Bioresource Technology 221 (2016) 666–670

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Response of anodic bacterial community to the polarity inversion for chloramphenicol reduction

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HIGHLIGHTS

- The biofilm established from bioanode reduced CAP effectively.
- The electroactive microbes adapted to the bidirectional electron transfer.
- Nitroaromatics-reducing genera dominated in the established biocathode.

ARTICLE INFO

Article history:
Received 23 July 2016
Received in revised form 8 September 2016
Accepted 11 September 2016
Available online 13 September 2016

Keywords:
Chloramphenicol reduction
Polarity inversion
Bacterial communities
Bidirectional electron transfer

ABSTRACT

Chloramphenicol (CAP) is a frequently detected environmental pollutant. In this study, an electroactive biofilm for CAP reduction was established by initially in the anode and then inverting to the cathode. The established biocathode could enhance the reduction of CAP to the nitro-group reduced CAP (AMCl2) and further dechlorinated form (AMCl), both had lost the antibacterial activity. The phylogenetic diversity of the acclimated biofilm was decreased after the polar inversion. Proportions of functional bacterial genera, including *Geobacter*, *Desulfovibrio* and *Pseudomonas* responsible for the bidirectional electron transfer and nitroaromatics reduction, had increased 28%, 104% and 43% in the cathode. The relatively high abundances (over 50%) of *Geobacter* in anode and cathode were rarely detected for the nitroaromatics reduction. This study provides new insights into the electroactive biofilm structure improvement by the polarity inversion strategy for refractory antibiotics degradation.

1. Introduction

The chlorinated nitroaromatic antibiotic chloramphenicol (CAP, 2,2-dichloro-*N*-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitro phenyl) ethyl] acetamide), which could inhibit both Gram-positive and Gram-negative bacteria, had been used for human and veterinary since the 1950's (Liang et al., 2013). Due to its biotoxicity, it has been banned by many developed countries. However, CAP is still in use in some developing nations. The insufficient removal during wastewater treatment led to the frequent detection of CAP residue in environments, especially in diverse water environments. For example, in livestock wastewater effluent, sewage treatment plant effluent and surface water, 3–47,400 ng/L CAP were detected (Kong et al., 2015). The emergence of CAP-resistant bacteria, resistance genes and even multiple-antibiotic resistance is another growing public concern (Tao et al., 2012). Thus, the degradation of such pollutants and elimination of their antibacterial activity is urgently necessary.

Bioelectrochemical system (BES) has been considered as an efficient and cost-effective approach for various pollutants removal. Electrochemically active bacteria (EAB) had been reported could utilize cathode as the terminal electron donor and accelerate the reduction of multiple organic and inorganic pollutants, such as azo dye, nitrobenzene, nitrate, perchlorate and heavy metals (Gregory and Lovley, 2005; Liang et al., 2014). CAP also had been successfully reduced with biocathode (Kong et al., 2014, 2015; Liang et al., 2013; Sun et al., 2013). However, these biocathodes were established with the anaerobic enrichment as cathodic inoculum, which was obtained by the targeted pollutant acclimation. The process was generally time-consuming and function limited. The electroactive species were unlikely to be maintained well to a large extent.

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http://dx.doi.org/10.1016/j.biortech.2016.09.047
0960-8524/

In this study, a biocathode was established with a bioanode firstly and then the polarity was inverted to change the electron flow direction. The hypothesis for this proposal was that communities with the anodic establishment primarily had more diversified electron transfer routes and oxidoreductases that can work on the backward electron transfer and had enhanced electron transfer ability (Rozendal et al., 2008). However, whether the bioanode to biocathode would influence the bacterial community structures for CAP reduction yet has not been documented. Whether the CAP reduction would be improved and how would be improved by the dominant genera was not clarified. To answer the above questions, the CAP reduction ability of the acclimated biofilm was confirmed before and after the polar inversion. Based on the 16S rRNA gene-based Illumina MiSeq sequencing, the structure and composition of bioanode and biocathode communities were compared, the dominant genera were thoroughly analyzed and their potential functions were discussed.

2. Materials and methods

2.1. Chemicals

Chloramphenicol (CAP, >98% purity) and high performance liquid chromatography (HPLC) grade methanol were purchased from Sigma-Aldrich (St. Louis, MO, U.S.). Other chemicals were at least of analytical reagent grade.

2.2. Reactor construction

The dual-chamber BES reactor was constructed as reported recently (Kong et al., 2015). Pre-treated carbon brushes (Kong et al., 2015) (4 cm in diameter by 3 cm in length, TOHO TENAX Co., Ltd., Tokyo, Japan) served as both anodic and cathodic electrodes. Saturated calomel electrode (0.247 V vs. standard hydrogen electrode (SHE), model-217, Shanghai Precise. Sci. Instru. Co., Ltd., China) and precision resistors (10 Ω) were connected in the circuit for potential and current acquisition. Potentials mentioned herein were all against SHE.

