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Electricity Generation by *Saccharomyces cerevisae* and *Clostridium acetobutylicum* via Microbial Fuel Cell Technology: A Comparative Study

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Abstract: A microbial fuel cell (MFC) is a device that converts chemical energy into electricity through the catalytic activities of microorganisms. In the present contribution, we demonstrated the comparative electricity production capacity of *Saccharomyces cerevisae* and *Clostridium acetobutylicum* in two chambered MFC using Artificial Waste water. Temperature, pH, substrate concentration and addition of complex waste (Molasses) were used as comparison indicators. Up to 10.89 mA and 10.45 mA current was generated by *Saccharomyces cerevisae* and *Clostridium acetobutylicum* in 10 days of operation.

Key words: Bioelectricity · Microbial fuel cells · *Clostridium acetobutylicum* · *Saccharomyces cerevisae*

INTRODUCTION

Energy is the prime mover of economic growth and is vital to the sustenance of a modern economy. Future economic growth crucially depends on the long-term availability of energy from sources that are affordable, accessible and environmentally friendly. It is important to find an alternative form of energy before the world's fossil fuels are depleted.

Recently, the microbial fuel cell (MFC) technology has been developed as a novel biotechnology, in which microorganisms are used to harvest energy from biodegradable materials [1]. In this way, chemical energy is converted directly into electrical energy [1-3]. Microorganisms gain energy for metabolism by transferring electrons from an electron donor, such as glucose or acetate, to an electron acceptor, such as oxygen. The anode electrode of a MFC takes the place of the bacteria's typical electron acceptor, moving the electrons into a circuit, through a resistor, to the cathode electrode of the MFC, generating electricity. Protons diffuse from the anode through proton exchange membrane (PEM) and join with oxygen to form water at the cathode completing the reaction.

Microorganisms can transfer electrons to the anode electrode in three ways: Exogenous mediators (ones external to the cell) such as Methylene Blue [2], or Neutral red [4]; using mediators produced by the bacteria; or by direct transfer of electrons from the respiratory enzymes (i.e., Cytochromes) to the electrode [5]. These mediators trap electrons from the respiratory chain and become reduced to transfer the electron to the electrode via outer cell membrane [6]; *Clostridium butyricum* [7] and *Saccharomyces cerevisae* [8] are reported to transfer electrons in mediated MFC while *Shewanella putrefaciens* [9], *Geobacter sulfurreducens* [10], *Desulfobulbus propionicus* [11] and *Rhodoferax ferrireducens* [12] have been shown to generate electricity in a mediator less MFC [13]. Bacteria present in mediator less MFCs have electrochemically active redox enzymes on their outer membranes that transfer the electrons to external materials and therefore, do not require exogenous chemicals to accomplish electron transfer to the electrode [14].

Most MFC studies have been demonstrated using pure compounds, such as acetate [10], glucose [7], sucrose [15], an amino acid (cysteine) [16], or a protein (bovine serum albumin) [17]. Waste water sources, utilized in MFC tests include domestic wastewater [18], swine wastewater [19], food processing wastewater [20], hydrogen fermentation reactor effluent [21], corn stover hydrolysates (liquefied corn stover) [22] and paper industry waste water [2]. Power densities obtained with these substrates vary with MFC architecture, but they are generally higher with pure compounds than tests with actual wastewaters.

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The Benefits of Using Microbial Fuel Cells Include: clean, safe, quiet performance, low emissions, high efficiency and direct electricity recovery.

In the present contribution, we demonstrated the electricity production capacity of *Saccharomyces cerevisiae* and *Clostridium thermohydrosulfuricum* using artificial waste water. Some operational parameters like temperature, pH, substrate concentration and addition of complex waste (Molasses) were used as comparison indicators.

Saccharomyces cerevisae (Yeast) is heterotrophic, found in a wide range of natural habitat, facultative anaerobe (grow rapidly under both aerobic and anaerobic conditions), have simple nutritional requirements and can utilize a wide variety of substrates which make it ideal for a MFC [23-24]. *Clostridium acetobutylicum,* (Gram Positive Bacteria) is obligate anaerobe, chemoorganotroph, found in a wide range of habitat, is mesophilic with optimal temperature range of 10-45°C and can grow on complex substrates, so may be an active agent in MFCs [25-27]. The basis of comparison of both microorganisms was their saccharolytic (can break down sugar) activity.

MATERIALS AND METHODS

Chemicals and Microorganisms: All chemicals used in this study were of analytical or biochemical grade. Microbial seeds of *Clostridium acetobutylicum* (MTCC-481) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Microbial seeds of *Saccharomyces cerevisae,* available in the laboratory used in present study.

