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Full Length Research Paper

Studies on the optimization of bioelectricity production from industrial and domestic waste using immobilization of microbial cells

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A unique type of fuel cell with a potential in long-term is the bio fuel. The data obtained from the studies undertaken reflect glucose yielding the peak voltage. However, sucrose is cheap, similar to glucose in the electricity production pattern. Using glucose in anaerobic culture conditions, the microbial fuel cell produced a peak voltage of 1.35 by organisms *Saccharomyces cerevisiae* but different current obtained were 1.9 and 2.5 mA respectively, and the catalyst was shown to affect the duration of the peak but not the peak voltage, ultimately, providing new insights for industrial waste recycling for an ongoing pursuit of the most productive microbial fuel cells.

Key words: Bioelectricity, biofuel, *Saccharomyces cerevisiae*, microbial fuel cell (MFC), molasses, waste recycling.

INTRODUCTION

A unique type of fuel cell holding a promise in the long-term is the bio fuel cell (Larminine et al., 2003; Liu et al., 2004; Lu et al., 2009; Min et al., 2005). Further work on cattle waste substrate for successful production of bioelectricity by MFCs has been reported (Zheng et al., 2010). In fact, the conversion of carbohydrate to hydrogen is achieved by a multienzyme complex system. In bacteria, it has been reported to involve the conversion of glucose to 2 mol of NADH, a reduced form of coenzyme 1, namely: b-nicotinamide adenine dinucleotide of the vitamin niacin, and 2 mol of pyruvate formed by Embden–Meyerhof pathway (Palmore et al., 1998). The immobilized microbial cells have been noticed to continuously produce H_2 under anaerobic conditions for a series of weeks, whereas non-immobilized bacterial cells were shown to be completely deactivated within less than two days. The electro-active groups responsible for the redox activity of enzymes present in the microbial cells were observed to be buried inside their prosthetic groups leading to poor electrical communication between the cells and the electrode surface (Willner et al., 1996). Low molecular weight redox species may probably assist the shuttling of electrons between the intracellular bacterial space and an electrode, referred to as mediators probably meeting the following requirements: (a) The oxidized mediator should easily penetrate through the bacterial membrane to reach the reductive species inside the bacteria, and the redox potential of the mediator should match the potential of the reductive metabolite; (b) The reduced mediator should easily escape from the cell through the bacterial membrane; (c) Both the oxidized and reduced states of the mediator should be chemically stable in the electrolyte solution (Willner et al., 1996). Microbes can be exploited to donate electrons to an electron mediator or electrode, and produce electricity (Cooney et al., 1996). Therefore, the present studies were undertaken to optimize the bioelectricity production from immobilized microbial fuel cells from certain number of microorganisms at defined

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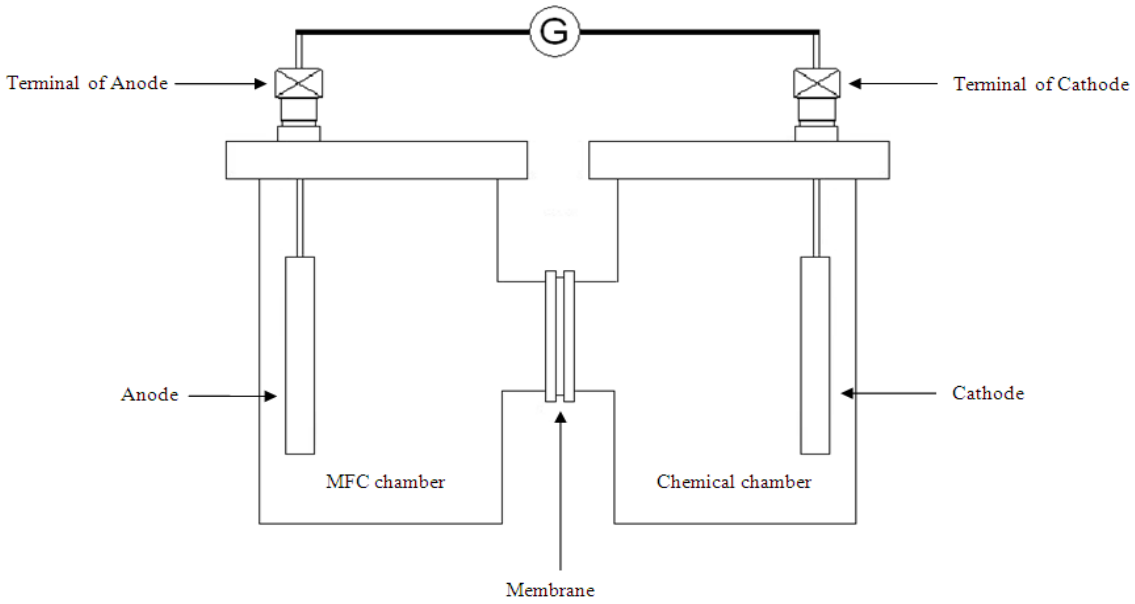


