Accelerated Reduction of Chlorinated Nitroaromatic Antibiotic Chloramphenicol by Biocathode

Bin Liang,^{†,‡} Hao-Yi Cheng,^{†,‡} De-Yong Kong,[†] Shu-Hong Gao,[†] Fei Sun,[‡] Dan Cui,[†] Fan-Ying Kong,[†] Ai-Juan Zhou,[†] Wen-Zong Liu,^{†,§} Nan-Qi Ren,[†] Wei-Min Wu,^{†,||} Ai-Jie Wang,^{†,§,*} and Duu-Jong Lee^{†,‡,*}

[†]State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin, 150090, P. R. China

[‡]School of Environmental and Civil Engineering, Jiangnan University, Wuxi, Jiangsu Province, 214122, P. R. China

[§]Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, P. R. China

^{||}Department of Civil & Environmental Engineering, and Center for Sustainable Development & Global Competitiveness, Stanford University, Stanford, California 94305-4020, United States

[‡]Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan

S Supporting Information

ABSTRACT: Chlorinated nitroaromatic antibiotic chloramphenicol (CAP) is a priority pollutant in wastewaters. A fed-batch bioelectrochemical system (BES) with biocathode with applied voltage of 0.5 V (served as extracellular electron donor) and glucose as intracellular electron donor was applied to reduce CAP to amine product (AMCl₂). The biocathode BES converted 87.1 ± 4.2% of 32 mg/L CAP in 4 h, and the removal efficiency reached 96.0 ± 0.9% within 24 h. Conversely, the removal efficiency of CAP in BES with an abiotic cathode was only 73.0 ± 3.2% after 24 h. When the biocathode was disconnected (no electrochemical reaction but in the presence of microbial activities), the CAP removal rate was dropped to 62.0% of that with biocathode BES. Acetylation of one hydroxyl of CAP was noted exclusive in the biocatalyzed process, while toxic intermediates, hydroxylamino (HOAM), and nitroso (NO), from CAP reduction were observed only in the abiotic cathode BES. Electrochemical hydrodechlorination and dehalogenase were responsible for dechlorination of AMCl₂ to AMCl in abiotic and microbial cathode BES, respectively. The cyclic voltammetry (CV) highlighted higher peak currents and lower overpotentials for CAP reduction at the biocathode compared with abiotic cathode. With the biocathode BES, antibacterial activity of CAP was completely removed and nitro group reduction combined with dechlorination reaction enhanced detoxication efficiency of CAP. The CAP cathodic transformation pathway was proposed based on intermediates analysis. Bacterial community analysis indicated that the dominate bacteria on the biocathode were belonging to α , β , and γ -Proteobacteria. The biocathode BES could serve as a potential treatment process for CAP-containing wastewater.

INTRODUCTION

After application of antibiotic for more than sixty years, the presence of antibiotic in environment, particularly in the receiving waters, poses adverse ramifications for human health by yielding emerging antibiotic resistant bacteria and antibiotic resistant genes.^{1,2} The chlorinated nitroaromatic antibiotic chloramphenicol (CAP) (2,2-dichloro-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide) was introduced into clinical practice in 1949 and also the first synthetic antibiotic to be applied at large scale,³ serving as an effective bacteriostatic antimicrobial to many Gram-positive and Gram-negative bacteria, including most anaerobic organisms. However, owing to the fatal toxicity of CAP to aplastic anemia and the potential carcinogenicity and genotoxicity of CAP to humans,^{3,4} the U.S. Food and Drug Administration banned its use on food-producing animals. Although the CAP is no longer

a first-line drug in the U.S. and other developed nations, it is still used in low-income countries owing to its low production cost and wide availability. In Asian countries including China, CAP concentration ranging 26–2430 ng/L in the influent and 3–1050 ng/L in the effluent of sewage treatment plants were noted.^{5–12} In specific studies the CAP concentration in sewage (Guiyang, China) was observed as high as 47.4 μ g/L.¹³ Upon CAP exposure the CAP resistant bacteria/genes were found in numerous water streams.^{14–16}

The nitro group of CAP is the essential functional group determining its antibiotic properties.^{17,18} The CAP toxicity can