Molasses sample was collected from Daurala Sugar Works, Meerut, India. This sample was left undisturbed for 24 hrs at 38°C under anaerobic conditions so as to settle the solid particulate contents.

Microbial Growth: *Clostridium acetobutylicum* was grown anaerobically at 37°C for 48 hrs in a medium [28] containing (g l^{-1}): 1.5 g KH_2PO_4, 2.9 g $K_2HPO_4.3H_2O$, 1.3 g $(NH_4)2SO_4$, 0.1 g $MgCl_2$ •$6H_2O$, 0.02 g $CaCl_2$, 5 g Yeast Extract, 25 µl 5% $FeSO_4$ solution, 1 ml 0.2% Resazurine, 0.5 g Cysteine hydrochloride and 1 g Glucose. The medium was adjusted to a pH of 7. *Saccharomyces cerevisae* was also grown anaerobically at 37°C for 36 hrs in a medium [29] containing (g l^{-1}): 3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone, 10.0 g glucose. The medium was adjusted to a pH of 7.0. 15% inoculum of these cultures was used to transfer into anode chamber of MFC for electricity production study.

Artificial Wastewater (AW): The Artificial wastewater was prepared by modifying previous method [13]. Filtered sterile glucose solution (15 ml) was mixed with trace mineral salt solution. The composition of trace mineral salt solution was (g l^{-1}): 450.0 mg $NaHCO_3$, 100 mg NH_4Cl, 10.5 mg K_2HPO_4, 6.0 mg KH_2PO_4, 64.3 mg $CaCl_2.2H_2O$, 18.9 mg $MgSO_4.7H_2O$, 10.0 mg $FeSO_4.7H_2O$, 6 mg $MnSO_4$, 0.5 mg $ZnSO_4.7H_2O$, 20 mg $CoCl_2.6H_2O$, 0.65 mg $CuSO_4.5H_2O$. 50 ml phosphate buffer (1 M, pH 7.0) and 0.5 ml methylene blue (as indicator) was added to this solution and volume made up to 1000 ml by adding ultrapure water (Milli Q System; Milipore Corp). This AW was autoclaved at 121°C for 20 min and cooled under oxygen free nitrogen gas before being mixed with filtered sterile glucose solution. The AW was made and maintained under an anaerobic atmosphere by connecting to Anaerogas pack (Hi Media Ltd. LE002A-5NO). The BOD of AW was 289 mg/L.

MFC Construction and operation: The MFCs were constructed from glass (10x10x10 cm) with a total working volume of 500 ml. Both anode and cathode were separated by a glass, containing hole (5x5 cm) which was covered with a proton exchange membrane (Nafion™ 117, DuPont Co.). Three electrode arrangements consisting of plain carbon paper (6x6cm) as anode and graphite (6x6 cm) as cathode were used in this study. The electrodes were attached using copper wire with all exposed metal surfaces sealed with a nonconductive epoxy. The anode chamber was filled (500 mL) with artificial waste water for study. The anode was continuously flushed with N_2/CO_2 (80:20) to maintain anaerobic conditions. Cathode chamber (aerobic chamber where oxygen was used as the electron acceptor for the electrode) was filled with 100mM Phosphate Buffer and pH adjusted to 7 by 0.5 N NaOH. The cathode chamber was provided with air that was passed through a 0.45µm pore size filter. Experiments were conducted using full-strength wastewater, at 35°C and pH 7. These results were considered as Test for further comparative studies.

Monitoring Electricity: Current (*I*) measurements were recorded using a Digital Multimeter (Kusam electrical industries, Model – 108) by connecting with 10Ω external circuit. COD measurements were conducted using standard methods [30].

Statistical Analyses: All experiments were conducted using 3 separate microbial fuel cells. When a single MFC was used, the experiments were repeated at least 3 times.

And results were presented as average values or a typical result. We found that the all data presented were statistically significant.

RESULTS AND DISCUSSION

Current Generation: After the inoculation with adopted *C. acetobutylicum* and *S. cerevisae* separately to the anode chamber of MFCs, the fuel cells were operated with AW at different conditions as feed to support the formation of biomass and subsequent generation of electricity. The Microbial Fuel Cells were continuously monitored during experiment and readings were taken after each 24 hrs, inoculation time was considered as time 0. Fuel Cells were operated for 15 days and readings were taken up to 10 days. Preliminary experiments conducted using MFCs showed that electricity could be generated using *C. acetobutylicum* and *S. cerevisae*. Stable current output was achieved after two cycles.