Figure 1. Schematic diagram of MFC fabricated chamber system with two chambers of 5.5 × 24 cm, anodic chamber containing MFC is interconnected with membrane (Nitrocellulose/Poly-vinyl Chloride) to cathodic chamber containing chemicals. The galvanometer (G) connected shows the deflection when the bioelectricity is produced after 24 h of incubation.

growth conditions.

MATERIALS AND METHODS

Sample preparation

The sample was maintained in anaerobic conditions so as to settle down the solid particulate contents, and aspirated for the analytical purposes. The sample used for bioelectricity production was prepared at the laboratory scale incorporating two different sugar sources, principally, glucose and sucrose. Sample preparation from domestic waste and industrial waste was differentially treated, made in duplicate and designated as given as: Sample (A): Plain diluted waste water without any treatment; Sample (B): 10% glucose solution of plain diluted waste water (Sample A); Sample (C): 10% glucose and 0.5% methylene blue solution of plain diluted waste water (Sample A); Sample (D): 10% sucrose solution of plain diluted waste water (Sample A); Sample (E): 10% sucrose and 0.5% methylene blue solution of plain diluted waste water (Sample A).

Chemicals and microorganisms

The wastewater samples were collected from the industrial areas of Moradabad, U.P. and India. In the present study all the chemicals used were of analytical grade and procured from Sigma-Aldrich Co., and microorganisms, namely: *Bacillus subtitles (MTCC-121),Clostridium acetobutylicum* (MTCC-481), *Escherichia coli* (MTCC-2939), *Sacharomyces aureus* (MTCC-96), *Saccharomyces cerevisiae* (MTCC-178), and *Proteus vulgaris* (MTCC-742) were grown, sub-cultured, maintained and inoculated in to substrate mediated culture medium, and then after revived in nutrient agar medium, followed by their transfer into anode chamber of MFC for study pertaining to optimization of electricity production.

S. aureus and *S. cerevisiae* were grown aerobically in a defined medium followed by an appropriate inoculation, and the samples were incubated for 72 h at 28°C. The samples were centrifuged for 5 min at 5000 ×g in a cooling centrifuge (Mehmet et al., 2010), the pellets thus obtained were weighed and processed for fatty acid analysis using GC Chromatography (Gehin et al., 1995). *Proteus vulgaris* was grown in aerobic conditions in defined medium with incubation at 37°C for 12 h (Korsten et al., 1996).

Substrate

Glucose rich molasses and sucrose rich domestic waste water were used as fermentation substrates in reaction mixture medium in the present study. In addition, four specific mediators namely, methylene blue, crystal violet, Commassie brilliant blue, and cresol red were incorporated with the 5 mM concentration. Three sets of experiments with triplicates in each set \were designed for optimization of voltage and current. Graphite and charcoal electrodes were used and Salt Bridge with either Graphite electrode or Carbon cloth was interconnected but separated through semi permeable membrane of nitrocellulose and poly-vinyl chloride membrane. The anode chamber was filled with MFCs and cathode chamber is filled with 4.0 M potassium Ferro cyanide $K_4Fe(CN)_6$ contained in 30 mM Tris buffer (pH 7). The chambers were constantly stirred at 1600 rpm.

