Fuel 143 (2015) 72–79

Contents lists available at ScienceDirect

Fuel

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

Enhanced power generation using controlled inoculum from palm oil mill effluent fed microbial fuel cell

E. Baranitharan [a], Maksudur R. Khan [a,*], Abu Yousuf [b], Wee Fei Aaron Teo [c], Geok Yuan Annie Tan [c], Chin Kui Cheng [a]

[a] Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang, 26300 Pahang, Malaysia
[b] Faculty of Engineering Technology, University Malaysia Pahang, 26300 Pahang, Malaysia
[c] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

HIGHLIGHTS

- A new form of inoculum called controlled inoculum was made from palm oil AS.
- Controlled inoculum consists of both fermentative and electrogenic microorganisms.
- The inoculum showed higher power density and coulombic efficiency than AS.
- EIS analysis showed significant reduction of anode R_{ct} using controlled inoculum.

ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 1 November 2014
Accepted 8 November 2014
Available online 20 November 2014

Keywords:
Microbial fuel cell
Polyacrylonitrile carbon felt
Controlled inoculum
Palm oil mill effluent
Electrogen

ABSTRACT

Enhancing the anode performance is a critical step for improving the power output of MFCs. This study deals with the dual chamber MFCs to increase the power generation using the controlled inoculum in Palm oil mill effluent (POME). Controlled inoculum (CI) was made using the predominant microorganisms such as *Pseudomonas aeruginosa*, *Azospira oryzae*, *Acetobacter peroxydans* and *Solimonas variicoloris* isolated from palm oil anaerobic sludge (AS) as well as from biofilm of MFC anode operated with AS and identified using BIOLOG gene III analysis, PCR, DGGE and sequencing. Biofilm formation on electrode was investigated by Fourier Transform Infrared spectroscopy (FTIR) and Thermogravimetric analayis (TGA). The MFC operated with Polyacrylonitrile carbon felt (PACF) anode and CI reached the maximum power density of 107.35 mW/m^2, which was two times higher as compared to MFC operated with usual anaerobic sludge as inoculum. The maximum coulombic efficiency (CE) of 74% was achieved from the MFC with CI, which was 50% higher than the CE with anaerobic sludge. But, it showed lower COD removal efficiency of about 32%, which might be due to the absence of required fermentative microorganisms in CI to utilize POME. The electrochemical activities have been investigated by electrochemical impedance spectroscopy (EIS). EIS and the simulated results showed the significant reduction of charge transfer resistance (R_{ct}) by ~40% during the operation of the cell with CI. EIS results provided evidence that there was a substantial improvement in electron transfer between the microorganisms and the anode with CI. These results demonstrate that the power output of MFCs can be increased significantly using CI.

1. Introduction

Energy needs in the world continue to increase and in an effort to aid energy independence, research initiatives are focused on alternate, renewable and carbon neutral energy sources. Production of electrical energy using microorganisms through microbial fuel cells (MFCs) is one such renewable and sustainable technology that is considered to be one of the most efficient [1,2] and carbon neutral energy sources [3]. MFCs are fuel cells that are capable of converting chemical energy available in organic substrates into electrical energy using bacteria as a biocatalyst to oxidize the biodegradable substrates. The fact that bacteria can oxidize the substrates to produce electricity makes MFCs an ideal solution for wastewater treatment and domestic energy production [4]. The MFC is still at an early research stage, but it can be a revolutionary breakthrough for capturing renewable energy sources [5]. Understanding the fundamental relationship between the microbiology and electrochemistry is critical to the advancement of this technology. It was found that the cell count, biofilm density and the type

* Corresponding author. Tel.: +60 9 5492872; fax: +60 9 5492889.
E-mail addresses: mrkhancep@yahoo.com, mrkhan@ump.edu.my (M.R. Khan).

http://dx.doi.org/10.1016/j.fuel.2014.11.030
0016-2361/

of communities in the anode biofilm had a direct influence on the power output of microbial fuel cells. Also the power density was directly dependent on the biofilm growth that increased significantly during the initial biofilm growth period [6,7]. Current outputs of microbial fuel cells (MFCs) are too low for most perceived practical applications. Most efforts for further optimization have focused on modifications of fuel cell architecture or electrode materials, with little investigation in the inoculum that are most essential for maximal current production [8]. Usually anaerobic sludge (AS) has been used as inoculum in the MFC experiments when wastewater is used as substrate but many unwanted microorganisms, which could reduce the MFC performance also present in the sludge. Besides that, in most of the MFC experiments, single substrate (Glucose, acetate) and known microorganisms (*Geobacter sulfurreducens*, *Shewanella oneidensis*) has been used which showed high power generation [9]. But the same high power producing microorganisms when used in wastewater showed less power density. This is due to the presence of different kinds of substrates in the wastewater, which makes specific microbes not suitable to be used as inoculum when wastewater is used as substrate.

