They are consumed reducing an oxidant at the cathode, usually oxygen which is supplied by aeration.

Many commonly occurring and easy to culture microorganisms can contribute to electricity production in microbial fuel cell. Recently, researchers have reported a range of electricity producing microorganisms that can use a wide variety of organic compounds and can effectively convert them into electricity in self sustaining microbial fuel cells [4,5]. These organisms, known as electricigens, can completely oxidize organic compounds to carbon dioxide, with an electrode serving as the sole electron acceptor, and thereby generating energy in the form of electricity from this electron transfer [1,6].

In a typical MFC, an anodic electrode potential is developed when the electrons from the oxidation of substrates by microorganisms are available to the electrode. Electrons cannot be transferred from the normal microbial electron transport systems to the electrode due to the nonconductive nature of the cell surface structures. Thus electrochemical mediators like methylene blue, potassium ferricyanide are employed to render electron transfer from inside of the microbial cells to the electrode [2].

Working principle

Microbial fuel cells explore metabolic potential of microbes for conversion of energy present in organic substrates into electrical energy by transferring electrons from cell to an external circuit [3]. Microorganisms can transfer electrons to the anode in three different ways, firstly, using exogenous mediators (those present outside the cell) such as potassium ferricyanide, thionine, methylene blue or neutral red. Secondly, using mediators produced by the bacteria and lastly by direct transfer of electrons from the respiratory chain enzymes i.e. cytochromes, to the outer cell membrane, which in turn is reduced and then leaving it in a reduced state to shuttle the electrons to the electrode [5,6]. Electrons donated to the anode pass through a resistor or other type of electrical device to the cathode. The cathode may be exposed to the air or submerged in aerobic water. Protons released from oxidation of the organic matter migrate to the cathode, often through the cation membrane that also limits diffusion of oxygen into the anode chamber. Electrons, protons and oxygen combine at the cathode surface to form water [7].

In mediated electron transfer machinery, microbes produce/acquire indigenous soluble redox compounds (quinones and flavins) or synthetic exogenous mediators (dyes) to shuttle electrons between terminal respiratory enzyme and anode surface [8, 9]. These mediators can divert electrons from respiratory chain by entering outer cell membrane, becoming reduced, and then leaving in a reduced state to shuttle electrons to the electrode [10]. Number of electrons and protons fabricated depends upon the substrate being utilized by microbes. Electrode reactions in a MFC compartments are as follows:

i) If glucose is used as substrate

Anodic reaction:
$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+$
Cathodic reaction:
$12O_2 + 24e^- + 24H^+ \rightarrow 24H_2O$
ii) If sucrose is used as substrate
Anodic reaction:
$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$

Cathodic reaction:
$12O_2 + 48e^- + 48H^+ \rightarrow 24H_2O$
iii) If acetate is used as substrate

Anodic reaction:
$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$
Cathodic reaction:
$4O_2 + 8e^- + 8H^+ \rightarrow 4H2O$

The experiments conducted in this study serve as key steps towards the goal of designing and developing a MFC that would be based on transducing the power of oxidative metabolism using microbial cells into electrical energy. In the first phase of the study, the function and current output of a MFC was investigated by using different substrates like glucose, sucrose, acetate and starch with two strains of bacteria *E. coli* and *B. subtilis* and the yeast, (*S. cerevisiae*). In the second phase, investigation was carried out for analysing the comparative power generation and power density by these substrates. The study also intended to find out the potential use of agar salt bridge as cation exchange membrane.

Material and Methods

The simplest type of MFC consists of two chambers separated by a material that conducts protons between the chambers but not the electrons. A simulation of this type of MFC was designed in the lab using two plastic bottles connected by a tube, containing a separator which is usually a cation exchange membrane (CEM), but in this study it was an agar salt bridge that was made by using again a plastic tube filled with 10% agar in 2M NaCl to serve the same function as a cation exchange membrane (Figure 1). It was prepared by first dissolving 11.6gm NaCl in 100 ml of Double Distilled Water (DDW) and then using this solvent to make 10 % agar (10 gm of agar agar in 100 ml of the solvent, first the solvent was heated and then the agar powder was added gradually while the heating continued until a clear suspension was obtained). Agar was poured hot in the tube which was sealed from both ends, once agar solidified on cooling, the seals were removed.

The stock solutions (SS) were prepared to accommodate different microorganism and different substrate at different times for the use in the experiments, in the otherwise common environment of buffers and mediators. The SS for anode chamber contained 500 ml of 100mM phosphate buffer (monobasic 6.5gm and dibasic 8.7gm in 500 ml DDW), 500 ml of DDW and 5ml of 100μM mediator (0.16mg methylene blue in 5ml DDW), to which different substrates (at final concentration of 20gm/lt) were added along with the 100ml of bacterial suspension prepared in 50mM phosphate buffer (pH 7.0) [5].

