Bioresource Technology 100 (2009) 5132–5139

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber

Sunil A. Patil [a], Venkata Prasad Surakasi [b], Sandeep Koul [a], Shrikant Ijmulwar [a], Amar Vivek [a], Y.S. Shouche [b], B.P. Kapadnis [a,*]

[a] Department of Microbiology, University of Pune, Pune 411 007, Maharashtra, India
[b] Molecular Biology Unit, National Centre for Cell Science, Pune 411 007, Maharashtra, India

ARTICLE INFO

*Article history:*
Received 14 March 2009
Received in revised form 18 May 2009
Accepted 19 May 2009
Available online 17 June 2009

*Keywords:*
Microbial fuel cell
Chocolate industry wastewater
Activated sludge
Wastewater treatment
16S rDNA microbial analysis

ABSTRACT

Feasibility of using chocolate industry wastewater as a substrate for electricity generation using activated sludge as a source of microorganisms was investigated in two-chambered microbial fuel cell. The maximum current generated with membrane and salt bridge MFCs was 3.02 and 2.3 A/m$^2$, respectively, at 100 Ω external resistance, whereas the maximum current generated in glucose powered MFC was 3.1 A/m$^2$. The use of chocolate industry wastewater in cathode chamber was promising with 4.1 mA current output. Significant reduction in COD, BOD, total solids and total dissolved solids of wastewater by 75%, 65%, 68%, 50%, respectively, indicated effective wastewater treatment in batch experiments. The 16S rDNA analysis of anode biofilm and suspended cells revealed predominance of *β-Proteobacteria* clones with 50.6% followed by *unclassified bacteria* (9.9%), *α-Proteobacteria* (9.1%), *other Proteobacteria* (9%), *Planctomycetes* (5.8%), *Firmicutes* (4.9%), *Nitrospora* (3.3%), *Spirochaetes* (3.3%), *Bacteroides* (2.4%) and *γ-Proteobacteria* (0.8%). Diverse bacterial groups represented as members of the anode chamber community.



## 1. Introduction

Use of fossil fuels has contributed to global climate change, environmental degradation and health problems. In this context, many researchers predict that biohydrogen and bioelectricity could play an important role as fuel in the near future. Biological hydrogen and electricity could team to provide attractive options in transportation and power generation. Microbial fuel cell (MFC) technology offers the possibility of harvesting electricity from organic waste and renewable biomass. It has been developed as a novel technique to gain energy with simultaneous wastewater treatment. Efforts have been dedicated by researchers towards the development of MFCs for the electricity generation in last decade. Various carbohydrates and industrial wastewaters have been tried for production of electricity (Catal et al., 2008; Liu et al., 2004). The MFC research is strongly growing with respect to alternative substrates, potent microbial cultures, electrodes, ion exchange membranes and anodic electron transfer mechanisms (Schroeder, 2007).

The pure microbial cultures have limitations for technical application because of necessity for highly sterile conditions, culture degradation and high cost. Mixed cultures or microbial consortia have been shown to be robust and more productive than single strains and their extraction can be easily achieved from natural sources (Ha et al., 2008). The microbial communities in MFCs with different types of substrates and microbial sources have been analyzed to study the involvement of microorganisms in electricity generation (Holmes et al., 2004; Jung and Regan, 2007). Several groups have characterized active microbial communities at anode to potentially improve MFC performance by addressing microbial constraints. Various phylogenetically diverse bacteria are reported to generate electricity in MFCs (Logan and Regan, 2006; Ha et al., 2008). However, there is much scope for improvement in the performance of MFCs using microorganisms from natural habitats.

Due to environmental concerns, there is a great interest in developing new methods either to reduce the treatment costs or to get value added products from wastes. The MFC technology proved to be a novel approach for treating wastewater with simultaneous production of electricity (Venkata Mohan et al., 2008). The chocolate manufacturing industry wastewater can be characterized as nontoxic because it is devoid of hazardous compounds, but with high content of total solids (TS), biochemical oxygen

* Corresponding author. Tel.: +91 20 25690643; fax: +91 20 25690087.
*E-mail address:* bpkapadnis@yahoo.com (B.P. Kapadnis).

0960-8524/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2009.05.041

demand (BOD) and chemical oxygen demand (COD). To our knowledge, there are no reports on electricity generation using chocolate industry wastewater with microbial consortia from natural habitats. Therefore, in the present work the chocolate industry wastewater was investigated as a substrate for generation of electricity in both salt bridge and proton exchange membrane MFCs using activated sludge as a source of microorganisms. The electricity generated using wastewater was compared with that of glucose as a substrate. The use of same wastewater was tried as a catholyte in the cathodic chamber and performance was compared with air cathode and $K_3[Fe(CN)_6]$. Phylogenetic analysis of microbial communities from anode chamber of the MFC was carried out to study the microbial diversity.

## 2. Methods

### 2.1. Substrate and source of microorganisms

The chocolate industry wastewater was collected from the Chocolate India Malted Food Division, Warananagar (India). The source of microorganisms was activated sludge from Municipal wastewater treatment plant, Kolhapur, India. All chemicals (analytical or biochemical grade) and other materials used in MFC experiments were purchased from Merck and SD fine.