Received: February 28, 2013

Revised: April 17, 2013

Accepted: April 22, 2013

Published: April 22, 2013

be effectively mitigated by anaerobic transformation of the nitroaromatics to aromatic amines, with the latter being less toxic and considerably easier to be mineralized than the former.^{19–21} Dehalogenation generally makes halogenated compounds less toxic and more readily biodegradable.²² Physical-chemical methods such as photocatalytic degradation,²³ Fenton process,²⁴ zerovalent bimetallic nanoparticles²⁵ and microwave radiation²⁶ were proposed for CAP removal at the expense of high energy and chemical costs. The bioelectrochemical system (BES) with biocatalyzed cathodic reduction is an emerging technology that could reductively degrade various pollutants including chloroethenes,^{27,28} chlorophenols,^{29,30} azo dyes,^{31,32} Cr(VI),³³ U(VI),³⁴ and perchlorate³⁵ in waters. Additionally, the BES was demonstrated effective on converting nitroaromatic nitrobenzene to aniline.^{19,36–38} Although the bioanode BES was claimed to be capable of degrading antibiotic such as sulfonamides, ceftriaxone, and penicillin,^{39–41} no report has been published on the feasibility of using biocathode BES technology for removing antibacterial activity and enhancing biodegradability of CAP.

This study aims at revealing the feasibility of cathodic reduction of CAP with BES and unveiling the reaction pathways. We demonstrated that the CAP removal efficiency in the BES with biocathode was significantly enhanced and CAP reduction rate accelerated using BES with an abiotic cathode as a control. The results have proven the effectiveness of removal of an antibiotic using biocathode BES.

MATERIALS AND METHODS

Reactor Setup. Same sized, dual chamber BES reactors made of polycarbonate were constructed by assembling two equal-size Lexan plates ($7 \times 7 \times 4 \text{ cm}^3$) with a cylindrical cavity (5 cm in diameter, 4 cm in length) (Supporting Information (SI) Figure S1). One set reactor was started as biocathode BES with microbial inoculation while another set was started as abiotic cathode BES without inoculation. For BES construction, the two plates were bolted together between two equal-size Lexan plates ($7 \times 7 \times 1 \text{ cm}^3$) and separated by a cation exchange membrane (Ultrex CMI-7000, Membranes International, U.S.). The working volume of each chamber was 85 mL. A graphite fiber brush (4 cm in diameter and 30 mm in length, TOHO TENAX Co., Ltd., Japan) was used as anode. For the CAP reduction tests, carbon cloth (5 cm in diameter, nonwet proof, YB-20, YiBang Technology Co., Ltd., China) was used as the cathode, which was pretreated by immersing them in 1 M hydrochloric acid for 24 h then immersed in deionized water for another 24 h. Titanium wire (1 mm in diameter, Baoji LiXing Titanium Group Co., Ltd., China) was connected onto the cathode as current collector. A high-precision resistor (10 Ω) with the power in series was employed for the connection. External power source (0.5 V) was applied to test CAP reduction during abiotic cathode and biocathode BES operation unless otherwise mentioned. Saturated calomel reference electrode (SCE, 0.247 V vs standard hydrogen electrode (SHE), model-217, Shanghai Precise. Sci. Instru. Co., Ltd., China) was inserted into the cathode chamber to measure the cathode potential. All of the potentials reported herein were against SHE. When the additional power source and the resistor were disconnected the BES was operated as opened biocathode mode. The tests with opened biocathode BES lasted for 120 h, hence should have minimal effects on the microbial communities in the biofilms.

Microbial Inoculum and BES Tests. Microbial consortium used as the inoculum of the biocathode was pre-enriched using activated sludge obtained from Taiping wastewater treatment plant, Harbin, China. Sludge (about 10 g dry weight) was mixed with 200 mL anaerobic medium which was prepared by boiling and purged with N_2 gas (99.9% purity) for 15 min. The medium consisted of CAP (32 mg/L), glucose (500 mg/L), and the nutrient medium previously described.³⁶ The mixed culture was incubated in a rotary shaker at 160 rpm and 25 °C for one week, and then was transferred to another fresh medium (180 mL) at 10% dilution once a week. This procedure was repeated four times. The CAP-removal efficiency of the enriched consortium was monitored by determine CAP concentration using HPLC described below. Finally, the enriched consortium, or CAP-reducing consortium, was capable of converting CAP to amine product by $88.0 \pm 4.7\%$ in 72 h.

