Response of Anodic Bacterial Community to the Polarity Inversion for Chloramphenicol Reduction

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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.

Keywords

Chloramphenicol reduction; Polarity inversion; Bacterial communities; Bidirectional electron transfer

1. Introduction

The chlorinated nitroaromatic antibiotic chloramphenicol (CAP, 2,2-dichloro-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) 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 & Lovley, 2005; Liang et al., 2014). CAP also had been successfully reduced with biocathode (Kong et al., 2014; Kong et al., 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.

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 ($23 \pm 3^\circ\text{C}$).

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; Liang et al., 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. $87.35 \pm 1.99\%$ of CAP was diminished within 24 h, while $60.54 \pm 5.15\%$ 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 $62.12 \pm 6.76\%$ and $32.55 \pm 4.40\%$ 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.

3.2 Richness and diversity of the communities at phylum, class and family levels

For each sample, more than 31,000 valid sequences were obtained. As shown in Fig. S1, both rarefaction curves had approached to plateau, which stated the representative of the sequencing results. In comparison with the biofilm from anode, the biodiversity indices were all relatively decreased after the polar inversion, such as Shannon index (H) from 2.80 to 2.20, and Invsimpson index (1/D) from 3.67 to 2.71, indicating a reduced phylogenetic microbial diversity for the inverted bioanode community (Fig. S2). Besides, the classified OTUs were 527 and 362 for the biofilms before and after the polarity inversion (Fig. S2), thereinto 312 (54.07%) of the total OTUs were shared

by them.

The relative abundances at phylum, class and family levels of bacterial communities were shown in Fig. 1. The diversities of the two communities were relatively high, which had 20 and 18 phyla detected for the bioanode and biocathode communities, respectively. The unclassified OTUs at phylum level only accounted for 0.26% and 0.40%, indicating that most of bacteria had taxonomic status. The dominant phylum for bioanode and biocathode were both *Proteobacteria*, which accounted for 84.51% and 82.48%, respectively. It is well known that exoelectrogens, like *Geobacter*, *Pseudomonas* and *Shewanella*, belong to such phylum. *Proteobacteria* was also detected as the dominant phylum in the bioanode for power production and the biocathode for nitrobenzene and CAP reduction (Liang et al., 2014; Liang et al., 2013). The second dominant phylum was *Bacteroidetes*, followed by *Firmicutes* and *Synergistetes*. The differences for *Bacteroidetes* and *Synergistetes* in the two communities were negligible. The relative abundance of *Firmicutes* had doubled after the polar inversion (5.43%), which may result from a more reductive circumstance under lowered potentials. Members of *Firmicutes* were also obviously enriched in the acclimated nitrobenzene-reducing biocathode communities as reported previously (Liang et al., 2014; Wang et al., 2011). More *Actinobacteria* (1.71%) was detected in the bioanode (Fig. 1a). More than 30 classes were detected in the two communities and the majority belonged to 10 classes (Fig. 1b). δ -*proteobacteria* covered 54.52% and 71.61% before and after the polar inversion, followed by α -*proteobacteria*, β -*proteobacteria* and γ -*proteobacteria*, which seemed to be affected by potential changes. *Sphingobacteria* and *Actinobacteria* had relatively higher abundances in the bioanode. *Clostridia*, *Bacteroidia* and *Flavobacteria* had increased abundances after the polarity inversion. At family level, about 100 families dropped to

84 after the polarity inversion. Except *Geobacteraceae* accounting for over 50%, *Bradyrhizobiaceae*, *Porphyromonadaceae*, *Comamonadaceae*, *Brucellaceae*, *Desulfovibrionaceae* and *Pseudomonadaceae* were subdominant in both communities (Fig. 1c). The sum of these families accounted for 24.78% and 16.47% before and after the polarity inversion, respectively. The community analysis clearly revealed that different establishment method and potentials selected and maintained different electroactive communities.

Fig. 1

3.3 The potential function of dominant genera

The number of genus which had relative abundance above 0.5% was 16 and 10 before and after the polarity inversion (Table 1). EAB *Geobacter* was rarely enriched to such high proportion for nitroaromatics-reducing biocathode communities though it is capable of reducing nitroaromatics into corresponding aromatic amines (Cai et al., 2012). Interestingly, *Geobacter* was dominant in both communities (over 50%), which had a 28.34% increase after the polarity inversion. EAB *Rhodopseudomonas* dropped to less than 5% from 11.34%, though it also could accept electrons from electrode for metabolism (Bose et al., 2014). *Brucella*, *Stenotrophomonas*, and *Comamonas* declined to a marginal level. The bidirectional electron transfer of the exoelectrogenic *Comamonas*. sp also had been reported, which had different routes for inward and outward electron transfer (Yu et al., 2015). In contrast, *Desulfovibrio*, *Pseudomonas*, *Petrimonas* and *Dysgonomonas* had increased by 2.04, 1.43, 1.47 and 1.88 times, respectively. Members of *Geobacter* were found to be electrochemically active both at the anode or cathode (Geelhoed & Stams, 2010). In the conditions of hydrogen production, nitrate, U (VI) and organohalide reduction, *Geobacter* was considered important for electrons acceptance from the cathode (Croese et al., 2011; Lovley,