Microbial Cultures used in the study were obtained as stock cultures from competent authorities and were first grown for 48 hrs in a basal medium (composition, 10 gm of glucose per liter, 5 gm of yeast extract per liter, 11.6 gm of $NaH_2PO_4.2H_2O$ per liter, 10 g of $NaHCO_3$ per liter, sterilized at 15 PSI for 15 minutes) at 37 ºC [5,11]. Once the culture was grown at maximum, which was observed as high turbidity in the medium and an optical density (OD) between 0.5-0.6 measured by colorimeter, resting cell suspensions were obtained by centrifugation at 3000 rpm for 15 minutes at 4ºC. Resting cells were resuspended in 100 ml of 50mM phosphate buffer (pH 7.0) for their later inoculation in the anodic compartment. All these operations and processes were done under strict aseptic conditions to prevent any contamination and maintain the absolute purity of the cultures[12]. The cathode compartment was filled with 500ml of 100mM phosphate buffer and 500ml of 50mM potassium ferricyanide solution (8.2gm in 500 ml of DW) *i.e*. catalyst in order to accelerate the reduction of oxygen in water[11,12].

The key to this design is to choose a bridge that allows protons to pass between the chambers, but optimally not the substrate or electron acceptor (typically oxygen) in the cathode chamber. In the study MFC was operated in batch mode carrying different substrate with different microorganism at a time, employing nickel plate electrode 15 cm X 1 cm in size for generation of power and to facilitate the proper electron and proton transfer with its large size. The electrodes were connected by copper wire with all surfaces coated with a non-conductive epoxy [11]. Four organic substrates that were used in the study included glucose, sucrose, starch and sodium salt of acetate. The power was calculated by using external resistance of 100Ω.

Calculation

Voltage (V) was recorded and used to calculate the power (P) and current (I) [13]:

For calculation of power density

$P = V^2/vR$

For calculation of current

$I = V/R$

For calculation of power

$p = VI$

Where,

P - Power density

p - Power

V - Voltage

v - Volume of Anode chamber

R - Resistant

I - current

Results and Discussion

Electricity in the two chambered MFC was generated using four different organic substrates namely, glucose, sucrose, starch and acetate along with inoculation of three species of microorganisms namely, *E .coli*, *B. subtilis* and *S. cerevisiae* in anode chamber (Table 1). The average voltage among the batches varied between 70 millivolts (mV) to 307 mV, depending upon the microorganism and substrate used in the anode chamber (Figure 2).

Figure 1: **Experimental design of MFC**

Table 1

Average voltage, current and power density produced by organic substrates through activity of three microbial species

Microorganisms	Substrate	Average Voltage (mV)	Average Current (mA)	Average Power Density (mW/cm^3)
Escherichia coli	Glucose	307	2.05	1.14
	Sucrose	248	1.57	0.65
	Starch	203	1.11	0.43
	Acetate	187	1.49	0.33
Bacillus subtilis	Glucose	298	2.14	1.07
	Sucrose	207	1.01	0.52
	Starch	170	0.70	0.38

	Acetate	166	0.72	0.37
Saccharomyces cerevisiae	Glucose	183	0.65	0.37
	Sucrose	170	0.64	0.32
	Starch	125	0.24	0.17
	Acetate	70	NIL	NIL

