Fuel 125 (2014) 30–35

Contents lists available at ScienceDirect

Fuel

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

Effects of different mediators on electricity generation and microbial structure of a toluene powered microbial fuel cell

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HIGHLIGHTS

- A toluene powered microbial fuel cell was constructed.
- Potassium ferricyanide and neutral red could elevate the power generation efficiency.
- Toluene degradation time of the mediated MFCs was increased.
- DGGE analysis showed a mediator-related characteristic of the MFC.

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 7 February 2014
Accepted 11 February 2014
Available online 22 February 2014

Keywords:
Microbial fuel cell
Mediator
Toluene
Electricity generation
Microbial community structure

ABSTRACT

Neutral red and potassium ferricyanide are two electron mediators that can increase the efficiency of microbial fuel cells (MFCs) via significantly different mechanisms. In this study, effects of the two mediators on electricity generation and microbial structure of a toluene powered MFC was first investigated. The maximum voltage (V_{max}) was 110.4 mV and the half-saturation constant (K_s) was 12.8 mg/L in the absence of mediators. Although the power generation efficiency was increased when adding modest amount of mediators to the anode, the toluene degradation time was 1.56–2.15 times longer than that of unmediated MFC's, relying on the mediator used. Denaturing gradient gel electrophoresis (DGGE) analysis showed a mediator-related characteristic of the microbial structure in the MFC. Results of this investigation can be used as a basis for future assessment and design of MFCs powered by xenobiotics-contaminated wastewater, such as toluene.

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1. Introduction

There has been a steep rise in the demand of the petroleum fuels because of increasing industrialization and motorization. Because current reliance on petroleum-based fuels is unsustainable due to limited resources, new alternative energy sources are urgently needed. In developing these alternatives, it is imperative that emphasis be put on sustainable yet environmentally friendly technologies in order to avoid supply problems and contributions to global warming.

It has been known for several years that bacteria can be used to generate electricity from many different substrates. The electricity produced biochemically can then be harvested in microbial fuel cells (MFCs) [1]. Virtually any source of biodegradable organic matter can be used as a fuel in an MFC for power generation. The flexibility of microorganisms to use a range of substrates makes the MFC an ideal technology for renewable bioelectricity generation from wastes and biomass [2]. To this end, research toward the aspect of simultaneous pollutant treatment and power generation has attracted more attention due to their green characteristics. A novel MFC design was described for petroleum hydrocarbon biodegradation in anaerobic sediment or groundwater with anaerobic overlying water, which could achieve voltages as high as 190 mV (2162 mW/m^3) [3]. MFCs using phenol or glucose–phenol mixtures as substrates (fuel) were also designed to investigate the biodegradation of phenol. The maximum power densities were 9.1 and 28.3 W/m^3 for MFCs using phenol and glucose–phenol mixture as the fuel, respectively [4]. The power generation potential of a MFC during the process of benzene biodegradation with potassium ferricyanide as the terminal electron acceptor had been evaluated by Wu et al. [5].

Several factors can affect the power generation efficiency of a microbial fuel cell, such as the community structure of

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http://dx.doi.org/10.1016/j.fuel.2014.02.018
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microorganisms [6], the material of electrodes [7] and the addition of mediators [8,9]. The basic requirement for the establishment of an MFC is the electron transfer from a microbial cell to an electrode, either via direct electron transfer or via mediated electron transfer [10]. For a direct electron transfer, a physical contact of the bacterial cell membrane or a membrane organelle with the fuel cell anode is involved, but no diffusional redox species is required for the electron transfer from the cell to the electrode. For a mediated electron transfer, exogenous (artificial) redox mediators, such as potassium ferricyanide (FeCN) [11], neutral red (NR) [8], thionine [12] and methylene blue [13] or secondary metabolites, such as phenazines and pyocyanine [14], represent an effective means to wire the microbial metabolism to a fuel cell anode.

The mechanism of how mediators can improve the performance of an MFC depends on many factors, such as their molecular structures, abilities to dissociate and polarities [15]. NR has a redox potential of −325 mV, which is similar to that of NADH (−320 mV), and is highly permeable to the lipid bilayer [16]. On the other hand, ferricyanide (FeCN) is unable to cross the plasma membrane [17,18]. Park et al. showed immobilizing NR on the anode can improve the performance of an MFC using *Escherichia coli* [19]. Similarly, Wang et al. used amidation procedure to increase the amounts of NR molecules immobilized on the anode and showed it can significantly increase the power output [20]. Barnett Cohen found that the capacity of a potentiostat-poised half-cell could be improved by introducing potassium ferricyanide as an electron mediator in the anode [21]. The addition of ferricyanide to carbon/graphite electrodes greatly improves the electron transfer with a performance comparable to platinum [22,23].

Although the influence of microbial community structures on power generation efficiency is a crucial issue, most studies focused on assessing the effect of different substrates, such as glucose [24], synthetic wastewater [25], and cysteine [26], on the microbial structures in MFCs. Effects of different mediators on the microbial structure in a MFC are rarely discussed. Therefore, it is difficult to relate the changes of microbial community structure with power generation outcomes when exogenous redox mediators are used. Herein, polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) was employed to assess the impact of two different widely used model mediators (FeCN and NR) on the microbial structure in a MFC fed with toluene, a typical pollutant commonly found in petroleum contaminated sites and media.