2011). Sulphate-reducing bacteria *Desulfovibrio*, another electroactive member from *Deltaproteobacteria*, is able to reduce nitroaromatics (Liang et al., 2016). The biofilm formed with *D. caledoniensis* or *D. vulgaris* both could produce hydrogen and grow with the support of electrode (Croese et al., 2011; Yu et al., 2011). In a set of methane-producing BES reactors, *Desulfovibrionaceae* was also dominant, and its attachment to the electrode may improve the electrons exchanges between the electrode and methanogens (Bretschger et al., 2015). *Pseudomonas* is also a model EAB, which was detected within the CAP-reducing cathode biofilm that established by inoculating the specific CAP-reducing consortium into the cathode (Liang et al., 2013). These electroactive bacteria, possessing capabilities of bidirectional electron transfer and nitroaromatics reduction, may decrease the overpotential or electron transfer resistance for CAP reduction in this study. This was confirmed with supplied potential compared with other researches (Liang et al., 2013; Sun et al., 2013). Cytochrome *c* and mediators like phenazines from these members may reinforce the electron conduction as well (Liang et al., 2016).

Table 1

The significantly higher *Geobacter* populations in the electrode biofilm was also likely to contribute to the reductive dehalogenation of AMCl₂ to AMCl (Strycharz et al., 2008). In the metabolic network, some genera like *Cloacibacillus* from *Synergistetes* are heterotrophic degraders under anaerobic conditions. Their metabolites may also support or maintain the viability and activity of the EAB and nitroaromatics-reducing species (Jumas-Bilak & Marchandin, 2014). Similarly, members belonging to *Firmicutes* are presumably oxygen scavengers who also disassimilated macromolecules (Jung & Regan, 2007). The interactions among the electroactive and fermentative partners were complex but effective approaches for the

stability and functioning maintenance of the electrode communities. The main reduction products AMCl₂ and AMCl had attenuated toxicity and antimicrobial inactivity (Kong et al., 2015; Liang et al., 2013), which had weakened impact on the eco-environments and could be easily removed compared with their parent compound. This study clearly demonstrated that the CAP-reducing communities with the anodic acclimation had powerful electron transfer ability and reductive function after the polarity inversion.

4. Conclusions

CAP reduction was investigated using biofilms enriched from anode and then suffered from the polarity inversion. The biofilms could adapt to the backward electron transfer. After the polarity inversion, the biofilm had significantly higher CAP reduction rate and efficiency. The phylogenetic diversity of the inversed bioanode community was decreased. Key EAB *Geobacter* (67.55%), *Desulfovibrio* (3.49%) and *Pseudomonas* (2.29%), possessing capabilities of bidirectional electron transfer and nitroaromatics reduction, were all enriched in the inversed bioanode community, which provides a prerequisite to obtain electrons from electrode for the accelerated reduction of CAP to the antibacterial inactive products AMCl₂ and AMCl.

Acknowledgements

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Table and Figure

Table 1 Taxonomic classification of 16S rRNA gene sequences (relative abundance > 0.5%) for communities at genus level before and after the polarity inversion.

Fig. 1 Taxonomic classification of 16S rRNA gene sequences for communities at phylum (a), class (b) and family (c) levels before and after the polarity inversion.

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Phylum	Class	Family	Genus (%)	anode	cathode
<i>Proteobacteria</i>	<i>δ-proteobacteria</i>	<i>Geobacteraceae</i>	<i>Geobacter</i>	52.65	67.55
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Bradyrhizobiaceae</i>	<i>Rhodopseudomonas</i>	11.34	4.83
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Brucellaceae</i>	<i>Brucella</i>	1.71	0.20
<i>Proteobacteria</i>	<i>γ-proteobacteria</i>	<i>Xanthomonadaceae</i>	<i>Stenotrophomonas</i>	1.71	0.29
<i>Proteobacteria</i>	<i>δ-proteobacteria</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i>	1.71	3.49
<i>Proteobacteria</i>	<i>γ-proteobacteria</i>	<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	1.61	2.29
<i>Synergistetes</i>	<i>Synergistia</i>	<i>Synergistaceae</i>	<i>Cloacibacillus</i>	1.42	1.24
<i>Proteobacteria</i>	<i>β-proteobacteria</i>	<i>Alcaligenaceae</i>	<i>Achromobacter</i>	1.49	0.02
<i>Bacteroidetes</i>	<i>Bacteroidia</i>	<i>Porphyromonadaceae</i>	<i>Petrimonas</i>	1.04	1.54
<i>Bacteroidetes</i>	<i>Bacteroidia</i>	<i>Porphyromonadaceae</i>	<i>Dysgonomonas</i>	0.90	1.70
<i>Proteobacteria</i>	<i>α-proteobacteria</i>	<i>Phyllobacteriaceae</i>	<i>Aquamicrobium</i>	0.86	0.25
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	<i>Chitinophagaceae</i>	<i>Terrimonas</i>	0.84	0.003
<i>Proteobacteria</i>	<i>β-proteobacteria</i>	<i>Comamonadaceae</i>	<i>Comamonas</i>	0.70	0.36
<i>Proteobacteria</i>	<i>β-proteobacteria</i>	<i>Rhodocyclaceae</i>	<i>Dechloromonas</i>	0.60	0.17
<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Dietziaceae</i>	<i>Dietzia</i>	0.65	0.01

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