When MFCs were inoculated with *C. acetobutylicum* and *S. cerevisae* with artificial waste water samples, there was about 24 h Lag phase followed by an increase in cell current. The initial increase of current here can be attributed to the presence of components that were easily utilized by microorganisms. When these easily degradable substrates were exhausted, the current outputs began to decrease. Meanwhile, degradation of complex components was taken place by which a lower current was still obtained. Fresh feed was supplemented when current drop was observed. A steady increase in current generation was observed with additional feed and might be attributed to the adaptation, phenomenon and development of the biofilm on the surface of the anode. Electrode fouling was not observed and the electrodes could be used in further experiments without remarkable activity loss.

Test: Two MFCs were operated at 35°C, pH 7 in full strength wastewater as test. Both cultures started fermentation and current generation after about 24 hrs of inoculation. *S. cerevisae* reached the maximum current output of 6.25 mA after about 4 days of operation. *C. acetobutylicum* achieved maximum current output of 6.83 mA. The current decreased after approximately 5 days and 6 days in the case of *S. cerevisae* and *C. acetobutylicum* due to substrate exhaustion in the medium. When 50% part of AW was replaced with a syringe through the anode by fresh AW, the current generation recovered quickly and reached a maximum value of 10.89 mA and 9.81 mA after 9 and 10 days of operation. Results clearly indicate that *S. cerevisae* performed better than *C. acetobutylicum* in oxidizing AW.

Effect of Temperature: To evaluate the effect of temperature on current generation, two MFCs were operated with *C. acetobutylicum* and *S. cerevisae* at 45°C (Figure 1). Experimental data indicate that performances of both MFCs were slightly decreased with increase of temperature from 35 to 45°C.

S. cerevisae started current generation after about 24 hrs and reached a peak value of 6.13 mA after 4 days of operation. The current fall observed after 5 days which was recovered when 50% AW was replaced with fresh AW. *S. cerevisae* followed same current generation pattern as at 35°C up to 6 days. After 6 days a current fall observed which continued up to 10^{th} day of operation. These results were not surprising as the most ambient temperature range for *S. cerevisae* activity is 30-37°C. Above this temperature range the microbial multiplication so substrate oxidization and ultimately current generation was less. *C. acetobutylicum* showed decreased yet competitive results at 45°C. The culture started current generation after about 24 hrs and reached a peak value of 7.36 mA after 5 days of operation. The current fall observed after 6 days which was recovered when 50% AW was replaced with fresh AW and reached a maximum value of 8.96 mA after 9 days. These competitive results were due to mesophilic nature of *C. acetobutylicum* which made *C. acetobutylicum* around equally affective at 45°C.

Effect of pH: To check the comparative electricity generation capacity by both microbes, four MFCs were operated with AW at pH 6 and 8. Figure 2 represents current generation by *S. cerevisae* and *C. acetobutylicum* at pH 6 and 8. As shown in figure, the highest current was observed at pH 7 (Test), while values were decreased at pH 6.0 and pH 8.0. Each microorganism involved in anaerobic degradation had a specific pH optimum in which it grew and performed best and generally microbial activity is lower at sub-optimal pH than an optimal pH.

At pH 6.0, *S. cerevisae* generated comparatively better results than *C. acetobutylicum*. This culture started current generation after about 24 hrs and reached maximum current output of about 5.18 mA and 8.54 mA after 4^{th} and 8^{th} day of operation which was higher than current response of *C. acetobutylicum* which produced 4.89 mA and 6.51 mA current after 5^{th} and 9^{th} day. *C. acetobutylicum* showed better results than *S. cerevisae* at pH 8.0. This culture started current generation after

