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Perchlorate reduction in microbial electrolysis cell with polyaniline modified cathode

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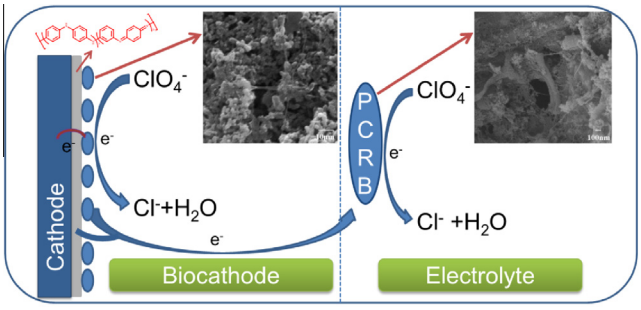
HIGHLIGHTS

- Excellent perchlorate reduction was obtained in a non-membrane MEC.
- PANI-cathode is conductive to the formation of biofilm.
- The biofilm mainly facilitated electron transfer from cathode to electrolyte.
- Pili-like were prone to formation with cathode as sole electron donor.

GRAPHICAL ABSTRACT



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ABSTRACT

Excellent perchlorate reduction was obtained under various initial concentrations in a non-membrane microbial electrolysis cell with polyaniline (PANI) modified graphite cathode as sole electron donor. PANI modification is conducive to the formation of biofilm due to its porous structure and good electrocatalytic performance. Compared with cathode without biofilm, over 12% higher reduction rates were acquired in the presence of biocathode. The study demonstrates that, instead of perchlorate reduction, the main contribution of biofilm is involved in facilitate electron transfer from cathode to electrolyte. Interestingly, hairlike structure, referred as to pili-like, was observed in the biofilm as well as in the electrolyte. Additionally, the results show that pili were prone to formation under the condition of external electron field as sole electron donor. Analysis of microbial community suggests that perchlorate reduction bacteria community was most consistent with *Azospiraoryzae* strain DSM 13638 in the subdivision of the class *Proteobacteria*.

1. Introduction

Perchlorate (ClO_4^-) is an emerging contaminant found in the surface water and groundwater, primarily used as ingredients in solid rocket fuels and missile propellants, airbag inflators (Oremland et al., 2013). Perchlorate, as an endocrine disrupting chemical (Pearce et al., 2010), diffuses rapidly in aquatic environments due to its high stability and mobility (Logan, 2001; Srinivasan and Sorial, 2009). The environment and health problems caused by perchlorate have raised serious extensive concern. It is typically treated via various methods such as ion exchange (Lee et al., 2008), ultrafiltration (Huq et al., 2007), biological treatments (Ryu et al., 2012) and electrochemical reduction (Láng et al., 2008). Perchlorate reduction bacteria activity can be facilitated through utilizing different electron donors. Therefore, engineered approaches for bioremediation of perchlorate are typically based on the continual addition of chemicals as electron donors to sustain microbial degradation activity, including inorganic

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electron-donating substrates such as acetate, citrate, ethanol, hydrogen gas and long-lasting, slowly biodegradable organic substrates (AH_2DS) (Ju et al., 2008; Van Trump and Coates, 2009). Addition of chemicals always be companied by by-product and high cost, which would causes even more problematic.

Electrochemical reduction is a promising method for removal of perchlorate as non-toxic Cl^-, whereas it is limited for the lack of effective catalysts (Srinivasan and Sorial, 2009). In the microbial electrolysis cells (MEC), biocathode utilizes electrochemical active microorganisms as catalysts, therefore it eliminates the needs for isolation of individual enzymes and allows active biomaterials under the condition close to their natural environment to convert pollutants (Kong et al., 2014). Especially, biocathode avoids the need for a noble metal catalyst, which is hard to be practical so far due to its high cost and low reaction rate for ClO_4^- reduction. However, conventional carbon-based biocathode has shown low power density and its performance is influenced by both the bioactivity and electrochemical characteristic (McLean et al., 2010; Wang et al., 2011). Moreover, the need for long-time preparation also limits its practical application (Butler et al., 2010). Polyaniline (PANI), as usually popular conducting polymer, was modified on the cathode to facilitate immobilization of bacteria, enhance the electron transport due to its porous structure, good electrochemical characteristic as well as simple process of synthesis (Gvozdenović et al., 2011).

In this study, a new PANI-biofilm composite biocathode was synthesized for perchlorate reduction without adding appended electron donors. Since mediators, which usually are costly and/or unsustainable compounds, are not necessary to actuate the extracellular electron transfer process, the process is very attractive *in situ* applications. Meanwhile, in order to enrich perchlorate reducing bacteria (PCRB), an external permanent potential was employed during the biofilm formation synthesis process. The microorganism of this PANI-biofilm composite biocathode was analyzed via biological techniques at the end of experiment.