MFC construction and operation

In the present study, MFC was principally designed with the aid of electrodes concomitant with two-chamber glass vessel interconnected with nitrocellulose membrane/poly vinyl chloride membrane/ salt bridge/ cotton cloth under define variable experimental conditions. Figure 1 shows the schematic MFC fabricated chamber designed configuration. Six MFCs were constructed with 1000 ml teflon bottles. The bottles were joined by

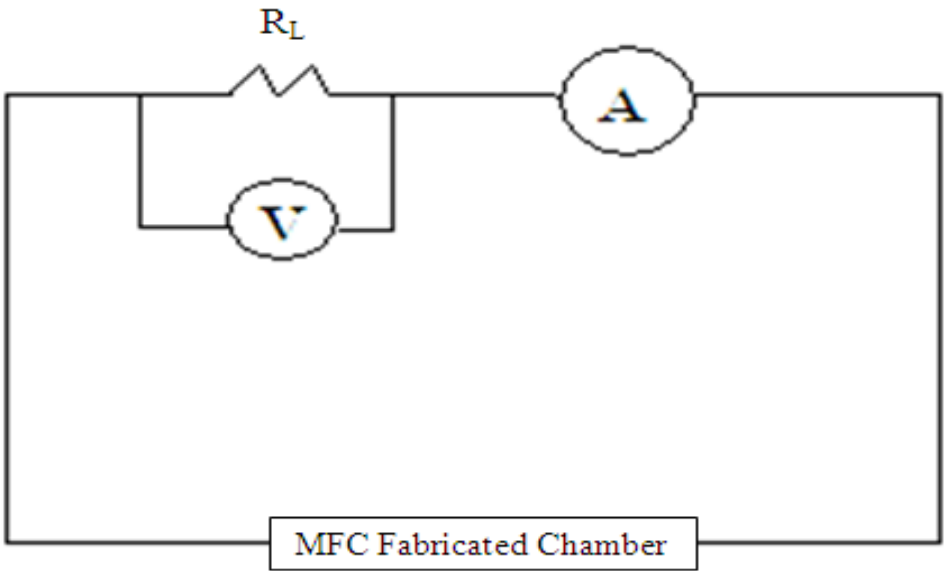


Figure 2. An ammeter and a rheostat are connected in series and a voltmeter is connected in parallel with battery. Here, R_L= Load Resistance (1 KΩ), V = Voltmeter; A = Ammeter.

a glass bridge containing a proton exchange membranes, namely Nitrocellulose and Polyvinyl Chloride held by a clamp between the flattened ends of the two glass tubes fitted with rubber gaskets. Electrodes were cleaned with 1.0 N NaOH followed by 1.0 N HCl. After each experiment it was stored in sterile distilled water before use. Water tight electrical connections were made. The liquid volume in each chamber was approximately 500 and 100 ml with a headspace of about 200 and 440 ml. In addition, the flushed for maintaining the anaerobic condition were used. In the fuel cell, the cathode chamber contained 30 mM Tris buffer (pH-7), which was continuously flushed with sterile, water-saturated air. For determination of power output a variable resistance (0.1 to 3.0 Ω) was used as external load. All experiments were carried out at a temperature of 37°C. The current and voltage were measured with Digital Multimeter.

Current and voltage measurements

After the deflection observed in the galvanometer (Figure 1), a load resistance of 1 KΩ was employed in parallel across the electrodes shown in Figure 2. Voltage and current were recorded by data acquisition device of Keithley millimeter. Data were recorded as varied on three consecutive days by three times for a single set of experiment and total nine set of trials were monitored for current and voltage data analysis.

