Bioresource Technology 180 (2015) 185–191

Contents lists available at ScienceDirect

Bioresource Technology

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

Effect of acclimatization on hexavalent chromium reduction in a biocathode microbial fuel cell

Xiayuan Wu [a,1], Xujun Zhu [b,1], Tianshun Song [a], Lixiong Zhang [a], Honghua Jia [a,*], Ping Wei [a]

[a] College of Biotechnology and Pharmaceutical Engineering, State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing Tech University, Nanjing 211816, PR China
[b] College of Horticulture, Nanjing Agricultural University, Nanjing 210095, PR China

HIGHLIGHTS

- An anode inversion method can be used to yield a Cr(VI)-reducing biocathode.
- The efficiency of the Cr(VI)-reducing biocathode significantly improved.
- Higher microbial density and less Cr(III) precipitates on the cathode were observed.
- Some species of exoelectrogenic–electrotrophic bacteria play the vital roles.

ARTICLE INFO

Article history:
Received 23 November 2014
Received in revised form 30 December 2014
Accepted 31 December 2014
Available online 8 January 2015

Keywords:
Acclimatization method
Microbial fuel cell
Biocathode
Cr(VI) reduction
Electricity generation

ABSTRACT

A simple acclimatization method for the reduction of hexavalent chromium (Cr(VI)) at a biocathode by first enriching an exoelectrogenic biofilm on a microbial fuel cell (MFC) anode, followed by direct inversion of the anode to function as the biocathode, has been established. This novel method significantly enhanced the Cr(VI) reduction efficiency of the MFC, which was mainly attributed to the higher microbial density and less resistive Cr(III) precipitates on the cathode when compared with a common biocathode acclimatization method (control). The biocathode acclimatization period was shortened by 19 days and the Cr(VI) reduction rate was increased by a factor of 2.9. Microbial community analyses of biocathodes acclimatized using different methods further verified the feasibility of this electrode inversion method, indicating similar dominant bacteria species in biofilms, which mainly consist of *Gamma-proteobacteria* and *Bacteria*.

1. Introduction

Hexavalent chromium (Cr(VI)) is one of the most toxic metal ions and is released into the environment by various industries including electroplating, leather tanning, mining and metal finishing (Agarwal et al., 2005). As a widely known mutagen, teratogen and carcinogen, Cr(VI) has high water solubility and mobility. In contrast, Cr(III) is less soluble in water, less mobile and less harmful (Niebor and Jusys, 1988). Therefore the most common mechanism for removing Cr(VI) from aqueous effluents is by reduction of Cr(VI) to Cr(III) followed by chemical precipitation as $Cr(OH)_3$. However, conventional physical and chemical treatments for Cr(VI) wastewater may be either economically or environmentally inefficient because of high energy input requirements and the generation of secondary pollution. Microbiologically-catalyzed reduction is regarded as a safe and sustainable process which gained extensive attention after the first report by Romanenko and Koren'Ken (1977). The addition of an external organic carbon source is always required, however, which increases the cost and reduces the efficiency due to competition between other microorganisms that easily grow in this heterotrophic environment and chromium-reducing bacteria for the carbon source (Molokwane et al., 2008).

An emerging technology combining microbiologically-catalyzed reduction using a biocathode MFC was recently shown to reduce Cr(VI) in an autotrophic environment and simultaneously harvest electricity during the treatment process (Tandukar et al., 2009). Biocathode MFCs utilizing electrochemically active microorganisms as catalytic centers at both the anode and cathode show great promise in Cr(VI) bioremediation because their operation is inexpensive, the catalysts can self-regenerate and the power supply is sustainable (He and Angenent, 2006; Huang et al., 2011c; Lovley, 2011). However the area is still in its infancy and further extensive research into efficient biocathodes for the enhancement

* Corresponding author at: Nanjing Tech University, No. 30 Puzhu Road(S), Nanjing 211816, PR China. Tel./fax: +86 25 58139368.
E-mail address: ijbtg2009@163.com (H. Jia).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.biortech.2014.12.105
0960-8524/

of Cr(VI) reduction and electricity generation in MFC is required. The promotion of the enrichment of cathodic biofilms and improving the performance of biocathode could decrease the internal resistance and therefore increase the Cr(VI) reduction efficiency (Huang et al., 2011a, 2011b, 2010).

Biocathodes using microorganisms as catalysts to transfer electrons from the cathode to electron acceptor, similar as the bioanode require an enriched electron-accepting (electrotrophic) biofilm on the surface, formed via acclimatization (Huang et al., 2011c). A common acclimatization method for Cr(VI)-reducing biocathodes (resulting in a mixed microbial community) was that inoculated with an anaerobic digester and enriched a biofilm with extraneous Cr(VI) in the cathode chamber (in-situ), where the biocathode acclimatization time was long (approximately 45 days) (Tandukar et al., 2009). Huang et al. (2011a, 2010) have applied an inoculum of some indigenous microbial consortia from Cr(VI)-contaminated sites and set the biocathode potential to promote the enrichment of the biofilm, which both improved the performance of biocathode and shortened the acclimatization period. However an indigenous inoculum is not always readily available, and changing the biocathode potential both increases the energy input and complicates the system.