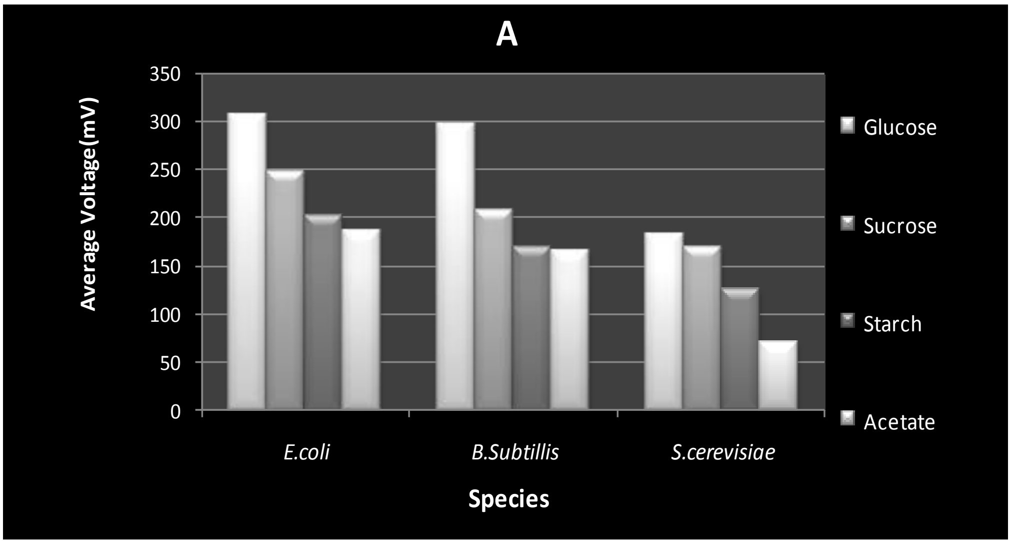

Figure 2: Comparative analysis of Average Voltage generated by organic substrates through metabolic activity of three microbial species

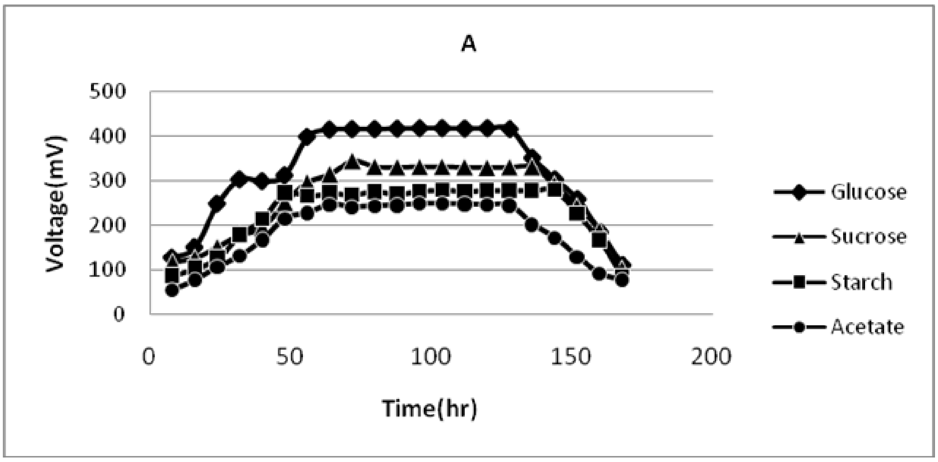

Figure 3: **Voltage (mV) generated by *E.coli* with time (hr)**

These results followed the same trends reported earlier by researchers, where individual microbes in conjugation with a known substrate, mostly sugars, were used in indigenous MFC. One study reported the production of a power density of 0.37 mw/cm^3 along with an average current of 1.25 mA and average voltage of 300 mV with the substrate glucose and mediator thionine, employing *Proteus vulgaris* in nafiane membrane MFC [16]. Similarly, in another study *E. coli* produced high power density of 3.06 mw/cm^3 along with a higher average current of 4.5 mA and a very high average voltage of 680 mV with the substrate glucose and neutral red as mediator in nafiane membrane MFC [9]. Similar results were obtained by several other workers, who have reported the electricity generation from a variety of microorganisms like *Shewanella putrefaciens*, *Geobacter sulfurreducens, Lactobacillus plantarum*, *Streptococcus lactis* and *Erwinia dissolvens*, employing several different nutritional sources [4, 5, 17, 18].