### 2.2. Experiments using salt bridge and membrane MFCs

The two-chambered MFCs were used throughout the study. The experiments were carried out in batch mode using salt bridge and proton exchange membrane MFCs composed of two 400 ml volume bottom flasks. The salt bridge MFC was constructed by joining two bottles with glass tube (5 cm length; 0.6 cm inner diameter) prepared using agar and KCl powder (1:1). In proton exchange membrane MFC, membrane (Nafion-117) of 2.5 cm diameter was clamped between anode and cathode compartments. The electrodes used were graphite rods (projected area of 16.485 $cm^2$). The anode part of the both MFCs was filled with 200 ml chocolate industry wastewater as substrate and 20 ml activated sludge as microbial inoculum, obtained from previously enriched MFC to reduce the start up time. The 100 mM $K_3[Fe(CN)_6]$ prepared in 100 mM $KH_2PO_4$ buffer (pH 7.5) was used in the cathode chamber of both MFCs. The anode chamber and cathode chamber with ferricyanide as catholyte were maintained at anoxic conditions. The voltage generated in MFCs was measured by using a voltammeter with an external resistor (100 Ω) connected across the electrodes and recorded after regular time intervals. The voltage values were recorded only after a steady state. Current ($i$) was calculated using relationship, $i = V/R$, where $V$ is the voltage and $R$ is resistance. Power ($P$) was calculated using the relationship, $P = iV$, and normalized by the surface area of the anode. All experiments were conducted at 28 ± 2 °C.

### 2.3. Electricity generation using synthetic medium with glucose as substrate

These experiments were conducted with proton exchange membrane MFC using synthetic medium containing glucose as a substrate instead of chocolate industry wastewater, with activated sludge in the anode chamber. The ingredients of medium included (g/l): KCl (0.74), NaCl (0.58), $KH_2PO_4$ (0.68), $K_2HPO_4$ (0.87), $NH_4Cl$ (0.28), $MgSO_4 \cdot 7H_2O$ (0.1), $CaCl_2 \cdot 2H_2O$ (0.1), and to this, 1 ml of trace element mixture was added (Zehnder et al., 1980). We used 2.0 g/l glucose as a carbon source in the synthetic nutrient medium. The pH of the medium was adjusted to 6.8. The other experimental conditions were same as mentioned earlier for previous experiments.

### 2.4. Testing of different catholytes in the membrane MFC

Different catholytes viz. $K_3[Fe(CN)_6]$, chocolate industry wastewater and phosphate buffered air cathode were tested in the cathode chamber and performance of the MFCs was studied with respect to these catholytes. Chocolate industry wastewater and activated sludge were used in the anode compartments of all these MFCs. The electrodes used in cathode chamber were graphite rods. The ferricyanide (100 mM) was prepared in 100 mM $KH_2PO_4$ buffer (pH 7.5). Phosphate buffer (10 mM $KH_2PO_4$) was used in air cathode experiments and it was continuously sparged with air applying sparger. In case of chocolate industry wastewater as catholyte, the cathode chamber was also sparged with air. These experiments were carried out with membrane MFC and other experimental conditions were same as mentioned earlier.

### 2.5. Analysis of various parameters of chocolate industry wastewater

The characteristics of chocolate industry wastewater before using it in MFC are presented in Table 1. Various parameters of wastewater viz., pH, COD, BOD, total solids, total dissolved solids, acidity and electrical conductivity, were experimentally determined before and after experimental runs using standard methods (APHA, 1995) to monitor progress of wastewater treatment. This study was conducted with the samples from proton exchange membrane MFC. All experiments were repeated and the mean values were considered for the all calculations.

### 2.6. Phylogenetic analysis of microbial communities from anode of MFC

#### 2.6.1. DNA extraction

The DNA was extracted from the scraped anode biofilm and suspended cells from anode chamber (at the completion of a batch cycle) of MFC using protocol explained earlier (Yeates et al., 1997). Some minor modifications were introduced such as cell pellet of 2 ml enrichment culture was suspended in extraction buffer (100 mM Tris–HCl (pH 8.0), 100 mM $Na_2EDTA$ (pH 8.0) with 20 mg/ml lysozyme (Sigma–Aldrich Co., USA) and incubated for 2 h at 37 °C with continuous shaking. Proteinase K (Invitrogen, USA) was added to a final concentration of 100 μg/ml and incubated further at 55 °C for 2 h with continuous shaking. The NaCl (0.5 M) was added and incubated at 72 °C for 30 min. Subsequently DNA was extracted by phenol and chloroform:isoamyl alcohol (24:1) method as per Sambrook et al. (1989). DNA pellet was washed with 70% ethanol. The product was dissolved in TE buffer (pH 8.0).

#### 2.6.2. Amplification, cloning, and sequencing of 16S rDNA

The 16S rDNA was amplified from extracted DNA using universal bacterial primers 530F and 1490R as described previously (Wani et al., 2006). In the above PCR reactions, the number of cycles was reduced to 25 so as to minimize PCR bias. The PCR

**Table 1**
Chocolate industry wastewater parameters before and after feeding the MFC.

| Parameter | Parameter | |
|---|---|---|
| | Before | After |
| pH | 5.76 | 6.35 |
| COD (mg/l) | 1459 | 368 |
| $BOD_3$ at 27 °C (mg/l) | 640 | 230 |
| Total solids (mg/l) | 2344 | 754 |
| Total dissolved solids (mg/l) | 404 | 170 |
| Acidity (mg/l) | 0.388 | 0.582 |
| Electrical conductivity (μmhos/cm) | 0.44 | 0.51 |