The study of EET strategies has involved around the energy flow from organic to electron referred as the process of bioanode, while little research has been published about the mediated electron transfer between bacteria and electrode in mixed cultures under the condition of cathode as primary electron donor. Herein, a MEC was established with PANI modified graphite electrode as cathode for perchlorate reduction. To our knowledge, this is the first study to report pili-like structure found in the suspended sludge in the electrolyte at a time when cathode served as sole electron donor. It may help us to further reshape an understanding of mechanisms involved in extracellular electron transfer.

2. Methods

2.1. Electrode preparation

Aniline (99%) was purified by distillation and stored at 4 °C before use. PANI was electrochemically modified on wax-impregnated graphite electrode (1 cm in diameter, 8 cm in length and 15.7 cm^2 in effective area). The wax-impregnated graphite electrode was prepared by immerging spectroscopically pure graphite (SPG) (Shanghai Yifeng Co., Ltd.) into melted wax, and then polished to a mirror-like surface. The electrochemical polymerization of PANI was carried out in 0.5 M H_2SO_4 + 0.1 M aniline (oxygen free) using the cyclic voltammetric technique Electrochemical measurements and the synthesis of PANI were performed by a CHI 760D electrochemical workstation (Shanghai Chenhua Company) with a conventional three-electrode system (Gao et al., 2011) (sweep between −0.4 and 1.2 V for two cycles, and between −0.4 and 0.85 V for 15 cycles, at the scan rate of 50 mV/s). After polymerization, the PANI/graphite electrode were washed in distilled water and dried in the air for 24 h to get a firm PANI film This three-electrode system consists of a wax-impregnated graphite electrode as working electrode, a platinum foil electrode as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode.

2.2. Electrochemical system and electrolyte

A non-membrane MEC was constructed with borosilicate glass. The working volume was 1000 mL. A permanent potential (−0.4 vs. SCE) was applied to the PANI modified graphite electrodes (PANI-cathode), and spectroscopically pure graphite electrodes (SPG) used as counter electrode (anode). Anode and cathode were both equipped with 6 electrodes shared of similar-sized and cathodic total effective area is roughly about 66 cm^2. Meanwhile, a SPG electrode without any modification was also linked to the cathode under the same condition (contrastive electrode). Here, anaerobic sludge was collected from a lab scale reactor fed with synthetic wastewater with sodium acetate as additional electron donor. The perchlorate reduction bacteria (PCRB) were acclimated throughout addition of perchlorate and 1000 mg/L $NaHCO_3$ as the sole inorganic carbon source. Afterwards, the sludge was transferred into the MEC and amended with external electron field as sole electron donor instead of sodium acetate gradually. The reactor was fed with 1000 mL electrolyte and flushed with ultra pure nitrogen for 15 min prior to setup to maintain anaerobic environment every 3 days. The composition of the electrolyte (oxygen free) was (mg L^{-1}): $NaClO_4$ 80, K_2HPO_4 47, NaH_2PO_4 H_2O 27, $NH_4H_2PO_4$ 16, $NaHCO_3$ 1000, $MgSO_4$ $7H_2O$ 3, Na_2EDTA 6, $CaCl_2$ $2H_2O$ 1, Na_2MoO_4 0.2, $CoCl_2$ $6H_2O$ 0.4, Na_2SeO_3 0.066, $NiSO_4$ $6H_2O$ 0.1, $ZnSO_4$ 1.4, $CuCl_2$ $2H_2O$ 0.2, $MnCl_2$ $4H_2O$ 0.85, and H_3BO_3 0.6. During the establishing of MEC, the system current rose from 0.05 mA at the first day to 0.68 mA at the 26th day gradually and finally reached stability. In the meantime, biofilm was formed on the surface of the PANI-cathode (biocathode) gradually.

2.3. Analytical methods

2.3.1. Chemical analysis

All experimental analyses were performed in triplicate to ensure reproducibility. Perchlorate anion was determined via ion chromatography (ICS-900, Dionex Corporation, Sunnyvale, CA) using an IonPac AS20 column (Dionex, 1998). In order to analyze perchlorate in the electrolyte, samples were filtered through 0.22 μm nylon membrane. The current was monitored by means of CHI 760D electrochemical workstation.

2.3.2. Characterization

The surface morphologies of both biocathode and suspended sludge in the electrolyte were characterized by scanning electron microscopy (SEM, JSM-6700F).

Fluorescence *in situ* hybridization (FISH) was performed twice to assess the relative abundance of PCRB distribution in the electrolyte (Zhang et al., 2005): (1) at the beginning of the startup phase; and (2) at the end of the experiment. The FISH was conducted according to an established method (Winkler et al., 2011). The biomass was fixed with 4% paraformaldehyde solution at 4 °C for3 h. After fixation, samples were centrifuged at 10,000 rpm for 5 min, and then were washed twice with 1× phosphate buffer saline (PBS). The mix sludge was harvested by centrifugation, resuspended in ethanol/PBS solution (1:1) and storage at 20 °C before hybridization. Two oligonucleotides for PCBR were designed, one for Dechloromonas (FITC-Monas1403) and another for Dechlorosoma (FITC-Soma1035). Cy3-EUB338 was used for total bacteria detection. Hybridized samples were observed with