2.3. Biofilm establishment and tests

The experiment was divided in three stages. In the first stage, to establish an electroactive bioanode, 5 mL anaerobic activated sludge from Taiping wastewater treatment plant (Harbin, China) was inoculated in the anodic chamber. The anolyte ingredients were described previously (Kong et al., 2014). The catholyte was 50 mM phosphate-buffered saline (PBS) supplied with 0.108 mM CAP. Anolyte and catholyte were replaced every 4–5 days until stable potential and current were obtained. In the second stage, to acclimate the anodic biofilm with CAP, the anolyte amended with 0.016 mM CAP was refreshed until the recovery of currents. The CAP concentration was then doubled applied and refreshed for another several cycles. The final supplied concentration of CAP was 0.108 mM. In the third stage, the CAP-acclimated biofilm was tested in the biocathode mode with a three-electrode potentiostat (WMPG1000K8 multichannel potentiostat, WonATech Co., Ltd., Seoul, South Korea) applied to precisely control the potentials, and −0.40 V (10 mM sodium bicarbonate working as carbon source), −0.20 V (20 mM sodium acetate supplied) and −0.40 V (20 mM sodium acetate supplied) were tested. The −0.20 V and −0.40 V were selected based on the initial potential achieved by the biofilm and the theoretical reduction potential of CAP without hydrogen evolution disturbance, respectively (Kong et al., 2015). Fresh anolyte containing 0.108 mM CAP was degassed, filled in the working electrode chamber (48 h for one cycle) and fully replaced before another cycle. The counter electrode chamber was filled with 50 mM potassium ferrocyanide in PBS. Each operation was at least in triplicate at ambient temperature (23 ± 3 °C).

2.4. Analytical methods

CAP and the corresponding reduction products were determined with a reverse-phase HPLC (model-e2695; Waters Co., Milford, MA, U.S.) as described previously (Kong et al., 2015). To compare the dynamic changes of the bacterial communities, biofilms from carbon brushes were randomly sampled from the acclimated bioanode and reversed bioanode as described elsewhere (Liang et al., 2014). The details for DNA extraction, 16S rRNA gene PCR amplification, Illumina MiSeq sequencing and data analysis were similar with previous researches (Liang et al., 2014, 2016). The calculation of CAP reduction efficiency and rate were as references (Kong et al., 2015).

3. Results and discussion

3.1. CAP reduction under different potentials with the acclimated biofilm

The well-developed electrogenic community could maintain potential at ca. −0.20 V at anode. CAP could severely inhibit the electricity generation of the biofilm even at a low concentration (0.016 mM). By acclimation with concentration-increased CAP, the biofilm could recover to the original potential after about 200 h. This implied the previous power production biofilm was successfully screened by CAP. To confirm the CAP reduction ability of the biofilm, the opened circuit was tested with sodium acetate as electron donor and carbon source. CAP (0.108 ± 0.006 mM) could be transformed within 48 h, and the main products, nitro-group reduced CAP (AMCl2) and partly dechlorinated AMCl2 (AMCl) were accumulated (Table S1). This illustrated the biofilm had a strong function on CAP reduction.

After the polarity inversion, whether the biofilm could adapt to the backward electron transfer and effectively use the electrode as electrons donor for CAP reduction was confirmed initially. After a period of starvation, the reversed biofilm was poised at −0.40 V and sodium bicarbonate worked as carbon source. About 99% of the initial CAP (0.110 ± 0.002 mM) was eliminated within 24 h (Table S1), which strongly indicated the biofilm could accept electrons from the electrode. Subsequently, to test whether the CAP reduction would be enhanced with the biofilm when both intracellular (sodium acetate) and extracellular (electrode) electrons were supplied, −0.20 V and −0.40 V modes were tested. At −0.20 V, the CAP reduction rate in the first 3 h was 1.3 times higher than that of the opened circuit. 87.35 ± 1.99% of CAP was diminished within 24 h, while 60.54 ± 5.15% for the opened circuit ($P < 0.05$). The AMCl yield could be 3 times more than the opened circuit within 6 h. When −0.40 V was applied, the CAP reduction rate was 7.19 ± 0.65 mM/h for the initial 3 h, compared to 3.62 ± 0.64 mM/h under potential of −0.20 V. Analogously, the CAP reduction efficiency was 62.12 ± 6.76% and 32.55 ± 4.40% for −0.40 V and −0.20 V, respectively. The maximum AMCl2 accumulation at −0.40 V was nearly twice of that at −0.20 V (Table S1). AMCl was formed since the 1st h under the potential of −0.40 V. Collectively, the biofilm could rapidly adapt to the polarity inversion from electron acceptor in the anodic mode to electron donor when extra negative potentials supplied. The more negative of the potential supplied, the more electrons can be accepted by the biofilm for the enhanced detoxification of CAP.