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Enhancement of power generation by toluene biodegradation in a microbial fuel cell in the presence of pyocyanin

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ABSTRACT

Toluene degradation and electricity generation were examined under varying toluene doses. Addition of pyocyanin was evaluated for effectiveness in improving electricity generation *via* toluene degradation, and the mechanisms of improved electricity generation were analyzed. Supplemental pyocyanin achieved a 3.64-fold increase in maximum power density from 4.69 to 21.76mW/m^2 and a 13-fold increase in Coulombic efficiency (CE) from 0.83% to 11.62%. Addition of pyocyanin significantly improved electricity generation by reducing system impedance, increasing electron density and substantially lowering the internal resistance of MFC (from 500 to 100 Ω). The results of this investigation not only demonstrated the possibility of adding pyocyanin for enhanced power production in a toluene-fed MFC but also can be used as a basis for future assessments of the power generation capacity of MFCs that were used to treat xenobiotics-contaminated wastewater, such as toluene.

1. Introduction

Toluene is a common solvent and gasoline additive and is also used as a raw material for producing benzene and xylene. Therefore, the global consumption of toluene has now reached 2×10^7 tons annually [1]. The production, application and conversion of toluene into benzene, xylene and various intermediates make it a common constituent of wastewater from many industrial processes [2]. Because toluene is a toxic, refractory and mutagenic pollutant, removing the resulting wastewaters is critical for safe water supplies [3]. Conventional toluene removal processes include adsorption by activated carbon, decomposition by advanced oxidization technique or biodegradation by certain aerobic or anaerobic microorganisms [3–6].

Microbial fuel cells (MFCs) are a recently emerging technology for converting chemical energy of substrates to electrical energy through catalytic reactions by electro-active microorganisms [7,8]. By oxidizing substrates, live microorganisms in the anode chamber of MFCs generate electrons, protons, and other metabolic products; electrical energy is then generated by transferring the electrons yielded by the microorganisms to the cathode *via* external circuits [9]. The MFCs are considered a promising sustainable technology that can help to meet increasing energy needs, especially since the wastewaters can be used as substrates for simultaneously generating electricity and treating wastewater [10]. It is well known that the readily degradable compounds such as glucose or wastewaters such as brewery wastewater were preferentially used as MFC fuel for generation of electricity. However, many recalcitrant wastes, including dyes, pesticides, explosives, polyalcohols, furan derivatives and phenolic substances, also can be used as the fuel substrates of MFCs for both biodegrading pollutants and for generating electricity in recent years [11,12]. The use of toluene as a MFC fuel for biodegrading pollutant and generating electricity has not been reported until Li et al. [13]. However, the enhancement of power generation by toluene biodegradation in MFCs in the presence of pyocyanin has not been fully explored.

Although MFCs offer the possibility of generating electricity from many organic wastes and from renewable biomass, current and power yields are relatively low, which limits their practical applications [11,12]. A major bottleneck in attempt to improve the power output of MFCs is electron transfer from the bacteria to the anode [14–16]. Electron transfer between bacteria and electrodes is generally achieved by membrane-associated cytochromes [17], conductive pilus-like appendages [18,19], or by mobile redox

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mediators [19,20]. Phenazines produced by some *Pseudomonas* species as metabolites function as electron shuttles from the bacteria to the anode. However, the enhancing effects of phenazines on biodegradation and energy conversion of recalcitrant pollutants, including toluene in MFCs, have not been reported.

To evaluate the potential use of toluene fuel for simultaneous toluene biodegradation and electricity generation in MFCs, this study had three objectives: (1) to examine MFC performance in terms of toluene degradation and electricity generation under varied toluene concentrations; (2) to evaluate the performance of toluene degradation and electricity generation when pyocyanin is used as electron shuttle in MFC; (3) to reveal the characteristics of toluene degradation and electricity generation by pyocyanin.

2. Materials and methods

2.1. Microorganisms and chemicals

Exoelectrogens consortia were used for toluene degradation and electricity generation. The consortia were isolated from activated sludge from the Oil Cracking Wastewater Treatment Plant, Nanya Plastics Co., Ltd., Yunlin County, Taiwan, which was subjected to long-term exposure to BTEX (benzene, toluene, ethylbenzene and xylene). Biodegradation of BTEX and generation of electricity by the consortia were performed in a single-chamber MFC with air cathode for more than one year. The consortia were grown in the same single-chamber MFC to enable regular maintenance and inoculation.

Toluene was of HPLC grade with purity of 99.9% (ECHO Chemical, Taiwan), pyocyanin was of HPLC grade with purity of 98% (Sigma–Aldrich, USA), and potassium ferricyanide was of reagent grade with purity of 98% (Showa, Japan). All other chemicals were of analytical grade.