RESULTS AND DISCUSSION

Development of MFCs is going on around the whole world, because of its significant characteristic of recycling the pollutants and on the other hand, producing the desirable amount of electricity. Therefore, working on the development of MFCs was one of the aims to join the path of developing MFCs amongst the others. In the process of developing a MFCs there are many factors, which can be considered and kept in mind so that a better MFCs can be developed. One should have the understanding of various factors related to the metabolic activity of various microorganisms. Utilization of the specific substrate by a microorganism, its activity towards the electrodes and other various factors solely depend upon the overall process being involved in. Developing of MFCs requires a thorough knowledge of electrochemistry (Yahiro et al., 1964). In general, an MFC functions same as a fuel cell acts for, and is developed on its fuel cell based principle, the difference here is, that the reactions are catalyzed by the microorganism and the fuel is, the growth medium and the substrate. The substrate being used can generally be any good source of carbohydrate (sucrose, glucose, sugarcane juice, molasses etc.) (Chaudhuri et al., 2003). The choice of substrate can be made by the process being involved (waste water utilization, type of microorganism, etc.) Generally glucose proves to be the best substrate. The data shown in the Figure 3 provided the platform in view of further extension of the studies pertaining to the electric evolution as a consequence of application of various biological strain sources. The comprehensive account is provided graphically in Figure 4. The data shown in the Figure 4 are obtained after two months experimentation, in accordance with the objectives mentioned. It was ascertained that no contamination in the MFCs affected the results obtained. However Tables 1 and 2 provides a comprehensive account of strain, membrane, substrate, electrode, mediator, voltage, and current bioelectricity production using various microorganisms as listed. The values observed are for three consecutive days by three times for a single set of experiment and total nine sets of trials were monitored for current and voltage data analysis.