To start up the biocathode BES, the cathode chamber was filled with a mixture of the CAP-reducing consortium and catholyte at 1:3 (v/v) and incubated for 4 days with the applied voltage of 0.5 V. Subsequently, the cathode liquid content was discharged and the cathode chamber was replenished with a fresh mixture. This procedure was repeated for five times to achieve the development of biofilm on the cathode. Two BES reactors with well-developed biocathode were operated using CAP as substrate for five batch cycles to collect data of biocathode mode. Subsequently, these two reactors were switched to opened circuit and disconnection of additional power source (0.5 V) for further test as opened biocathode mode. Under this condition, the microbial population of biocathode conducted bioreduction of CAP with glucose as electron donor but no electron flowed from anode to the biocathode. Other two BES reactors with abiotic cathode, as well as the control experiment (no current and microbes) were operated for three cycles. The catholyte was prepared anaerobically by aerating N_2 gas and filtered via a 0.22 μm filter during all the experiments. To maintain sufficient levels of electron donor acetate in the anodic chamber, anolyte was replenished when the catholyte was refilled. All tests were conducted at room temperature (26 ± 2 °C).

Chemicals and Analytical Methods. Chloramphenicol (>98% purity) and HPLC grade methanol were purchased from Sigma-Aldrich. Ethyl acetate and chloroform used were of analytical grade.

Purification of major products during CAP transformation was conducted using thin layer chromatography (TLC) technology. The effluent (200 mL) of biocathode BES was taken at 4 and 48 h of an operational cycle, and extracted by shaking with 200 mL of ethyl acetate at 150 rpm for 2 h, respectively. The former contained mainly CAP-acetyl and CAP while the latter contained mainly AMCl₂ and AMCl. Then the upper ethyl acetate layer was dried using a rotary evaporator at 45 °C and the residues were dissolved into 2 mL methanol. The TLC with silica gel 60 HF254 adopted methanol/chloroform (20:80; v/v) as the mobile phase. The TLC purified samples were analyzed using a reversed phase liquid chromatography (Agilent 1200) with a quadropole time-of-flight mass spectrometry (QTOFMS) (Agilent 6520) detection in the negative ion mode, which performed on an Agilent Zorbax XDB C18 column (1.8 μm ; $2.1 \times 100 \text{ mm}$) with methanol/ H_2O (55:45; v/v) as the mobile phase at a flow rate of 0.15 mL/min with UV detection at 250 nm.

The determination of chloride ion was performed by an ion chromatography (IC) (ICS-3000, Dionex, Sunnyvale, CA) equipped using an IonPac AG20 guard column (2×50 mm I.D.) and an anion AS11-HC (2×250 mm I.D.) analytical column with KOH at a flow rate of 1.0 mL/min (0–7 min, 20 mM KOH; 7–15 min, 30 mM KOH) as the mobile phase. Samples were injected into the 100 μ L sample loop using an AS autosampler (Dionex).

Samples obtained from the cathodic chamber were filtered through a 0.45 μ m filter. Concentrations of CAP and transformation products were analyzed using a reverse-phase HPLC (model e2695, Waters Co., Milford, MA) with UV detection at 250 nm for AMCl₂ and AMCl, 275 nm for CAP and CAP-acetyl, and 310 nm for NO and HOAM intermediates, respectively. The separation of CAP and transformation products was performed with a C18 column (5 μ m; 5×250 mm, Waters Co.) and a mobile phase of methanol/H₂O (55:45; v/v) at a flow rate of 0.8 mL/min at 30 °C. To identify the products during CAP transformation, HPLC-MS/MS tests were performed. The samples of catholyte were collected at 10, 36, and 48 h for biocathode BES tests and at 0 and 96 h for abiotic cathode BES tests. The collected catholytes were centrifuged at 13 000g for 30 min and the supernatants were diluted by methanol prior to measurement. The HPLC-MS/MS (ion trap) (Thermo Finnigan LCQ Deca XP Max LC/MS, Germany) was equipped with an electrospray ionization source and operated in the positive/negative polarity mode. Separation was performed with a C18 column under the same condition as described above. The intermediates scanned in the normal mass range from 50 m/z (mass to charge ratio) to 500 m/z were separated and confirmed first by first-order MS with the full scan mode and further verified by tandem MS/MS with single reaction monitoring mode.