2.2. Media and culture of the consortia

The growth media for maintaining and harvesting the exoelectrogens consortia were prepared by adding 28.0 mg/L BTEX with approximately equal concentrations (7.0 mg/L) of each compound into inorganic salt media containing 0.95 g NH_4Cl, 4.27 g K_2HPO_4, 3.48 g KH_2PO_4, 0.53 g $MgCl_2{\cdot}6H_2O$, 0.972 g $FeCl_3{\cdot}6H_2O$, 0.018 g $CaCl_2{\cdot}2H_2O$, 0.01 mg $CuCl_2{\cdot}2H_2O$, 0.2 mg $CoCl_2{\cdot}6H_2O$, 0.047 mg $ZnCl_2$, 0.03 mg $MnCl_2{\cdot}4H_2O$, 0.03 mg $Na_2MoO_4{\cdot}2H_2O$, 0.02 mg $NiCl_2{\cdot}6H_2O$, 1.0 L of deionized water at pH 7.0. The consortia were grown in the single-chamber MFC at a room temperature of approximately 28 °C. Twenty microliter of cell suspension (about 28.35 mg/L in wet weight; about 0.032 in optical density) was collected by centrifugation and washed three times with sterile water to remove the remaining BTEX. The resulting biomass pellets served as inocula for toluene degradation and electricity generation in the two-chamber MFC.

The electricity-generated media using toluene as sole carbon and energy source were obtained by adding toluene into the above inorganic salt media to obtain initial concentrations of 11–55 mg/L in the batch experiments. One milligram per liter of pyocyanin was further added into the media containing 55 mg/L of toluene to evaluate the enhancing effects of phenazines on biodegradation and energy conversion. A control sample of pyocyanin without addition of toluene was used to investigate the biodegradability of pyocyanin by the exoelectrogens consortia.

2.3. MFC configuration and operation

The two-chamber MFC was a modification of the fuel cell reported in Logan et al. [8]. Serum bottles of 0.8 L were used for both chambers. Three holes were drilled in the cap of each bottle for electrode connection, substrate injection and sample extraction. The DuPont™ Nafion® 117 (reaction area, 2.3 cm × 2.3 cm × 3.14) was used as the proton exchange membrane (PEM), which was heated in 10% hydrogen peroxide solution at 80 °C for 1 h and subsequently washed several times using deionized water at room temperature prior to use. The anode and cathode (dimensions 8 cm × 5 cm), both of which were composed of carbon cloth (B1B, Hephas Energy Co., Ltd., Taiwan), were connected to a 1 kΩ external resistor in a loop. Before use, the carbon cloth was soaked in 15% nitric acid solution and heated for 1 h to remove any organic matter. After sealing the PEM between the anode and cathode chambers with a silicone gasket, the two-chamber MFC was assembled by tightly clamping the side-arms of the two serum bottles to the PEM and silicone gasket.

The toluene media used to generate electricity and the 50 mM of potassium ferricyanide solution used as electron acceptor were injected into the anode and cathode chamber, respectively. After a 24-h period to stabilize the system, the toluene biodegradation and electricity generation processes were initiated by inoculating the biomass pellets of the exoelectrogens consortia into the anode chamber at a room temperature of 28 °C. During the process of electricity generation by toluene biodegradation, data collection included voltage, polarization curve, power density curve, cyclic voltammetry (CV), toluene dose, pyocyanin content, total organic carbon (TOC), pH and oxidation–reduction potential (ORP) of the anode chamber.

2.4. Electrochemical analysis of the MFC

The MFC anode was periodically sampled for CV analysis with a constant potential rectifier (SP150, Biologic, France). Voltage measurements of the two-chamber MFC were performed with a digital electronic multimeter (CHY-48R, CHY, Taiwan) with the external resistance set at 1 kΩ. When the MFC reached the maximum voltage, the external resistances were varied within a range of 1000–50 Ω at 20 min per resistance to obtain voltage variation curve as function of resistances. The current was calculated using the Ohm Law $I = E/R$, where I is the current (A), E is the voltage (V) and R is the external resistance (Ω). Current density (I_d, A/m^2) was calculated by dividing the calculated current by the anode surface area (m^2). The power density (P, W/m^2) was calculated based on the equation $P = I_dE$. Thus, the polarization curve reflecting the relationship between E and I_d and the power density curve reflecting the relationship between P and I_d were plotted; the internal resistance was obtained using the polarization slope method as described by Logan [21].

As described by Liu et al. [22], Coulombic efficiency (CE) was calculated using the equation $CE = (C_E/C_T) \times 100$, where C_E, the actual electrical charge, is obtained by integrating the current over the operating time as $C_E = \int I(t)dt$, where t is the operating time (s). The equation used to calculate C_T, which is the electrical charge that can theoretically be produced by substrate biodegradation, is $C_T = (F \times n \times W)/M$, where F is the Faraday constant (96,485 C per mole), n is the number of moles of electrons produced per mole of substrate, W is the weight of removed substrate (expressed in grams), and M is the molecular weight of the substrate.

2.5. Chemical analysis of the MFC

During the toluene biodegradation and electricity generation processes, the pH and ORP of the anode chamber were measured with a multi-function water quality monitor (MU-SX751, Major Science, Taiwan). Samples periodically taken from the MFC anode were centrifuged to remove the biomass. The remaining pyocyanin concentration was then determined by HPLC (BETA 10, ECOM