2. Materials and methods

2.1. MFC construction and operation

As shown is Fig. 1, the dual-chambered MFC was modified from Logan [1]. Both chambers, each with a volume of 0.8 L, were made from serum bottles. DuPont™ Nafion1 117 was used as the proton exchange membrane (PEM) with an actual reaction area of 16.6 cm^2 (2.3 cm × 2.3 cm × 3.14). The side-arms of the two serum bottles were held tightly to the PEM and silicone washers using a clamp. The PEM was heated at 80 °C for 1 h in 10% hydrogen peroxide solution, washed several times using deionized water, then dipped in 1 M sulfuric acid for 1 h to remove organic residues and stored at room temperature before use. Three holes were drilled into each bottle cap for electrode connection, substrate injection, and sample extraction. Both anode and cathode were carbon cloth (B1B, Hephas Energy, Taiwan) of the dimension 8 cm × 54 cm and were connected to a 1 kΩ external resistor in a loop. The cathode was used without modification with metals such as Pt to avoid an interference of the MFC operation by hydrogen production. The carbon cloth was soaked in 15% nitric acid solution and heated for 1 h to remove any organic matter before use [27].

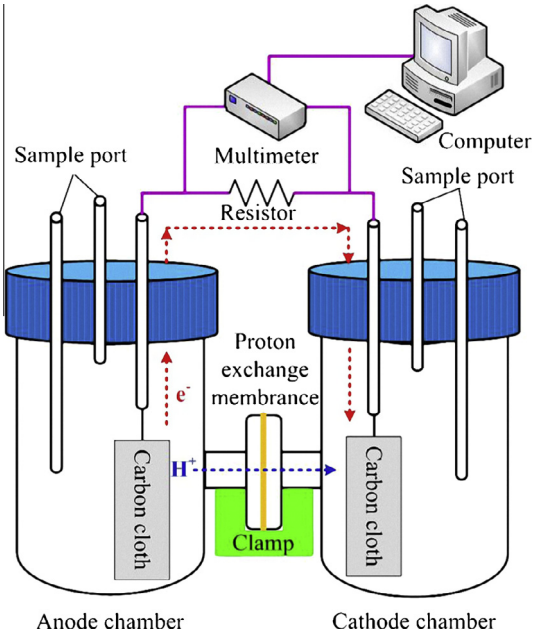

Fig. 1. Schematic diagram of the dual-chambered microbial fuel cell used in this study.

The cells were assembled by sealing the PEM between the anode and cathode chambers using silicone washers.

2.2. Microorganisms and medium

Sludge samples were obtained from the oil cracking wastewater treatment plant of Nan-Ya Plastics (Six Naphtha Cracking Industry Site at Mailiao of Yunlin County, Taiwan). The sludge samples were obtained using a sterilized stainless steel core tube and were then transported back to the laboratory and inoculated into an air cathode-type MFC at room temperature (about 30 °C) for pre-culture. Nitrogen gas was used to flush the MFC system to make the environment anoxic with a dissolved oxygen concentration of <1.0 mg/L. BTEX (benzene, toluene, ethylbenzene and xylene) of 7 mg/L each was used as the sole carbon source in a long-term acclimation culture to obtain a mixed culture that degrades BTEX effectively. Phosphate buffer was used to maintain a pH of 7 to ensure that the environment was suitable for bacterial growth. Each liter of the medium/anolyte contained K_2HPO_4 1.75 g, KH_2PO_4 2.145 g, NH_4Cl 10 mg, $MgCl_2{\cdot}6H_2O$ 100 mg, $CaCl_2$ 45 mg, $FeCl_3{\cdot}6H_2O$ 1 mg, $CuCl_2{\cdot}2H_2O$ 0.25 mg, $CoCl_2{\cdot}6H_2O$ 0.25 mg, $ZnCl_2$ 1 mg, $MnCl_2{\cdot}4H_2O$ 1 mg, $Na_2MoO_4{\cdot}2H_2O$ 0.1 mg, $NiCl_2{\cdot}6H_2O$ 0.02 mg. The MFCs was operated in a temperature-controlled lab at 30 °C. The same solution amended with up to 50 mM of potassium ferricyanide was used as the catholyte.

2.3. Chemicals and analysis of toluene concentration

Benzene was purchased from Echo Chemical (Germany), toluene was obtained from Fisher Chemical (USA), and both ethylbenzene and xylene were obtained from Tedia Company (USA). The BTEX compounds were of HPLC purity, the remaining compounds were of analytical purity. All chemicals were of analytical grade and were used without further purification.

As toluene is a highly volatile organic compound, 250 μL headspace gas was sampled via gas-tight syringe (Hamilton, USA), and then analyzed using a GC apparatus that was equipped with a flame ionization detector (GC-FID, Shimadzu Corp., GC-14B, Japan) and a capillary column (15 m × 0.53 mm ID × 3 mm film thickness,

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