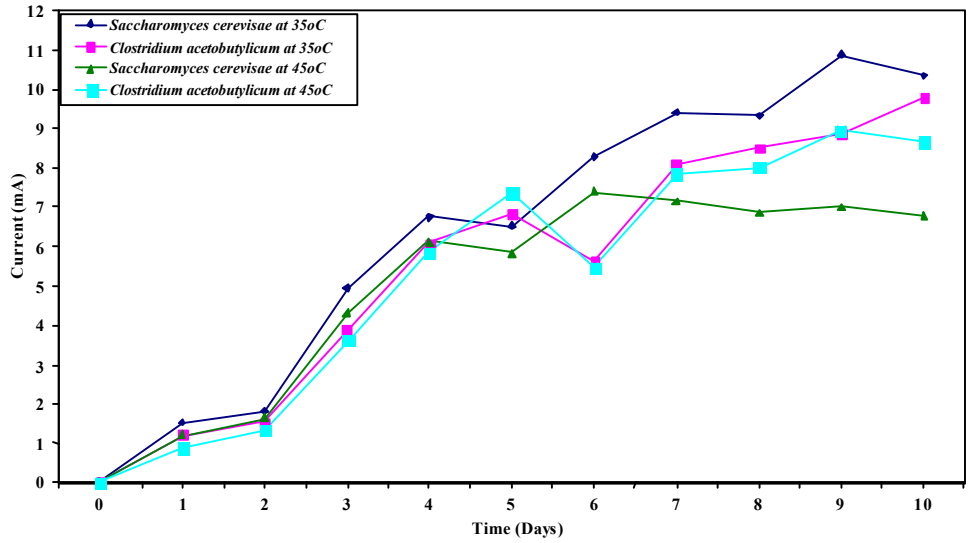

Fig. 1: Current Generation by *C. acetobutylicum* and *S. cerevisae* at 35 and 45°C

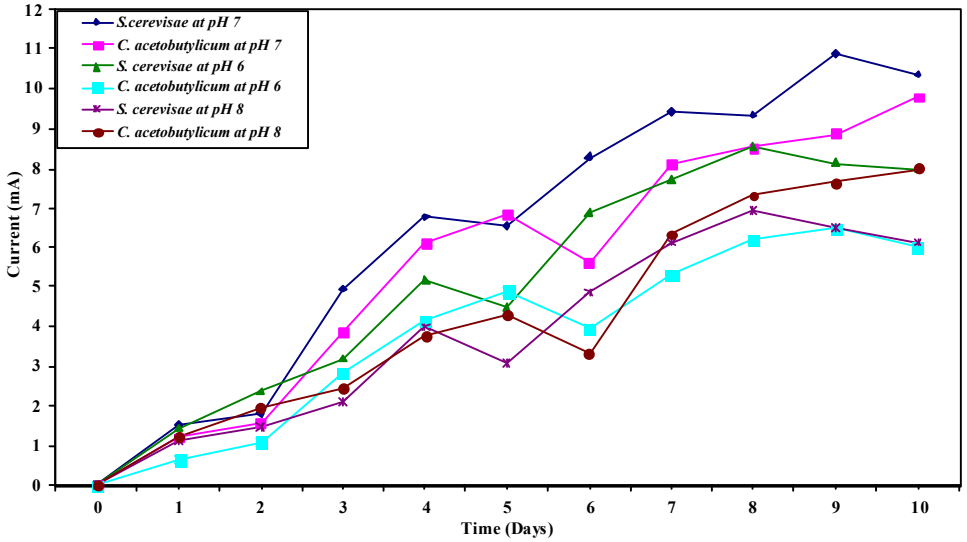

Fig. 2: Current Generation by *C. acetobutylicum* and *S. cerevisae* at pH 6, 7 and 8.

about 24 hrs and reached maximum current output of 4.29 mA and 7.98 mA after about 5 and 10 days. While *S. cerevisae* showed maximum current generation of 3.98 mA and 6.95 mA after 4th and 8th day of operation.

Effect of waste water concentration: Figure 3 shows the effect of waste water concentration (100% and 50% (by addition of 50% Ultra pure water)) on electricity generation by *S. cerevisae* and *C. acetobutylicum*. *S. cerevisae* started current generation after about 24 hrs and reached 6.98 mA and 9.83 mA after 4th and 9th day of operation respectively. This current generation was less than obtained at 35°C throughout except at 4th day. This may be due to comparative much affinity of *S. cerevisae* towards substrate than *C. acetobutylicum*. Current generation was less in 50% AW, possibly due to less availability of biodegradable substrates in 50% waste water samples than that of full strength waste water (100%) leading to competitive inhibition in microorganisms.

As the *C. acetobutylicum* exhibited less affinity towards full strength AW, possibly due to substrate inhibition, so in 50% AW, it showed better current response throughout than that of full strength waste water. It started current generation after about 24 hrs and reached a maximum output of 8.96 mA after about 6 days.