The current (I) and CAP removal efficiency (Er_{CAP} , %) were calculated as described previously.^{36,37} The kinetics of CAP transformation was assumed to follow first-order reaction model as $C = C_0 e^{-kt}$, where C is CAP concentration (mg/L) at time t (h) and C_0 is initial CAP concentration (32 mg/L). The rate constant k (1/h) was calculated by SigmaPlot v.11.0 software analysis. The half-life time ($t_{1/2}$) of CAP was calculated using an equation: $t_{1/2} = 0.693/k$.⁴²

Antibacterial Activity Measurement. After reaction of 160 h, most CAP in catholyte was consumed. Catholyte (300 mL) was withdrawn from the BES and then centrifugated at 13 000g for 30 min to collect supernatant. The supernatant was then dried by lyophilization. The residues were dissolved into 10 mL methanol, which was then concentrated to a final volume of 600 μ L. The concentrated methanol solution was filtered via a sterile 0.22 μ m filter prior to antibacterial activity tests. The antibacterial activity of CAP and AMCl to bacterial strains was evaluated based on their toxicity on the viabilities of *Escherichia coli* strain DH5 α (a typical Gram-negative bacterium) and *Shewanella oneidensis* strain MR-1 (an electrogenic bacterium). Cells of both strains were harvested in their exponential growth phase, washed for three times using sterile phosphate buffer solution (PBS) (50 mM, pH 7.0), and then inoculated into 50 mL of 3-fold-diluted Luria–Bertani (LB) medium with the same cell concentrations based on optical density at 600 nm ($OD_{600\text{ nm}}$) by a Shimadzu spectrometer (UV2550, Japan). Both the CAP and the AMCl were added to the 3-fold-diluted LB medium at the same concentration of 32 mg/L. The same medium without CAP and AMCl served as

control. The antibacterial activity assay was performed in triplicates.

During the antibacterial activity assay tests, samples were collected every 3 h. Biomass was collected by centrifugation of 1 mL cell suspension sample at 13 000g for 10 min and the pellets were resuspended in the 1 mL PBS (50 mM; pH 7.0) to measure $OD_{600\text{ nm}}$ (Shimadzu UV2550 spectrometer, Japan).

Cyclic Voltammetry Analysis. Analysis of cyclic voltammetry (CV) was performed using an electrochemical workstation (model-660D, CH Instruments Inc. U.S.) equipped with three-electrode system. The cathode/biocathode and the anode of BES were used as the working and counter electrode, respectively. The SCE was the reference electrode. Concentration of CAP and glucose was 32 mg/L and 500 mg/L, respectively, if applied. Cyclic voltammograms were recorded with the scan rate of 5 mV/s at 25 °C in all CV tests.

Electrochemical Dechlorination Mechanism Analysis. To verify the dechlorination mechanism of abiotic cathode BES, one abiotic cathode reactor with catholyte containing only 5 mM PBS and 200 mg/L CAP were tested at -0.4 V (producing no H or H₂) or at -0.7 V (same as the present fed-batch tests) cathodic potential (WMPG1000K8 multichannel potentiostat, WonATech Co., Ltd., Korea). The standard Hg/Hg₂SO₄ reference electrode (R0401, Tianjin Aidahengsheng Technology Co., Ltd., China) was used for Cl[−] measurement to avoid disturbance by Hg₂Cl₂ and KCl in the SCE. The cathode effluents (160 h) under two voltages were analyzed by IC and HPLC.