Generally speaking, it is more difficult to grow electrotrophic biofilms on cathodes under anaerobic conditions than it is to produce an exoelectrogenic biofilm on an anode (Morita et al., 2011). Rozendal et al. (2008) first demonstrated that bioanodes could electrically invert to produce efficient hydrogen-evolving microbial electrolysis cell (MEC) biocathodes in aqueous solution. Biofilms that develop on cathodes in MFCs under oxic conditions have been shown to sustain current generation when the function of a cathode is switched (i.e.: the cathodes operating as anodes) (Cheng et al., 2010; Strycharz et al., 2010). This indicates that there is no strict boundary between exoelectrogens and electrotrophs and that some bacteria may be both exoelectrogenic and electrotrophic. For example, *Shewanella* has been shown to be exoelectrogenic by functioning at bioanodes to oxidize organics and electrotrophic by functioning at biocathodes to reduce Cr(VI) in MFCs (Fitzgerald et al., 2012; Xafenias et al., 2013). However, it was not clear if the bioanode (mixed microbial community) would function as a biocathode in a Cr(VI)-reducing MFC similar to that achieved in MEC (Pisciotta et al., 2012; Rozendal et al., 2008). Therefore, in the present study, we have tested the hypothesis that the bioanode could function as a Cr(VI)-reducing biocathode by direct electrode inversion. This is the first attempt to establish an ex-situ (acclimatizing biocathode in anode chamber) biocathode acclimatization method in a Cr(VI)-reducing MFC.

The aim of this study was to enhance the performance of Cr(VI)-reducing biocathode by optimizing the acclimatization method. We investigated the effects of a simple electrode inversion method to acclimatize biocathodes on the electricity production and anaerobic Cr(VI) reduction in MFCs, in comparison to a control MFC using a common acclimatization method. Extensive characterization including biocatalytic activities, surface morphology and microbial community analyses of the biocathodes were conducted to validate the feasibility of this new acclimatization method.

2. Methods

2.1. MFC construction and operation

The dual-chamber MFC was constructed from two plexiglass cubic chambers (liquid volume of each chamber was 70 mL) and both chambers were kept gastight. The chambers were separated by a proton exchange membrane (38.5 cm^2; Nafion117, Dupont Co., USA). A sheet of graphite felt (5.0 × 5.0 × 0.5 cm; Hunan Jiuhua Carbon Hi-Tech Co., Ltd., China) was used as the anode, and also the cathode. The bioanode with a stable anode potential used in the present study was developed in the anodic chamber of a previous MFC, which had been acclimatized for glucose oxidation and continuously operated for 8 months. This pre-acclimatization of the bioanode could shorten the acclimatization period of the biocathode in the MFC (Huang et al., 2010). The original anodic inoculation was from anaerobic digester sludge. The anolyte consisted of the following: 0.31 g/L NH_4Cl, 2.452 g/L $NaH_2PO_4{\cdot}H_2O$, 4.576 g/L Na_2HPO_4, 0.13 g/L KCl, and 1 g/L $C_6H_{12}O_6{\cdot}H_2O$ at pH 7.0. The MFCs were operated in a batch-fed mode at a constant temperature (25 ± 0.5 °C) connected to a 1000 Ω external resistance. All experimental reactors were performed in triplicate to ensure reproducibility.

2.2. Acclimatization methods on the biocathode

The cathode inoculum was a mixed bacterial culture enriched in the presence of Cr(VI) with anaerobic digester sludge. In addition to the inoculation, the cathode medium (0.78 g/L KCl, 2.772 g/L $NaH_2PO_4{\cdot}2H_2O$, 11.53 g/L $Na_2HPO_4{\cdot}12H_2O$, 0.28 g/L NH_4Cl, 0.2 g/L $NaHCO_3$, pH = 7.0) containing various concentrations of Cr(VI) (prepared by dissolving $K_2Cr_2O_7$ in deionized water) was added to the cathode. The startup of biocathode was treated with Cr(VI) several times, increasing the initial Cr(VI) concentration from 6 to 20 mg/L (Tandukar et al., 2009). After 49 days, a stable Cr(VI) reduction current was observed which implied successful acclimatization of the biocathode in the MFC. This method is denoted in-situ acclimatization in this study.

The cathode electrode was first inserted into the anode compartment of a dual-chamber MFC and inoculated with anaerobic digester sludge, then this electrode was taken as an anode for acclimatization. The cathode chamber of this MFC was filled with 40 mM ferricyanide and 50 mM phosphate buffer solution (2.452 g/L $NaH_2PO_4{\cdot}H_2O$, 4.576 g/L Na_2HPO_4, 0.13 g/L KCl; pH 7.0), other conditions were as above. The media in both chambers were refreshed when the voltage of the MFC decreased to less than 100 mV. After 30 days the MFC achieved the same maximum voltage for two consecutive batch cycles which, implying the formation of a mature biofilm on the anode. This anode was then removed from the chamber and gently rinsed with deionized water to minimize carryover of residual glucose and other organic substances (Pisciotta et al., 2012). After cleaning the electrode was transferred to the cathodic chamber to function as the biocathode in a Cr(VI)-reducing MFC. This method is denoted ex-situ acclimatization in this study.

Besides above experimental MFCs, open circuit controls were also prepared. Control 1 and Control 2 represented the MFCs used in-situ and ex-situ acclimatization on biocathodes which were run in the open circuit mode (disconnected electrode) for Cr(VI) reduction, respectively.

2.3. Microbial community analyses

Biocathode samples for molecular analyses were collected after completion of in-situ and ex-situ acclimatization. Microbial communities were analyzed using a polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE). Total genomic DNA extraction was conducted using a Rapid Extraction Kit of Silt genomic DNA (Baisaike Co. Ltd., Beijing, China) according to the manufacturers instructions. Amplification of 16S rDNA gene fragments was performed using the universal primers 338F (5′-CCT ACG GGA GGC AGC AG-3′) and 518R (5′-ATT ACC GCG GCT GCT GG-3′). The products were then amplified a second time using the primer 338F containing a GC clamp (5′-CGCCCGGGGCGC GCCCCGGGGCGGGGCGGGGGCGCGGGGGG CCT ACG GGA GGC