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Bioelectricity production from paper industry waste using a microbial fuel cell by *Clostridium* species

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Abstract

Renewable energy is an increasing need in our society. Microbial fuel cell (MFC) technology represents a new form of renewable energy by generating electricity from what would otherwise be considered waste. It is possible to directly generate electricity-using bacteria while accomplishing wastewater treatment in processes based on microbial fuel cell technologies. When bacteria oxidize a substrate, they remove electrons. Electric current generation is made possible by keeping bacteria separated from oxygen, but allowing the bacteria growing on an anode to transfer electrons to the counter electrode (cathode) that is exposed to air. In this report, the cellulosic waste was utilized for the bioelectricity production by *Clostridium acetobutylicum* and *Clostridium thermohydrosulfuricum*.
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Keywords: Bacteria, *Clostridium acetobutylicum*, *Clostridium thermohydrosulfuricum*, Electricity, Microbial fuel cell.

Introduction

Microbial fuel cells are not new – the concept of using microorganisms as catalysts in fuel cells was explored from the 1970s (Suzuki 1976; Roller et al. 1984), and microbial fuel cells treating domestic wastewater were presented in 1991 (Habermann and Pommer 1991). A microbial fuel cell (MFC) is a device that uses bacteria to catalyze the conversion of organic matter into electricity (Suzuki et al. 1978; Wingard et al. 1982; Allen and Bennetto 1993; Kim et al. 2002; Bond and Lovley 2003; Gil et al. 2003; Liu et al. 2004; Liu and Logan 2004; Oh et al. 2004). Bacteria generate electrons and protons at the anode by oxidizing substrate. Electrons are transferred through an external circuit while the protons diffuse through the solution to the cathode, where electrons combine with protons and oxygen to form water (Delaney 1984; Lithgow 1986).

Microorganisms can transfer electrons to the anode electrode in three ways: Exogenous mediators (ones external to the cell) such as Thionine, or Neutral red; Using mediators produced by the bacteria; or By direct transfer of electrons from the respiratory enzymes (i.e., Cytochromes) to the electrode(Bond and Lovley 2003; Min et al. 2004). These mediators trap electrons from the respiratory chain and become reduced to transfer the electron to the electrode via outer cell membrane (Bennetto 1990).

Clostridium butyricum (Niessen et al. 2004), *Saccharomyces cerevisae* (Reed and Nagodawithana 1991) and *Proteus vulgaris* (Bennetto 1990) are reported to transfer electrons in mediated MFC while *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Geobacter metallireducens* and *Rhodoferax ferrireducens* have been shown to generate electricity in a mediator less MFC (Bond and Lovley 2003). 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 (Oh et al. 2004).

MFCs have operational and functional advantages over the technologies currently used for generating energy from organic matter. First, the direct conversion of substrate energy to electricity enables high conversion efficiency. Second, MFCs operate efficiently at ambient temperature. Third, an MFC does not require gas treatment because the off-gases of MFCs are enriched in carbon dioxide and normally have no useful energy content. Fourth, MFCs do not need energy input for aeration provided the cathode is passively aerated (Liu et al. 2004). Fifth, MFCs have potential for widespread application in locations lacking electrical infrastructures and can also operate with diverse fuels to satisfy our energy requirements. Some recent developments allow high conversion rates and high conversion efficiencies of simple carbohydrates like glucose (Rabaey at al. 2003), and complex carbohydrate like starch (Niessen et al. 2004) and cellulose (Niessen et al. 2005). Although MFCs generate a lower amount of energy than hydrogen fuel cells, a

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combination of both electricity production and wastewater treatment would reduce the cost of treating primary effluent wastewater.

The utilization of cellulose or cellulosic waste for microbial electricity generation is much more difficult than that of low-molecular carbohydrates, or of the storage carbohydrate starch, as the ß (beta)-glycosidic bonds of the structural carbohydrate cellulose are highly resistant against hydrolysis. On top of this chemical stability, its insolubility as well as the association with lignin makes cellulose a recalcitrant substrate for enzymatic or microbial hydrolysis. So, even though MFCs are generally referred to as devices in which biomass is converted to electricity, so far their operation has been demonstrated only with low molecular, soluble substrates such as simple carbohydrates and low-molecular organic acids. Aerobic and anaerobic microorganisms use different strategies to feed on cellulose. Whereas aerobes generally make use of single-enzyme components, excreted in high concentrations and in concert in order to hydrolyze the ß-glycosidic bonds linking the glucose units, some anaerobes have evolved a more elaborate way – by using an extra cellular multi-enzyme complex, the so called cellulosome Schwarz 2001.

The aim of present study was to investigate the possibility of better treatment of cellulosic waste for microbial electricity generation. To our best of knowledge it is for the first time that cellulosic waste is reported to serve as fuel in Microbial Fuel Cells. Two strains of *Clostridium* geneus, *Clostridium acetobutylicum* and *Clostridium thermohydrosulfuricum* were used as biocatalyst for microbial electricity generation.

Materials

Waste Water

Six Wastewater samples of 1L were collected from a Paper industry, near Kanpur, (UP, India).