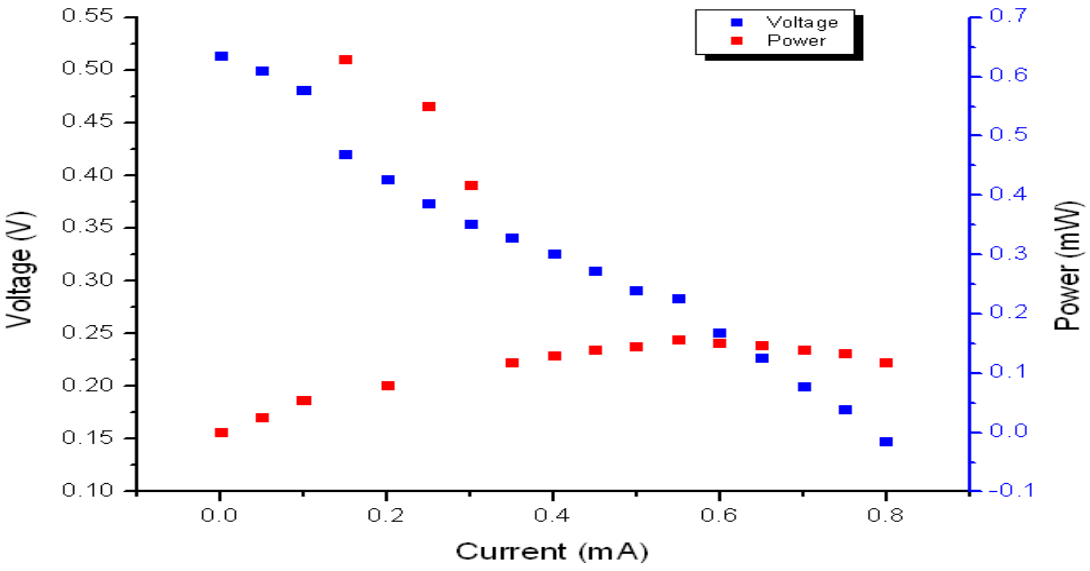
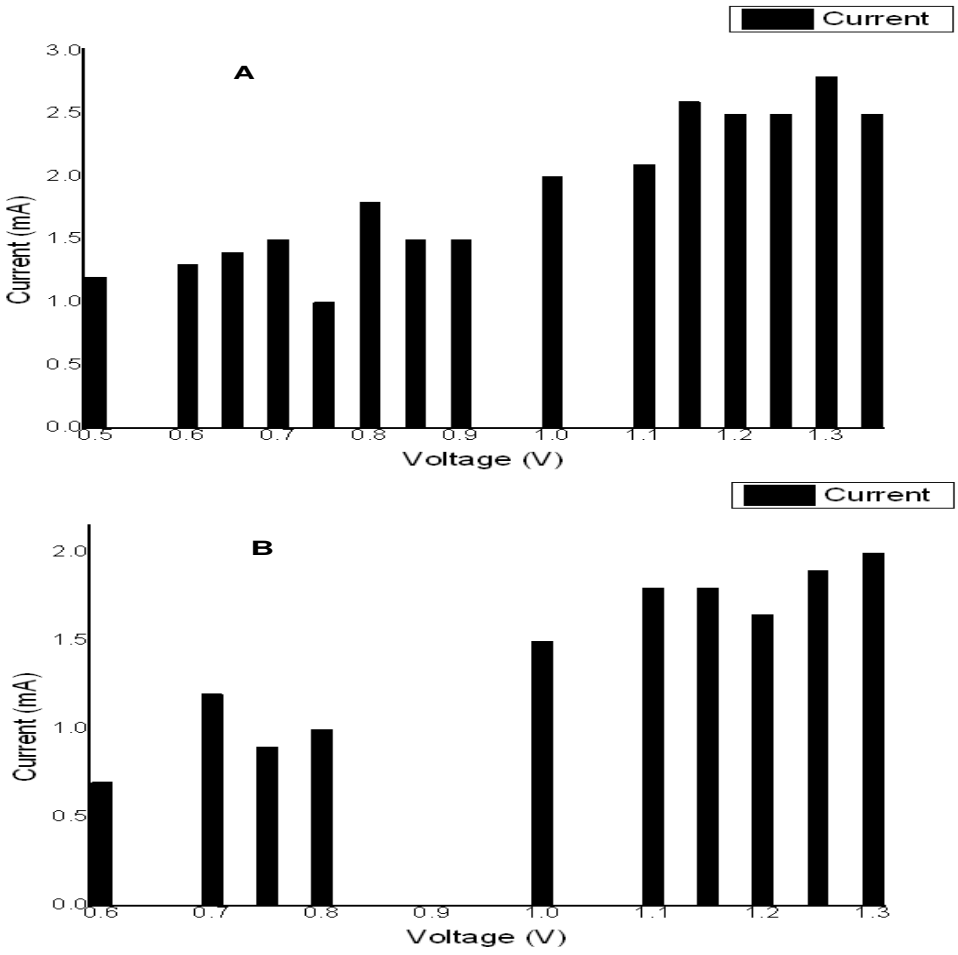


Figure 3. Graphical representation of voltage current and power using *Escherichia coli* culture from MFCSs. The shows the comparative account of voltage, power and current in different circuits namely: open circuit voltage/current, and the closed circuit voltage/current. Also, calculating these values we get the internal resistance of the system, which lets one improve on the various factors which would decrease the internal resistance of the system. The different readings were obtained connecting an ammeter and a rheostat in series and a voltmeter in parallel as shown in the Figure 2.