Microbial Community Analysis. The samples of CAP-reducing consortium (10 mL) and the biofilm on biocathode collected at the end of test were used for microbial community analysis. The total genomic DNA of the collected samples was extracted using a UNIQC-10 DNA Isolation Kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The PCR-denaturing gradient gel electrophoresis (DGGE) analysis was performed to target the V3 region of bacteria as described previously.⁴³ Fourteen bands were sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster, CA) by Sangon Biotech (Shanghai, China). The obtained sequences were deposited to GenBank with accession numbers being JQ319508 to JQ319522. Analysis of 16S rRNA gene sequences was conducted according to previous method.³⁶

■ RESULTS AND DISCUSSION

Bioelectrochemical Reduction of CAP. Three different modes were tested for comparison in this study: biocathode BES with closed circuit and external power source (biocathode in short), BES with biocathode at opened circuit (opened biocathode), and BES with an abiotic cathode (abiotic cathode). The sequence of CAP removal rate (k_{CAP}) was: biocathode > opened biocathode > abiotic cathode (Figure 1). In abiotic BES control (without current and microbes), the CAP concentration was declined slightly without detectable intermediates/products. The slight decline in CAP concentration was attributable to CAP adsorption onto carbon cloth and/or plastic surface of reactor parts. Conversely, the Er_{CAP} of the biocathode BES was $87.1 \pm 4.2\%$ at 4 h and $96.0 \pm 0.9\%$ at 24 h. Er_{CAP} of the abiotic cathode BES was only $73.0 \pm 3.2\%$ at 24 h. This indicated that more CAP was removed in biocathode BES within 4 h than abiotic cathode within 24 h. The BES with abiotic cathode and biocathode had similar cathode potentials (about -0.7 V vs SHE) but the initial current of biocathode

Figure 1. Reduction of CAP under different operating conditions in fed batch BES. Lines were fitted to apparent first order reaction model for kinetic analysis. The respective CAP removal rate (k) and half-life time ($t_{1/2}$) under different conditions are: biocathode, $R^2 = 0.9798$, $k = 0.5197 \pm 0.0533$, $t_{1/2} = 1.33\text{h}$; abiotic cathode, $R^2 = 0.9986$, $k = 0.0520 \pm 0.0008$, $t_{1/2} = 13.33\text{h}$; and open biocathode, $R^2 = 0.9309$, $k = 0.3222 \pm 0.0652$, $t_{1/2} = 2.16\text{h}$. Test conditions: ambient temperature ($26 \pm 2^\circ\text{C}$); initial CAP concentrations of 32 mg/L.

was 3.2 times as that with abiotic cathode (SI Figure S2). Further comparison was done with the opened biocathode BES to verify the role of electrochemical reaction. The k_{CAP} of biocathode was 1.6 times to that of opened biocathode although both had identical biofilm (Figure 1), indicating that

the generation of electron from anode and applied external voltage of 0.5 V did enhance the CAP reduction. Among the three operational modes, biocathode mode showed the highest k_{CAP} and E_{CAP} . The electrode likely provided a persistent extracellular electron while glucose in cathode acted as intracellular electron donor and carbon source for cathophilic bacteria that involved in CAP reduction metabolism.

Reduction Products of CAP. The products of CAP transformation with biocathode (in both closed and opened modes) were very different from those of produced with abiotic cathode (Figure 2). AMCl2 was produced rapidly as CAP was reduced in the biocathode BES. Conversely, AMCl2 was accumulated slowly in the abiotic cathode BES with nitroso (NO) and hydroxylamino (HOAM) as the additional intermediates (Figures 2A and 2D). The peak concentrations of AMCl2 produced from CAP in the abiotic cathode BES and opened biocathode was much lower than that in biocathode BES (Figure 2A). One intermediate, CAP-acetyl, was found only in both opened and closed biocathode BES (Figure 2C), indicating that it was produced from bioactivity since CAP acetyltransferase catalyzes acetylation of 3-hydroxyl of CAP while 3-hydroxyl acetylated CAP can also be transformed reversibly to 1-hydroxyl acetylated CAP.⁴⁴ In prolonged test, the produced AMCl2 was further reductively dechlorinated to AMCl under all BES modes (Figure 2B). The products mentioned herein were identified with HPLC-MS/MS, LC-QTOFMS and assisted with IC as demonstrated below.

