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Characterization and interactions of anodic isolates in microbial fuel cells explored for simultaneous electricity generation and Congo red decolorization

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HIGHLIGHTS

- Strains were isolated from MFCs for power production and azo dye decolorization.
- *Aquamicrobium* was firstly reported as exoelectrogen and dye degrading bacterium.
- Specific interaction could contribute to enhanced performance of MFCs.

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ABSTRACT

To investigate functions and interactions of predominant microorganisms in microbial fuel cells (MFCs) for simultaneous electricity generation and Congo red decolorization, four strains were isolated from the anodic biofilm, and identified as *Pseudomonas* (M-P and I-P), *Bacillus* (M-B) and *Aquamicrobium* (I-A). Higher maximum power density (by 158.2% and 58.1%) but lower Congo red decolorization rate (by 3.2% and 5.9%) were achieved in MFCs using pure cultures I-P and M-P as inoculums than those using I-A and M-B, respectively. By comparing MFCs using co-cultures with those using pure cultures (M-P&B versus M-B and M-P, I-P&A versus I-A and I-P), the maximum power density of MFCs using co-cultures increased 82.0%, 15.1%, 94.6% and −24.6% (minus meant decreased), but decolorization rate decreased 33.3%, 29.4%, 7.9% and 5.0%, respectively. The results indicated specific interaction could enhance the performance of MFCs and might benefit the development of bio-process controlling.

1. Introduction

Microbial fuel cell (MFC), which is classified as bioelectrochemical system (BES), has been developed as a promising solution to both wastewater treatment and electricity harvesting (Osman et al., 2010). In MFCs, microorganisms could convert substrates such as wastewaters into electricity directly through the metabolic activity and interaction with electrodes using electrons (Pant et al., 2010).

Recently, due to development of biotechnology, microorganisms in MFCs have gained more and more attention. Thus, exoelectrogen, which is also called electrochemically active microorganism, becomes a research focus of MFCs (Logan, 2009). So far, many strains of exoelectrogens have been isolated from different systems, such as *Shewanella putrefaciens* (Kim et al., 1999), *Shewanella oneidensis* (Ringeisen et al., 2007; Bretschger et al., 2007), *Desulfuromonas acetoxidans* (Bond et al., 2002), *Geobacter sulfurreducens* (Bond and Lovley, 2003), *Rhodoferax ferrireducens* (Chaudhuri and Lovley, 2003), *Pseudomonas aeruginosa* (Rabaey et al., 2004), *Ochrobactrum anthropi* (Zuo et al., 2008).

Microorganisms play an essential role in the performance of MFCs, especially those for wastewater treatment. Many studies have been conducted to investigate the community composition in MFCs, revealing that successful conversion of complex substrates into electrical current requires syntrophic interactions among a microbial consortium with diverse phenotypic characteristics (Kiely et al., 2011). However, identification of specific syntrophic processes among the dominant microorganisms remains

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largely unclear, which is the key to investigate controlling possibilities to improve the performance of MFCs from microbial ecology.

Azo dye, which is one of recalcitrant organic contaminants, has been explored as substrates or co-substrates in MFCs recently (Sun et al., 2009; Li et al., 2010). Compared to traditional technologies, MFCs have been promising for their outstanding performance, innovative features and environmental benefits in decolorization of azo dye (Pant et al., 2012; Solanki et al., 2013). Previous studies showed that two preponderant groups of microorganisms existed in the anodic chamber and were responsible for Congo red degradation and electricity generation, respectively. These bacteria were supposed to interact with each other and contribute to the performance of MFCs together (Hou et al., 2011a). Accordingly, preliminary understanding of the performance and mechanism of electricity generation and Congo red degradation (Sun et al., 2012) were achieved. However, in addition to the resultant effect, it is necessary to isolate each dominant microorganism to investigate the individual effect of each strain and the interactions between those strains. In this study, isolation, identification, electrochemical and decolorization characterization of the predominant bacteria were conducted with MFCs supplied with Congo red and glucose. Pure and defined co-cultures of the isolates were examined in comparison to investigate the interactions between each microorganism.

2. Methods

2.1. General conditions

All MFCs were established for simultaneous electricity generation and Congo red decolorization. All microbiological and electrochemical experiments were conducted under strictly sterile and anoxic conditions at constant temperature (30 ± 1 °C). If not stated otherwise, all chemicals were of analytical or biochemical grade. All potentials provided in this article referred to the saturated calomel electrode (SCE, +0.242 V vs. SHE). All reported data were based on at least three replicates per biofilm, and average values were reported.