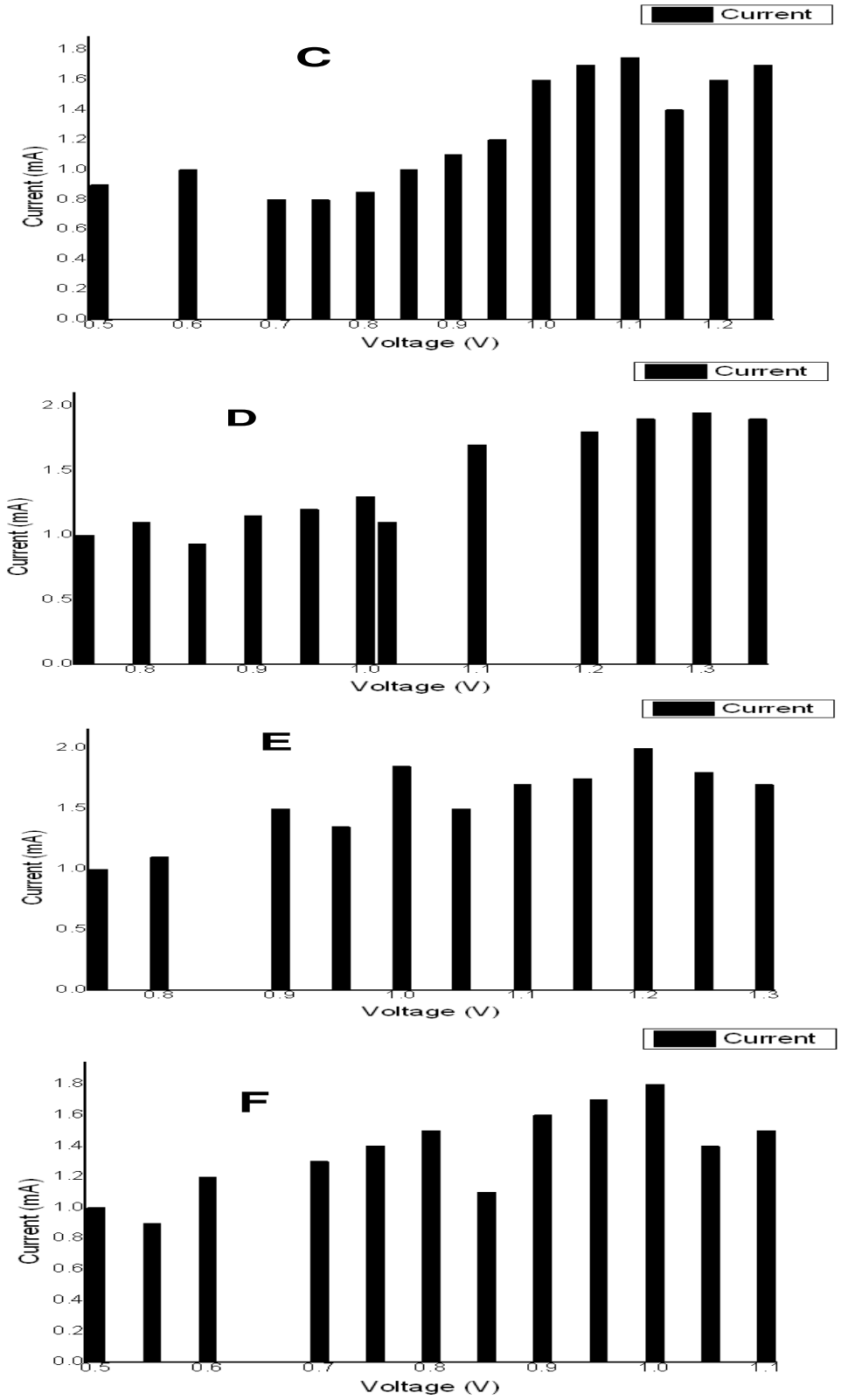


Figure 4. Comparative representation of membrane, substrate, electrode, mediator, voltage, and current for (A) *Saccharomyces cerevisiae* (B) *Bacillus subtilis (*C) *Staphylococcus aureus* (D) *Clostridium acetobutylicum,* (E) *Escherichia coli* (F) *Proteus vulgaris* for bioelectricity production. The values are mean of three sets of experiments with triplicates in each set. V = Voltage (V); I = Current (mA).

Table 1. Details of strain, membrane, substrate, electrode, mediator, voltage, and current bioelectricity production using various microorganisms as listed. The values are mean of three sets of experiments with triplicates in each set.