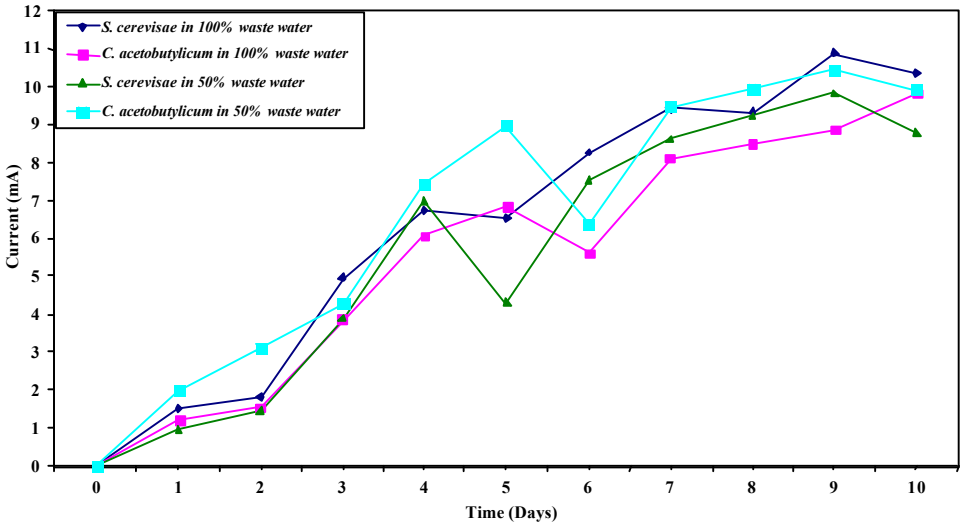

Fig. 3: Current Generation by *C. acetobutylicum* and *S. cerevisae* in different waste water concentrations

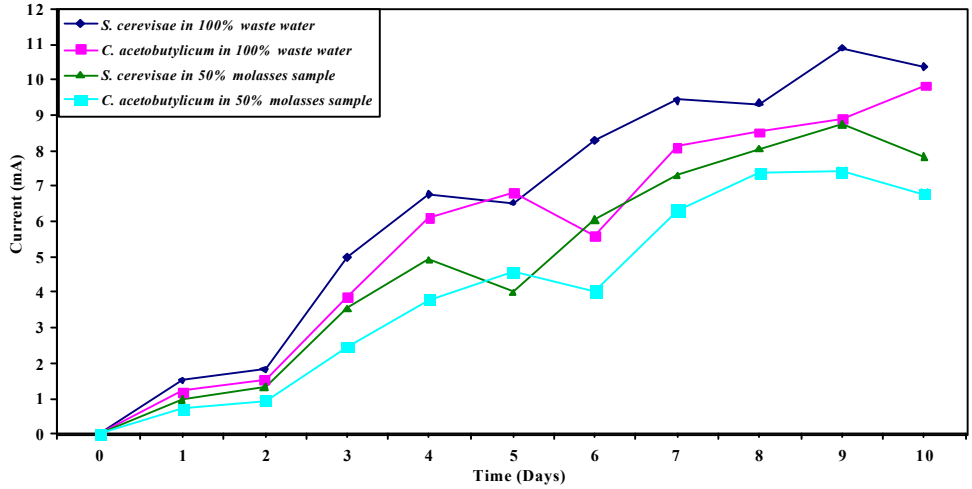

Fig. 4: Current Generation by C. acetobutylicum and S. cerevisae in 100% waste water and 50% molasses sample of waste water

Rapid current fall observed due to substrate exhaustion which was recovered after replacement of 50% fresh feed and reached a peak value of 10.45 mA after about 9 days of operation.

Effect of Complex Waste Water: To observe the effect of complex waste to the electricity production by both the microorganisms, a molasses sample was made by addition of 50-50% AW and molasses respectively and used as feed for *S. cerevisae* and *C. acetobutylicum*. A satisfactory current response was obtained by both the microorganisms. *S.* cerevisae generated 4.92 mA and 8.72 mA current after 4 and 9 days respectively and C. *acetobutylicum* generated 4.59 mA and 7.43 mA current after 5 and 9 days of operation. Although time taken for carbon source exhaustion was relatively more in molasses sample, which ultimately resulted in low current response (this could be due to complex nature of molasses sample (50% AW and 50% molasses) than chemically defined sample (100% AW)), yet as per cost factor analysis, this current response was still superior as the molasses was a waste product from Sugar industry and had no or very less economical value.

CONCLUSION

S. cerevisae and *C. acetobutylicum* both are good candidate for bioelectricity production via Microbial fuel cell technology. Their easy availability, anaerobic nature and wide nutritional requirements make them a suitable

catalyst for electricity production. As the microbes gave good response towards waste product, thus, the combination of wastewater treatment along with electricity production may help in saving money as a cost of wastewater treatment at present.

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