Chemicals and Microorganisms

All chemicals used in this study were of analytical or biochemical grade. Microbial seeds of *Clostridium acetobutylicum* (MTCC-481), and *Clostridium thermohydrosulfuricum (MTCC 115)* were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Methods

Waste Water Sample Preparation

Each wastewater sample was left undisturbed for 24 hrs. at 38°C under anaerobic conditions so as to settle the solid particulate contents. The 900 ml supernatant of each was taken, per analysis. Each sample was diluted 4 times, and diluted sample was equally divided into 5 aliquots of about 700-ml. Each aliquot was differentially treated, made in duplicate and designated as given below:

- **Sample A:** Plain diluted waste water without any treatment.
- **Sample B1:** 10% glucose solution of plain diluted waste water (Sample A).
- **Sample C1:** 10% glucose and 0.5% methylene blue solution of plain diluted waste water (Sample A).
- **Sample B2:** 10% sucrose solution of plain diluted waste water (Sample A).
- **Sample C2:** 10% sucrose and 0.5% methylene blue solution of plain diluted waste water (Sample A).

Microbial Growth

Clostridium acetobutylicum was grown anaerobically at 37°C for at least 48 hrs. in a medium (Gehin et al. 1995) containing (per liter): 1.5 g KH_2PO_4, 2.9 g $K_2HPO_4.3H_2O$, 1.3 g $(NH_4)_2SO_4$, 0.1 g $MgCl_2 \cdot 6H_2O$, 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 Cellulose. The medium was adjusted to a pH of 7.3. *Clostridium thermohydrosulfuricum* was also grown anaerobically at 46°C for 3 days in a medium containing (per liter): 1.3 g $(NH4)_2SO_4$, 2.6 g $MgCl_2 \cdot 6H_2O$, 1.43 g KH_2PO_4, 7.2 g $K_2HPO_4 \cdot 3H_2O$, 0.13 g $CaCl_2 \cdot 2H_2O$, 6 g Na-ß-Glycerophosphate, 1.1 g $FeSO_4 \cdot 7H_2O$, 0.25 g Gluthathione (reduced form), 4.5 g Yeast extract, 1 mg Resazurine, and 3 g Cellulose. 15% inoculum of these cultures was used to transfer into anode chamber of MFC for electricity production study.

MFC construction and operation

Five MFCs were constructed with 1000 ml Aspiratory bottles (BOROSIL®; 700 mL capacity). The bottles were joined by a glass bridge containing a proton exchange membrane (Nafion™ 117, Dupont Co.) held by a clamp between the flattened ends of the two glass tubes fitted with rubber gaskets. Three electrode arrangement consisting of Anodes made of plain carbon paper, while home made cathode, coated with a Pt catalyst (0.50 mg/cm^2) on both sides of the carbon paper (Hogarth 1995) 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 (600 mL) with various Samples respectively for separate 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.

For determination of power output a variable resistance (0.1Ω to 3.0Ω) 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 (Kusam electrical industries**,** Model – 108).

Results and discussion

After the inoculation with adopted *C. acetobutylicum* and *C. thermohydrosulfuricum* to the anode chamber of MFCs, the fuel cells were operated with different designed waste water 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 3 hrs, inoculation time was considered as time 0. Fuel Cells were operated for 48 hrs. and readings were taken up to 36 hrs. Experimental data showed the feasibility of electricity generation from waste water (Fig. 1).

All samples showed about same current generation pattern. The cultures started fermentation and current generation after about 3 hrs. of inoculation, they reached the maximum current output of

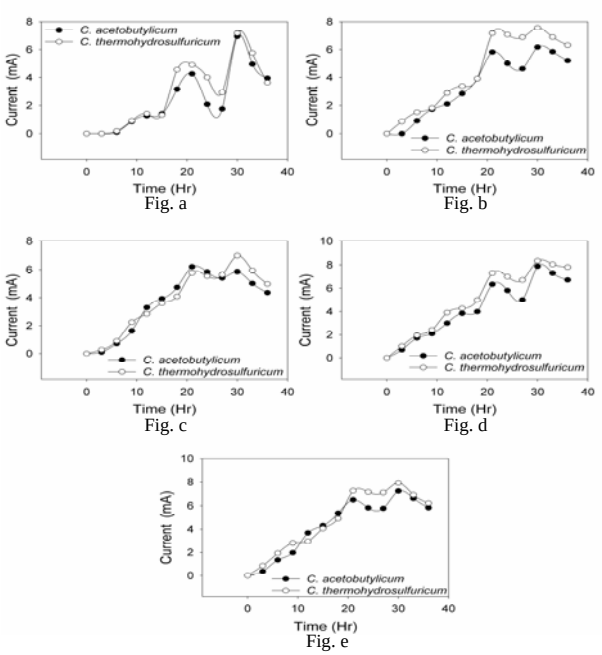

Figure 1: Current Generation by *C. acetobutylicum* and *C. thermohydrosulfuricum* in Sample A (Fig. a), Sample B1 (Fig. b), Sample B2 (Fig. c), Sample C1 (Fig. d) and Sample C2 (Fig. e)

about after 21 hrs. The current decreased after approximately 22 hrs. due to substrate exhaustion in the medium, but cellulose was still present in system (the cellulose being mostly precipitated at the bottom of the system). When 50% part of bacterial medium was replaced with a syringe through the anode by 10% glucose, the current generation recovered quickly. Electrode fouling was not observed and the anode could be used in further experiments without remarkable activity loss.