Strain	Membrane	Substrate	Electrode	Mediator	Voltage mV	Current mA
E. coli	Nitro-Cellulose membrane	Glucose	Graphite	Methylene Blue	0.8	1.7
S. Cerevisiae	Nitro-Cellulose membrane	Glucose	Graphite	Commassie Brilliant Blue	1.35	2.5
Candida Albicians	Salt Bridge	Sugarcane Juice	Carbon Cloth	Methylene Blue	0.9	2.1
E. coli	Nitro-Cellulose membrane	Sucrose	Graphite	Methylene Blue	0.9	2.1
Domestic waste water	Nitro-Cellulose membrane	Sugarcane Juice	Carbon Cloth	Commassie Brilliant Blue	0.5	1.3
Industrial waste water	Salt Bridge	Glucose	Graphite	Methylene Blue	1.3	2.8

The best results were shown by *Saccharomyces cerevisiae*, up to 1.35 V voltage and 2.5 mA current produced by applying the Nitrocellulose membrane, Graphite electrode and glucose as substrate for this particular organism.

Table 2. Details of membrane, substrate, electrode, mediator, voltage, and current for *Proteus vulgaris, Escherichia coli, Cmoltridium acetobutylicum, Staphylococcus aureus, Saccharomyces cerevisiae,* and *Bacillus subtilis* in bioelectricity production. The values are mean of three sets of experiments with triplicates in each set.

Membrane	Substrate	Electrode	Mediator	*Proteus vulgaris* (MTCC-742)		*Escherichia coli* (MTCC-2939)		*Clostridium acetobutylicum* (MTCC-481)		*Staphylococcus aureus* (MTCC-96)		*Saccharomyces cerevisiae* (MTCC-178)		*Bacillus subtilis (MTCC-121)*	
				V	I	V	I	V	I	V	I	V	I	V	I
Nitrocellulose membrane	Glucose	Graphite	Methylene Blue	1.1	1.5	1.3	1.7	1.35	1.9	1.25	1.7	1.35	2.5	1.25	1.9
			Crystal Violet	1	1.5	1.2	1.5	1.2	1.7	1.1	1.6	1.2	2.4	1.15	1.8
			Commassie brilliant blue	1.05	1.4	1.25	1.8	1.3	1.8	1.2	1.5	1.25	2.45	1.3	2
			Cresol red	0.9	1.2	1.2	1.6	1.1	1.6	1.15	1.3	1.1	2	1.1	1.5
		Charcoal	Methylene Blue	0.9	1	1	1.3	0.95	1.2	1	0.9	0.85	1.5	0.8	1
			Crystal Violet	0.8	1	0.9	1.25	0.8	1.1	0.8	0.85	0.7	1.4	0.7	1.2
			Commassie brilliant blue	0.9	0.95	1	1.3	0.9	1.15	0.9	0.95	0.8	1.8	0.75	0.9
			Cresol red	0.9	0.9	0.95	1.2	0.75	1	0.75	0.8	0.7	1.3	0.6	0.7
	Sucrose	Graphite	Methylene Blue	0.9	1.2	1.25	1.8	1.3	1.95	1.2	1.6	1.3	2.8	1.2	1.65
			Crystal violet	0.8	1.1	1.1	1.6	1.2	1.8	1.1	1.2	1.15	2.6	1	1.5
			Commassie brilliant blue	0.9	1.3	1.2	1.7	1.25	1.9	1.15	1.4	1.25	2.5	1.1	1.8
			Cresol red	0.85	1.1	1.15	1.75	1.1	1.7	1	1.1	1	2	1	1.5
		Charcoal	Methylene Blue	0.7	1	0.9	1.2	1	1.2	1	1.1	0.9	1.5	0.8	1
			Crystal violet	0.6	0.9	0.8	1.1	0.9	1	0.8	0.7	0.8	1.1	0.7	0.8
			Commassie brilliant blue	0.7	0.85	0.95	1.35	1.1	1.5	0.9	0.9	0.85	1	0.75	0.9
			Cresol red	0.6	0.7	0.8	0.9	0.9	1.1	0.75	0.8	0.65	1.2	0.6	0.6

Table 2. Contd.

