Potential Effect of Sugar Mill waste water as Substrate for Bio-Electricity Generation using Laboratory Scale Double Chamber Microbial Fuel Cell

M Siddique¹, A S Jatoi², M H Rajput³, S A Soomro³, S Aziz³, F Mushtaq¹, G Khan¹, M A Abro⁴, M Najam Khan¹, A K Shah⁵ and S K Sami¹

¹Chemical Engineering Department, Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS), Quetta, Pakistan.
²Chemical Engineering Department, Dawood University of Engineering and Technology (DUET) Karachi, Pakistan.
³Chemical Engineering Department Mehran University of Engineering and Technology (MUET) Jamshoro, Sindh, Pakistan.
⁴College of Chemical Engineering, Beijing University of Chemical Technology, Beijing 100029, P. R. China.
⁵Metallurgy and Materials Engineering Department, Dawood University of Engineering and Technology (DUET) Karachi, Pakistan.

Email: siddiqnasar786@gmail.com

Abstract: Bio-electrochemical system for power generation getting attention due to utilization of waste material. Based on that study was made to convert sugar industry waste water for bio-electricity generation using double chamber microbial fuel cell. Different organic load in form of substrate concentration and parametric effect were tested to optimize the best condition for electricity generation. From 100g/l to 300g/l with step size 100g/l, for aeration rate from 100-250ml/min with step size 50ml/min, and for as pH from 4.5 to 6.5 with step size 0.5pH. The maximum power generation were observed at pH 6, aeration rate 200ml/min and organic load 200g/l about 820mA. Regarding above results that found favourable condition on environment as well as waste reduction.

Keywords. Sugar mill waste water, bio-electricity, double chamber microbial fuel cell.

1. Introduction
The waste water generated during the sugar manufacturing process of sugarcane contain lots of organic compound in the form of carbohydrates like glucose, sucrose etc. This can be treated as well as used as substrate for the generation of power in Microbial fuel cell. For the treatment of waste water, we design Microbial fuel cells (MFCs). In Microbial fuel cells (MFCs) there are two chambers which are anode and cathode and separated by polymeric proton exchange membrane. To utilize the substrate and convert it into electricity we introduce bacteria in anode chamber. Most MCFs contain aqueous cathode in which dissolved oxygen can be provided to electrode by mean of water bubbled with air. To make MFCs more economical and energy efficient, we investigate the power generation in an air-cathode MFC having carbon electrode during the absence and presence of the polymeric proton exchange membrane (PEM). The waste water and glucose were used as substrate and the biocatalyst were the bacteria which are in the domestic waste water. The power density is much higher than that of the normally reported aqueous cathode MFCs, using a maximum of $262 \pm 10 \text{ mW/m}^2$ (6.6 ± 0.3 mW/L, liquid volume). The maximum power density $494 \pm 21 \text{ mW/m}^2$ (12.5 ± 0.5 mW/L) is achieved by eliminating PEM. The Coulomb efficiency is 40-55% of PEM, and the efficiency of removing PEM is 9-12%, which indicates that in the absence of PEM, the oxygen in the anode compartment diffuses in large amounts [1]. The chemical energy of the substrate in waste water is converted into electrical energy by utilization of bacteria in Microbial fuel cells (MFCs). In this cell electron are being transfer by the bacteria to the electrode rather than directly to an electron acceptor. Its technical feasibility has recently been tested, and the scientific
community has great enthusiasm for MFC's ability to generate “green power” sources by using household and industrial waste to generate energy. Acetate, lactate, and glucose are used as the feed stocks for MFCs. By using the Microbial fuel cell, we can generate the electricity from the domestic waste water and at the same the biological treatment of waste water has also been done such as removal of chemical oxygen demand; COD. These tests are carried out in the single chamber microbial fuel cell (SCMFC) with eight graphite electrodes (anodes) and a single air cathode. Primary clarified effluent which is taken from the local wastewater treatment plant was used in the system at continuous flow conditions. The waste water COD removed up to 80% while generating the electricity (maximum of 26 mW m⁻²) in the prototype SCMFC reactor. Power generated was proportional to the hydraulic retention time (3−33 hour) and to the influent wastewater strength (50−220 mg/dm³ of COD) [2]. Fuel cell and voltammetry were utilized for understanding the mechanism of electron transfer with the addition of Shewanellaputrefaciens. The bacterium’s electrochemical activity without any electrochemical mediators were determined by the both methods. By the studies of cyclic voltammetric, electrochemical activities were showed by the Shewanellaputrefaciens anaerobically, but for another condition regarding aerobically there was not enough. The activates measured regarding electrochemical system is nothing but the related closely with electric potential and current generation [3]. From the various operating condition a mediator less microbial fuel cell was optimized. pH, resistance, electrolyte used, and dissolved oxygen concentration in the cathode compartment are the several factors on which current generation depends. In neutral solution (pH=7) generation of current was maximum. The proton transfer and dissolved oxygen supply limited the cathode reaction at lower resistance than 500 Ω. Under the operating condition, the rate determining factor was resistance at over 500 Ω. Up to some extent proton limitation were reduced by high strength buffer solution. At the DO (dissolve oxygen limited condition the concentration of DO was around 6 mg l⁻¹) The fact that oxygen limitation was observed at high DO concentration is believed to be due to the poor oxygen reducing activity of the electrode used, graphite [4]. Some of bacterial culture use glucose as carbon source to identify the power generation with respect to glucose utilization. With the utilization of glucose dose 0.5−3 g l⁻¹ d⁻¹ the power output about 3.6 W m⁻² and 89% electron recovery in terms of electricity reported by [5].