Figure 1 shows current generation by *C. acetobutylicum* in Sample A, B1, C1, B2 and C2. For Sample A (Fig 1a), the cultures started fermentation and current generation after about 3 hrs and reached the maximum current output of 4.26 mA and 4.91 mA respectively after 21 hrs. The major feature was a drop of the current after approximately 24 hrs. The potential drop resulted from the cellulose fermentation by both *Clostridium* species as for all fermentative oxidative substrate degradation processes, the lack of inorganic electron sink (like oxygen) requires parts of the substrate to become partially or fully reduced. The current generation recovered quickly when 50% part of bacterial medium was replaced with a syringe through the anode by 10% glucose, and reached upto maximum value of 6.95 and 7.18 mA respectively after 30 hrs.

For Sample B1 (Fig. 1b), which was made by addition of 10% glucose in plain diluted waste water (Sample A), both cultures showed active results from 3 hrs and reached the maximum current output of 5.82 mA and 7.18 mA respectively after 21 hrs. A major current drop examined in *C. acetobutylicum* culture while *C. thermohydrosulfuricum* showed slow current drop after approximately 24 hrs. The current generation recovered quickly when 50% part of bacterial medium was replaced with a syringe through the anode by 10% glucose, and reached upto maximum value of 6.18 mA and 7.55 mA respectively after 30 hrs.

Results show that Sample C1 (Fig. 1c) is favorable for both of the organisms. Both culture started current generation after about 3 hrs and reached the maximum current output of 6.35 mA and 7.31 mA without much fluctuations up to 21 hrs. This may be due to rapid electron transfer capacity of Methylene Blue which is used as mediator in mediated Microbial Fuel Cells. A major current drop observed after 24 hrs., which recovered quickly when 50% part of bacterial medium was replaced with a syringe through the anode by 10% glucose, and reached upto maximum value of 7.88mA and 8.35 mA respectively after 30 hrs.

For Sample B2 (Fig. 1d), which was 10% sucrose solution of diluted plain waste water (Sample A), both cultures started fermentation after 3 hrs. Curves show that this sucrose sample was more suitable for *C. acetobutylicum* than *C. thermohydrosulfuricum*, as after 9 hrs. *C. acetobutylicum* showed more current generation than *C. thermohydrosulfuricum*, and both reached to a maximum current output of 6.21 and 5.78 mA for *C. acetobutylicum* and *C. thermohydrosulfuricum* respectively. A major current drop observed in *C. acetobutylicum* after 24 hrs., while *C. thermohydrosulfuricum* showed a minor fall of current. Current recovered quickly when 50% part of bacterial medium was replaced with a syringe through the anode by 10% glucose. Now *C. thermohydrosulfuricum* proved much efficient than *C. acetobutylicum*, and both cultures reached upto maximum value of 5.78 and 6.99 mA respectively after 30 hrs.

For Sample C2 (Fig. 1e), which was made by addition of 10% sucrose and 0.5% methylene blue in plain diluted waste water (Sample A), both cultures started fermentation after 3 hrs. Curves show random suitability of sucrose for *C. acetobutylicum* and *C. thermohydrosulfuricum* both, but *C. acetobutylicum* proved much efficient for this sample up to 18 hrs. Both cultures reached to a maximum current output of 6.5 and 7.25 mA for *C. acetobutylicum* and *C. thermohydrosulfuricum* respectively after 21 hrs. A major current drop observed in *C. acetobutylicum* after 21 hrs., while *C. thermohydrosulfuricum* showed a minor fall of current. Current recovered quickly when 50% part of bacterial medium was replaced with a syringe through the anode by 10% glucose. Now as in Sample B2 *C. thermohydrosulfuricum* proved much efficient than *C. acetobutylicum*, and both cultures reached upto maximum value of 7.23 and 7.92 mA respectively after 30 hrs.

Conclusion

Although Cellulose and Cellulosic wastes are not ideal substrates for electricity generation in MFCs as their insolubility and chemical stability limits the rate of microbial substrate decomposition and thus the current output that can be achieved, yet they provide good results with readily oxidizable substrate like glucose, starch. Methylene blue acts as an electrophore and support current generation. As in the experiments, Sample C1 which was made by addition of 10% glucose and 0.5% methylene blue solution in plain diluted waste water gave best results. Major issue to be solved for practical application is to overcome the activity loss and incomplete cellulose fermentation. Such activity losses are typical for bacteria of the *Clostridium* family and are most likely caused by the increasing concentrations of acidic fermentation products, leading to changes in the fermentation path away from hydrogen synthesis towards alcohol generation and finally to the inhibition of further substrate decomposition (Desvaux et al. 2000; Hawkes et al. 2002; Valdez-Vazquez et al. 2004). Study with some other anaerobic bacteria for same purpose may solve the problem.

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