Poly vinyl chloride	Glucose	Graphite	Methylene Blue	1	1.8	1.2	2	1.2	1.6	1.2	1.5	1.25	2.2	1.25	1.9
			Crystal Violet	0.9	1.6	1.1	1.55	1.1	1.3	0.85	1	1.1	2.1	1.15	1.8
			Commassie brilliant blue	0.95	1.7	1.2	1.75	1.25	1.5	1.1	1.3	1.2	2.5	1.3	2
			Cresol red	0.95	1.6	1	1.6	1.1	1.2	1	1.2	1.15	2.1	1.1	1.5
		Charocal	Methylene Blue	0.8	1.5	1	1.85	1	1	0.95	1.2	0.8	1.3	0.8	1
			Crystal Violet	0.75	1.4	0.9	1.5	0.85	0.9	0.9	1.1	0.7	0.9	0.7	1.2
			Commassie brilliant blue	0.8	1.45	1	1.65	0.95	1.1	0.95	1	0.75	1	0.75	0.9
			Cresol red	0.75	1.35	0.9	1.5	0.85	0.93	0.9	0.8	0.6	1.1	0.6	0.7
	Sucrose	Graphite	Methylene Blue	0.8	1.4	1.1	1.7	1	1.3	1.1	1.75	1.2	2.1	1.2	1.65
			Crystal Violet	0.75	1.3	1.05	1.5	1.02	1.1	1	1.6	1.15	1.9	1	1.5
			Commassie brilliant blue	0.9	1.35	1.15	1.6	1	1.2	1.05	1.7	1.2	2.2	1.1	1.8
			Cresol red	0.7	1.3	1	1.5	0.9	1.1	1.1	1.2	1.1	1.8	1	1.5
		Charcoal	Methylene Blue	0.6	1.1	0.9	1.2	0.9	1	0.9	1.1	0.7	1.5	0.8	1
			Crystal Violet	0.5	1	0.8	0.9	1.1	0.95	0.7	0.8	0.6	1.3	0.7	0.8
			Commassie brilliant blue	0.6	1.2	0.9	1.1	1	1.2	0.6	1	0.65	1.4	0.75	0.9
			Cresol red	0.55	0.9	0.75	1	0.95	0.9	0.5	0.9	0.5	1.2	0.6	0.6

Utilization of membrane is another crucial factor in the development of MFCs; the membrane is the responsible factor for the transfer of the required ions instead of the undesired ones. Various different types of membranes are principally poly-vinyl chloride membranes, nitrocellulose membrane, etc. These membranes are utilized keeping in mind, their cost and efficiency. Also the use of salt bridge can be made, but it is rather less efficient as compared to the membranes. Another potential factor is the use of mediators, there are several chemicals that can be used for this purpose, and some of those are methylene blue, crystal violet, commassie brilliant blue, and cresol red, etc. Electrodes employed in MFCs are many in numbers and is utilized according to its efficiency and cost involved, various electrodes utilized for this purpose are graphite, carbon cloth, charcoal, platinum etc. In fact, platinum is a costly electrode but its efficiency is the highest. The efficiency of MFCs depends on various factors altogether, but some points are more consi-derable, like the surface area of the electrodes, the internal resistance of the entire system, open circuit voltage and the closed circuit voltage. Therefore, during the development phase of the MFCs, all the above-mentioned factors should be taken in to consideration in view of developing an overall efficient MFCs system. Taken together the results obtained from the studies (Lu et al., 2009; Min et al., 2005; Zheng et al., 2010) the present model reflects an efficient MFCs system for bioelectricity production taking in to consideration with waste recycling. Further, comprehension pertaining biotechnological applications on the relevant notions and objectives is in the progress in over laboratory.

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