Lab-scale double chamber MFC was used for power generation. Electric current was produced from the sewage organics using the Saccharomyces cerevisiae sp. as a mediator and potassium ferricyanide as an oxidizing agent. The anodic and cathodic solutions were introduced in batch and continuous way the cells were connected in series. The cathode in continuous modes and anode in batch-feed were used to operate the cell. A maximum voltage of 830mV (3.3 mA) was obtained per liter of the sludge under optimize operating parameters such as oxygen flow rate, pH and substrate concentration [6]. Two dehydrogenases, cellobiose dehydrogenase and pyranosyl-capsule heat dehydrogenase, Agaricusblazei, used surface display system of yeast to show the first surface in Saccharomyces cerevisiae. The use of dehydrogenases displayed on the surface of microbial fuel cells produces high power output. The potential midpoint - 28 mV at pH (vs. Ag/ AgCl electrode) = 6.5 represents the dehydrogenase shown on the surface of the microbial fuel cell and the output power of 3.3 μWcm produced - 2 using lactose as its use without The fuel in the anode compartment of the mediator. In microbial fuel cells, pyranose dehydrogenase is exposed on the surface and produces high output efficacy by using different substrates. The maximum power of D-xylose is 3.9 μWcm⁻². These results indicate that cellobiose dehydrogenase and pyranose dehydrogenase exposed on the surface can be successfully used in microbial bioelectrochemical systems [7].

Protection and perseverance of our environment and energy crises are the two major problems that have played havoc with our lives. The conversion methods of other renewable energy contain transitional conversion into mechanical power but Microbial fuel cell (MFC) offers an advantage that transmit chemical energy directly into the electrical energy. In this context, the sewage sludge which is the sources of major environmental pollution but also contains high levels of organic compounds is utilize to generate power in the MFC. By using the microbial fuel cell, we converted the chemical energy from the substrate of sewage sludge into the electricity by using mediator which is methylene blue and biocatalyst which is Saccharomyces cerevisiae sp. By varying the agarose’s concentration in the salt
bridge of MFC we can examined the efficiency of MFC. Concentration of agarose salt which range from 7% to 12% was utilized for analyzes of the best possible concentration was noticed being 10% because it exhibited maximum voltage generation of 0.97V [8]. The utilization of the fossil fuels and pollutant producer must be reduced to make the world more feasible and conventional for living. These types of problems can be solved by treating the waste in an appropriate manner, such as sewage sludge. As we know that the sewage sludge is full of organic compound so it can be used as a perfect substrate for the power generation. According to the study it was found that dual compartment MFC with the bio cathode was used to generate the voltage from the sewage sludge (2L). An internal resistance of 36-46 ohm were developed by the bio cathode to obtained maximum voltage (2.5 V) Saccharomyces cerevisiae sp. was used as biocatalyst. Methylene blue (10 ml) was used a mediator and potassium ferricyanide (350 ml) was used as an oxidizing agent for the conversion of sewage sludge into voltage generation [9]. The results show that although the substrate has a high impedance, all the general parameters on the 5th day prove to be the maximum, and then the trend is observed after 6 days. The corresponding maximum values of the generated parameters are 0.825 V, 0.0113 μA, 0.009223 μW and 0.000000947 mW/m², respectively. It has also been found that the obtained voltage, current, power and power density have similar patterns [10]. This work is related with the potential use of sugar mill waste water for electricity generation using microbial fuel cell.

2. Materials and Methods

2.1 Microorganisms

Pure strain of Saccharomyces Cerevisiae was used as a biocatalyst [11]. The work of Saccharomyces Cerevisiae is same as other yeasts do, which is capable of transferring electron from biodegradation of substrate, which are acetate or glucose. Acetate and glucose work as substrate on the behalf of containing carbon source which dissociate into electron and proton [12, 13]. Saccharomyces cerevisiae sp. as a biocatalyst and methylene blue as mediator were used for the conversion of [9] sugar processing water into bio-electricity. This could make direction towards the implication of microorganism for degradation of substrate into electron, which make continuous flow of electron.