2.2. Strain isolation, cultivation and identification

The isolate resources were the anodic biofilm of two MFCs (MFC-I and MFC-M), which had been operated for simultaneous electricity generation and Congo red decolorization for more than 6 months. Anaerobic sludge from Xinzhou Industrial (printing and dyeing) and Liede Municipal Waste Water Treatment Plant (WWTP) (Guangzhou, China) were used as original inoculums, respectively. Both reactors were fed with Congo red (300 mg/L), glucose (200 mg/L), mineral salts (12.5 mL/L) and vitamins (12.5 mL/L) in a 50 mM phosphate buffer solution (PBS) as described previously (Lovley and Phillips, 1988).

The nutrient agar (NA) medium (pH 7.0) was used to isolate different functional exoelectrogens from MFCs. The microorganisms on the anodic biofilm of both reactors were suspended and diluted in sterilized DI water and cultivated at 30 ± 1 °C for 48 h in an anaerobic incubation chamber (YQX-II, CIMO Medical Instrument Manufacturing Co., Ltd., Shanghai, China). Repeated transfers and purification were conducted with fresh NA media until pure cultures were obtained. And the Luria–Bertani (LB) medium (pH 7.0) was employed for enrichment of the pure isolates. To all mediums, oxygen was removed by repeating evacuation and flushing with nitrogen gas.

Identification of the isolates was performed using 16S rRNA gene sequence analysis by a commercial service provided by TaKaRa Biotechnology (Dalian) Co., Ltd. Genomic DNA was extracted and purified, followed by PCR amplification of 16S rRNA genes with TaKaRa 16S rDNA Bacterial Identification PCR Kit (TaKaRa using TaKaRa Thermal Cycler Dice TP600). Amplification was carried out in a 50 μL reaction volume which contained 1 μL of genomic DNA, 25 μL PCR premix, 0.5 μL forward primer (20 pmol/μL), 0.5 μL reverse primer2 (20 pmol/μL), and 23 μL 16S-free H_2O. The amplification conditions consisted of an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min and finally an elongation step at 72 °C for 5 min. The sequencing reactions were run on ABI-PRISM™ 3730XL DNA Sequencer using BigDye® Terminator V3.1 Cycle Sequencing Kit. The nucleotide sequence analysis of the sequence was conducted at BLAST-n site at NCBI server (www.ncbi.nlm.nih.gov/BLAST). The sequences determined in this study have been deposited in GenBank under accession numbers KC853422 to KC853425.

2.3. Setup and operation of MFCs with pure and co-cultures

Air-cathode single chamber MFCs used for pure and co-cultures were constructed as reported previously (Li et al., 2012), consisting of cylindrical anode chamber (5 cm in diameter, 2.1 cm in length; effective volume of 40 mL), proton exchange membrane (Zhejiang Qianqiu Group Co., Ltd. China), graphite felt electrode (anode; projected surface area of 10.17 cm^2) and 40% Pt/C electrode (air-cathode, 5 cm × 5 cm; Hesen, China). Prior to use, graphite felt were cleaned by soaking in acetone overnight and then washed thoroughly with sterilized DI water. All materials including the chamber, electrode and membrane were sterilized by high temperature or soaking in 75% ethanol. MFCs were established and inoculated with pure or co-cultures in the anaerobic incubation chamber after ultraviolet disinfection. The anodic medium was periodically refreshed when the voltage dropped below 20 mV. Titanium wires were employed to connect the circuit with an external resistance of 1000 Ω.

2.4. Bioelectrochemical characterization

The voltage of each MFC was recorded every 6 min using a data acquisition system (Model 2700, Keithly Instruments, USA). For bioelectrochemical characterization, MFCs were allowed to equilibrate at open circuit for ~4 h till the open circuit voltage (OCV) stabilized. (The time required to establish steady OCV for all the tested MFCs were approximately the same.) To obtain the power density and polarization curves as a function of current, the external circuit resistance was varied in the range of 2000–80 Ω when the voltage output approached a plateau. The anode and cathode potentials were measured by placing a SCE in the anode chamber for reference. Current density (mA/cm^2) was calculated from $I = U/(RA)$, and power density (mW/m^2) was calculated from $P = UI \times 10^4$, where R was resistance, U (V) was cell voltage, and A (cm^2) was the projected area of the anode.

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were employed to determine the electrochemical characterization of each isolate through a potentiostat (Model 2273, Princeton Applied Research). The three-electrode setup consisted of a working electrode (the anode of MFC), an SCE (saturated KCl) reference electrode, and a counter electrode (the cathode of MFC). CV was performed between 1.2 V and −0.7 V. EIS test was conducted over a frequency range of 10 kHz to 5 mHz, with a sinusoidal perturbation of amplitude 10 mV.

2.5. Decolorization characterization

Decolorization efficiency of Congo red (300 mg/L) was measured by monitoring the decrease in absorbance at the maximum