In the recent MFC studies, it was found that biofilm plays an important role in electricity production [10,11] but predominant microorganisms present in it was not identified which are crucial for the high power generation. Moreover, in order to utilize wastewater, fermentative microorganisms are greatly needed in the inoculums. Katuri et al. [12] operated MFC with anaerobic sludge as inoculum for batch studies, after 3rd cycle of operation; substrate was refilled without anaerobic sludge. During that refilling period, current density increased and within a short period it retained back to its previous current density level. This shows that the necessary fermentative bacterial population needed to maintain power is retained in the anodic biofilm. But unfortunately fermentative microorganisms cannot completely oxidize the substrate and therefore high power cannot be achieved. To achieve complete oxidation, electrogenic microorganisms are needed [13] however, electrogenic microorganisms cannot utilize all the substrates. Therefore, the presence of atleast one electrogen in the inoculum is an important requirement for microbial fuel cells to oxidize organic substrates effectively to achieve maximum electricity. So far, only few studies have been reported about the use of biofilm as inoculum from the MFC run with AS [12,14] which is essential to enhance MFC performance. However, upto our knowledge, there is no study reported the use of combination of fermentative and electrogenic microorganisms as inoculum in MFC operation.

In this work, we prepared the controlled inoculum which consists of both fermentative and electrogenic microorganisms. Fermentative bacteria consume complex organic substrates and produce intermediate products. Whereas, electrogenic microorganisms can consume those intermediate products produced by fermentative organisms and also certain molecules like acetate and hydrogen and completely oxidize them. This synergistic interaction plays an important role in electricity production and wastewater treatment in MFC. Moreover, the performance of double chamber MFC with CI and also its power generation, COD removal efficiency and Coulombic efficiency were investigated. Electrochemical impedance spectroscopy (EIS) was used to study the correlation between the controlled inoculum and the electrochemical performance of microbial fuel cells operated for 14 days.

2. Materials and methods

2.1. MFC construction

MFCs consisting of two cylindrical compartments were made using glass material (borosilicate) and fabricated in China (Shangai sunny scientific, China). A piece of Nafion 117 membrane (Dupont Co., USA) was placed between the anode and cathode chambers to electrically separate the anode and cathode compartments while permitting proton transport. PACF (Polyacrylonitrile carbon felt) was purchased from Shangai sunny scientific, China and used as electrode material for all the experiments. Equal size of PACF (3.8 cm × 0.9 cm × 4 cm) was used as electrode in each compartment of dual chamber MFC. Prior to use, PACF was washed several times with water to remove impurities and Nafion membrane was drenched overnight in dilute HCL followed by washing with DI water for several times. The anode chamber was filled with 450 mL of POME which was collected before it discharged into the collection pond or mixing pond from palm oil industry effluent outlet and inoculated with CI isolated from AS of Felda Panching Timur, Kuantan, Malaysia. The cathode chamber was filled with 450 mL of potassium permanganate solution and its concentration was kept constant throughout the experiment. All MFC materials were sterilized prior to use by autoclave, bleach, or ethanol treatment and the cell components were assembled under sterile water. The anode and cathode electrodes were connected by using copper wires with a resistor to form a circuit. All experiments were carried out at the constant temperature (29 ± 0.5 °C) with a 1 kΩ external resistance connected unless otherwise specified.

2.2. Measurement and analyses

The voltage (V) and current (I) across an external resistor (1 kΩ) in the MFC circuit was continuously monitored (15 min intervals) using a multimeter with data logger (Fluke 289 True RMS Multimeter, USA) connected to the computer through USB cable adapter. To obtain polarization data, the external resistance was varied from 50 to 20,000 Ω. Current density (I) was calculated from $I = V/R$, and normalized by surface area. Power density normalized by surface area (P_A, W m^{-2}) were measured and calculated using the following equations

$$P = VI \quad (1)$$

$$P = V^2/AR \quad (2)$$

COD was periodically checked by taking small amount of sample from the anode effluent for every 24 h. The COD removal efficiency (η) was calculated as described by Baranitharan et al. [15] and the CE of the complex substrates was calculated following Logan et al. [5].

2.3. DNA extraction, PCR, cloning and sequencing

Extraction of total genomic DNA from the biofilm was done using a soil extraction kit (Nucleospin soil, Macherey – Nagel, Germany). For the identification of bacterial community, the bacterial 16S rRNA genes were amplified with primer pair 27f and 1492r [16] using the Veriti® 96-Well Thermal Cycler (Applied Biosystems, USA). Amplification was carried out in 50 μl PCR reaction mixtures consisting 100 ng of DNA template, 1.5 mM $MgCl_2$, 0.2 mM nucleotide mix, 0.2 μM of each primer and 1.25U of GoTaq® DNA Polymerase (Promega, USA) with the following cycling condition: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 30 s; and a final extension at 72 °C for 10 min. PCR products were then cloned with TOPO® TA Cloning® Kit for Sequencing, with One Shot® TOP10 Chemically Competent *Escherichia coli* (Invitrogen™, USA). Clone libraries were analysed by PCR with M13 forward and reverse primers, and nested PCR with primer pair GC341f and 517r were carried out for DGGE analysis [17] Nested PCR products were analyzed by DGGE with a denaturing gradient