2.2 Fabrication and Operation of double chamber MFC

Microbial fuel cell were fabricated with two plastic bottle attach with salt bridge having volume of 3liter of each chamber. Microbial fuel cell made of two chambers with different operation condition, one of them maintains at anaerobic condition which is anode and other chamber cathode maintained at aerobic condition. Figure 01 shows typical diagram of microbial fuel cell which consists of salt bridge with two chambers. In addition with them one pinhole drilled at top of the chamber in order to connect electrode with resistors for maintain the flow of electron from anode to cathode. As proton were concerned, it oxides with the addition of air in cathode chamber. During operation of microbial fuel cell substrate degrade by microbes, which split substrate into electron and proton.

2.3 Salt Bridge

Salt bridge or proton exchange membrane constructed through use of agar salt and different salt like NaCl and KCl for conducting proton from anode chamber by degradation of substrate. The typical composition of salt bridge is 8-13% agarose, 8g NaCl and 150ml of distilled water. The mixture of salts monitored continuously during heating and pouring into PVC pipe. The maximum power output observed by using 10% agarose concentration. Then mixture of salts and agarose were left for cooling in order to making it gel type media. After that PVC pipe attach with anode and cathode chamber for making circuit complete.

| Table 1. Dimension of salt bridge, anode and cathode chamber |
|--------------|--------|--------|
| Items        | Length (in) | Dia (in) | Height(in) |
| Cathodic chamber | 5      | 10     |          |
| Anodic chamber  | 5      | 10     |          |
| Salt bridge   | 1      | 0.5    | -        |
2.4 Anode and Cathode
Anode and cathode chamber made with two different conditions based on air supply. In anode chamber degradation of substrate in absence of oxygen and cathode chamber oxidize proton that emit from anode chamber with the use of different air ratio. Carbon cloth was utilized as electrode for conducting electron during conversion of organic matter into electron and proton. The power output was observed through use of multimeter. The total volume of anode and cathode chamber is 800ml with working volume of 500ml.

2.5 Assembling the Fuel Cell
The sample of sugar mill waste water put into anode chamber with different concentration and power were observed after 24 hour due to utilization of substrate and substantially growth in cell for microbial growth. Cathode chamber maintained pH with addition of 10%H$_2$SO$_4$ dilute solution for maintains the pH and 10%NaOH for anode chamber. Electrode was connected with lid inserted for receiving electron from anode chamber. The current is generated by moment of electron from anode to cathode chamber. Electric current were measured by multimeter which maintained at mode of fuel cell output voltage.

![Figure 1.Typical schematic diagram of Microbial Fuel cell](image)

2.6 Electrical parameters and measurements
Electric power was measured during experiments of degradation of sugar mill waste water into electron and proton. Electron flow through external resistors from anode to cathode chamber. The current and voltage measured after 24hour, periodically after 20min.

3. Results and Discussion
Microbial fuel cell for electricity generation gained huge interest in field of renewable energy resources. Microbial fuel cell is a device which utilizes organic matter for electricity generation with the help of electron and proton. Microbial fuel cell has also application in bioremediation for treatment of waste water [14]. The electricity generation was monitored by different operational condition for both season’s summer and winter. Anodic and cathodic chamber optimization regarding maximum current and maximum power is about 0.784 mA and 0.645 mA explained by [15] at organic concentration in solutions about 720 mgL$^{-1}$ which produces maximum power density of 768 mWm$^{-2}$ and minimum 556 mWm$^{-2}$.

Determined voltage generated from Microbial fuel cell and organic concentration of raw material for degrading seems to maintain the Monod type equation. Performance of microbial fuel cell related with molecular weight and structures of eight amino acids [15]. Electricity generation from sugar mill waste water using microbial fuel cell were under study to optimize the parametric effect on that, to measure potential of sugar mill waste water different operational parameter were under study.
Table 2. Current, voltage, power, power and current density at various aeration rate, pH, and substrate