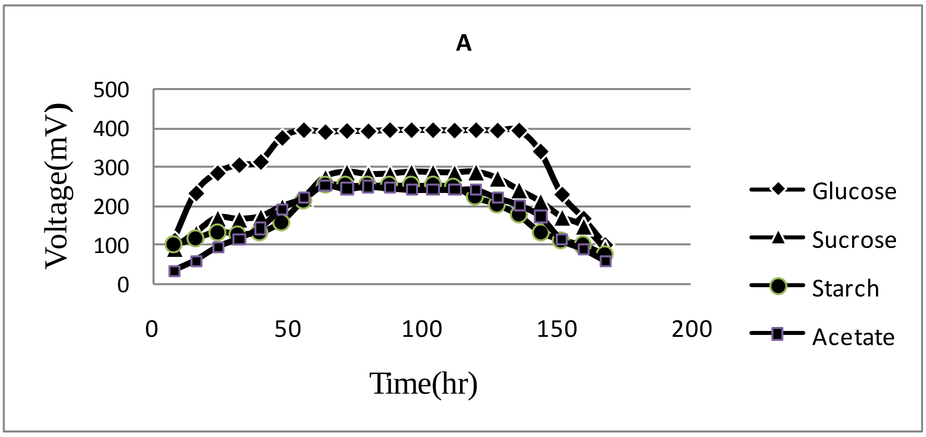

Figure 4: **(A) Voltage (mV) generated by *B.subtillis* with time (hr)**

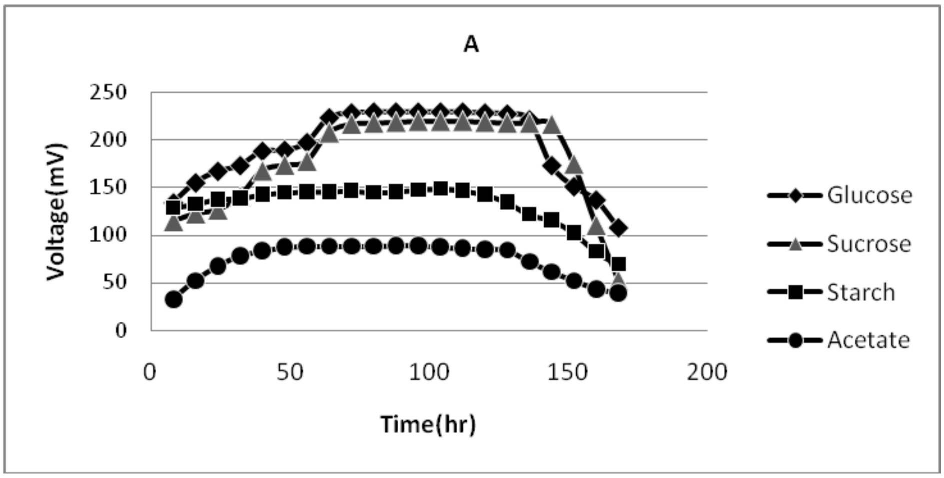

Figure 5: **(A) Voltage (mV) generated by *S.cerevisae* with time (hr)**

The lower current production in the study indicates that some electrons are consumed by mechanisms other than expected cathode reactions and use of salt bridge as proton exchange membrane [19, 20]. It is plausible that under the conditions of limiting electron disposal through the circuit with a high resistance, the electrons are consumed in the anode to reduce other electron acceptors such as sulphate and nitrate or the oxygen diffused from cathode compartment or dissolved oxygen present in the influent, or methane production in the anode compartment [21, 22].

The use of *S. cerevisiae* in MFC has been very meagre and sporadic and very few reports are there to support it or to claim it as an electricigen like bacteria [23, 24], further research employing variety of other substrates, mediators and PEMs is required to be done in this connection.

Unlike PEM which are prone to fowling and need timely replacements, the agar salt bridge is the most economical and viable component in the MFC, but the disadvantage is its high internal resistance and the maximum power output can be achieved when the internal resistance is equal to the external resistance [25]. Several workers have reported the feasibility of salt bridge based MFC with the production of power density and current in the same range employing sugars present in an effluent alone or an effluent blended with some substrates like glucose or lactose [24, 26]. In this study the agar salt bridge at 2M salt concentration in conjugation with glucose as the substrate gave the higher average current with *B. subtilis* and *E. coli*. Several studies have been done to optimise the salt concentration in the MFC with a salt bridge set up and these have revealed that, with the increase in molar concentration the average current decreases [27, 28]. Molar concentration of salt is critical since the transfer of protons through the salt bridge is facilitated by the dissociated ions in it. Cost reduction and improved yield are the key factors to the successful commercial use of MFC.

Further, to address the effluent problem in an economically viable and feasible manner, researchers have been trying to generate electricity by employing selected microbes in waste water treatment plants. There are interesting studies and exciting reports that can serve dual purpose of environment cleansing along with energy generation [7, 14, 29, 30], once adopted it can go a long way in meeting the demand of green energy in a sustainable and viable way.

Conclusion

In the present times where generation and consumption of energy is the most significant deriving fact to access the development quotient of any economy and its state of affairs, the generation of energy through renewable and ecofriendly means, can contribute all that is required for the sustainance. The energy generation through MFCs largely relies upon renewable organic sources and the easily culturable microbes that mimic the bioelectrochemical systems. This experimental setup of MFC with agar salt bridge working as cationic membrane, where four different organic sources and three commonly available microorganisms were used, showed promising and exciting results, although the power and voltage generation was lower in comparision to membrane based MFCs. The maximum power and voltage generation was observed in case of *E.coli* with glucose as substrate whereas the lowest was recorded in case of *S. cerevisiae* with starch as the substrate. Glucose as a substrate provided higher power and voltage output in comparision to starch, sucrose and acetate. The MFCs have recently emerged as an exciting and promising technology which can cater to the needs of modern world that is constantly looking towards such replenishable and safe energy generators.

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