| Parameter               | Oxygen flow rate (ml/min) | pH value | Substrate % w/v |
|-------------------------|---------------------------|----------|-----------------|
|                         | 100 | 150 | 200 | 250 | 5 | 6 | 7 | 8 | 25 | 50 | 75 |
| Current (mA)            | 0.81 | 0.89 | 0.94 | 0.98 | 0.79 | 0.83 | 0.86 | 0.89 | 0.75 | 0.79 | 0.83 |
| Voltage (volts)         | 0.77 | 0.86 | 0.95 | 0.97 | 0.75 | 0.79 | 0.82 | 0.85 | 0.72 | 0.74 | 0.77 |
| Power (mW)              | 0.62 | 0.77 | 0.89 | 0.91 | 0.59 | 0.66 | 0.71 | 0.76 | 0.54 | 0.58 | 0.64 |
| Power density (mW/m²)   | 50.43 | 61.53 | 71.98 | 78.42 | 49.38 | 54.64 | 58.77 | 63.04 | 45.0 | 48.72 | 53.3 |
| Current density (mA/m²) | 67.29 | 73.98 | 78.97 | 82.48 | 65.83 | 69.17 | 71.67 | 74.17 | 62.5 | 65.83 | 69.2 |

3.1 Effects of oxygen flow rate on electricity generation

During running of microbial fuel cell different ranges of oxygen flow rate were tested to investigate the optimize condition for electricity generation from sugar mill waste water. Regarding 500 Ω resistance dissolved oxygen supply and proton transfer decrease to cathode reaction [4] also explain the strategies behind dissolved oxygen, on the basis of that aeration rate had significantly fact on current generation. The microbial fuel cell might be used as a biochemical oxygen demand (BOD) sensor [4]. During degradation of organic matter in anode chamber, that splits into electron and proton for electricity generation from 100-250ml/min were analyzed periodically to measure the voltage generation using 50Ω resister with the help of digital multimeter. In figure 2, it is clearly observed that when oxygen flowrate increasing up to the level of 200ml/min voltage generation were also increased up to the 360 mv/l after 24-hourperiod of time for microbial growth.

![Figure 2. Effects of oxygen flowrate on electricity generation](image)

3.2 Effects of pH on electricity generation

The maximum current and voltage generation were observed at pH ranges between 8 and 10 when compared with lower pH ranges. Bacterial growth inhibited by buffer and changed electrolyte. In microbial fuel cell anode chamber gave favorable results with neutral pH ranges and minimum at lower pH ranges explained by [16]. The highest current was generated at a pH of 6.5 in the anode chamber containing 4% CE. The greater pH difference between the anode and cathode electrolytes favors higher currents and voltages. In the analyzed COD range (100-600 mg / l), a linear relationship was observed between the current and the substrate removed [17]. Microbial growth of saccharomyces cell during
running of microbial fuel cell affected by pH due to tolerable pH ranges of microbes specie increased. From 5-8 were tested with step size 01 using buffer solution to maintain the pH ranges according required range of pH under 48 hr running period of microbial fuel cell. The maximum voltage generation from microbial fuel cell by utilizing sugar mill waste water. In Figure 3, effects of pH on electricity generation clearly observed and maximum Voltage generated at pH 8 and about 276mv/l.

![Figure 3. Effects of pH on electricity generation](image)

3.3 Effects of substrate utilization on electricity generation

It was observed that the amount of power generation increased with increasing substrate concentration as shown in Figure 4. Starting from about 25% substrate concentration, the power obtained at this substrate concentration was 0.725V. At a substrate concentration of 75%, power generation increased by 1550mV. Further increases in the concentration up to 100 % resulted in the decrease in power production by more than 100% when it reached the value of 1V. This may be due to various factors such as pH-induced decrease in enzyme activity. This also shows that higher concentrations of substrates can affect the performance of the anode, which can lead to lower simultaneous generation of electricity.

![Figure 4. Effects of Substrate on electricity generation](image)
3.4 Effects of substrate utilization on current and power densities

Different substrate concentration was utilized to produced protons and electrons for making complete circuit between anode and cathode chamber. In microbial fuel cell electrodes were provided for acceptance of electron from anode chamber. With the use of bio-catalyst *Saccharomyces cerevisiae* substrate degraded into protons and electrons, where proton get oxidation in cathode chamber with the use of aeration rate provides through fish pump. Figure 6 clearly show that when increase in substrate content, there is significant change of power and current density. It is due to increase in organic load present in sugar waste water, because during processing of sugarcane some starch and glucose were remained in waste water. Figure 5 shows the maximum power and current density were obtained at 100w/v% about 58mW/m² and 74mA/m².

![Figure 5. Effects of substrate on current and power density](image)

4. Conclusion

Microbial fuel cell getting importance for treating waste water for power generation. From 100g/l to 300g/l with step size 100g/l, for aeration rate from 100-250ml/min with step size 50ml/min, Maximum electricity generation at 200ml/min about 360 mv/l and for as pH from 4.5 to 6.5 with step size 0.5pH. The maximum power generation was observed at pH 6 about aeration rate 200ml/min and organic load 200g/l about 820mA. Regarding above results that found favorable condition on environment as well as waste reduction.

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