Antibacterial Activity Elimination Due to CAP Reduction. The antibacterial activity assay was performed using two selected bacterial strains (*E. coli* strain DH5 α and *S. oneidensis*

Figure 2. Major CAP transformation products in fed-batch BES with abiotic cathode and biocathode (opened and closed circuit). AMCl2: reduced amine product; NO: nitroso intermediate; HOAM: hydroxylamino intermediate; AMCl: dechlorinated AMCl2; CAP-acetyl (acetylation of 1-hydroxyl or 3-hydroxyl of CAP to form CAP-acetyl).

strain MR-1). The growth of both strains was completely inhibited in the presence of CAP at 32 mg/L, but was not in the presence of AMCl at the same concentration (Figure 3).

Figure 3. Effect of CAP (32 mg/L) and AMCl (32 mg/L) on the growth of *E. coli* DH5α and *S. oneidensis* MR-1. The normal growth of two tested strains in the 3-fold-diluted LB medium was also evaluated.

This observation suggests that the end product AMCl has much less antibacterial activity than CAP. The similar observation indicated that AMCl₂ lacked antibacterial activity.¹⁸ As AMCl is less toxic to microorganisms and relatively easier to be biodegraded, thus, the reduction of CAP to AMCl would have ecological significance in the elimination of the selection pressure of antibiotic for the generation of antibiotic resistant bacteria/genes in the environments.^{16,45} On the other hand, HOAM and NO, the intermediates of CAP reduction in abiotic cathode BES, are toxic to microorganisms,⁴⁶ and can retain their antibacterial activity in another kind of nitroaromatic antibiotic nitroimidazoles.⁴⁷ As mentioned above, neither

HOAM nor NO was detected in the biocathode BES, therefore, the biocathode is likely superior to abiotic cathode in the elimination of antibacterial activity from CAP-containing wastewaters.

CAP Reduction Pathways. The reaction products of CAP reduction were identified by the HPLC-MS/MS, LC-QTOFMS and IC analysis. The detailed analytical procedures for the identification are available in the SI (Figures S3–S8). In the abiotic cathode BES, CAP was reduced to AMCl₂ with HOAM and NO as accumulated intermediates. In the biocathode BES, CAP was selectively converted to AMCl₂ with one identified intermediate, acetylated CAP (CAP-acetyl) (SI Figure S5), which was accumulated only in the biocathode BES (peaked at 2 h) and opened biocathode BES (peaked at 4 h) (Figure 2C). This observation indicated that the acetylation of 3-hydroxyl of CAP occurred with formation of CAP-acetyl as part of the metabolism by the microbes in biocathode. Some bacteria have CAP acetyltransferase which catalyzes acetylation of 3-hydroxyl group.⁴⁴ The CAP-acetyl was further reduced to its amine form and then be rapidly converted to AMCl₂ within 48 h in biocathode BES (Figure 2C).

Two amine products of CAP (AMCl₂) and a novel dechlorinated product (AMCl) were identified in this study (SI Figure S6). In both the opened and closed biocathode BES CAP was reduced to AMCl₂ and then was dechlorinated to AMCl (Figure 2B). Diverse dehalogenase in target chlorinated alkanes and alkanolic acids (similar as the side chain of CAP)⁴⁸ may be involved in the dechlorination process in the biocathode BES. We used the abiotic cathode BES with catholyte containing only 5 mM PBS and 200 mg/L CAP to analyze abiotic electrochemical dechlorination mechanism. At -0.4 V cathodic potential, after 160 h reaction in the abiotic cathode BES, 98.5% of CAP was transformed but failed to effectively dechlorinate AMCl₂. Conversely, CAP was effectively reduced to AMCl₂ and then to AMCl at -0.7 V

Figure 4. Proposed pathway of the cathodic CAP reduction in the BES. Green bar represents the abiotic cathode and biocathode (attached diverse cathodophilic bacteria). Electrode and glucose are both the electron donor for cathodophilic bacteria for reduction of CAP and glucose also works as carbon source for the cathode biofilm metabolism. Purple arrows indicates the occurred reduction reaction was presumed in this study. CAP-acetyl was the unique product found in biocatalyzed process, while NO and HOAM intermediates were only detected in abiotic electrochemical process. Dehalogenase likely catalysis of AMCl₂ to AMCl as pure microbial reduction also has the dechlorination activity. Electrochemical hydrodechlorination responsible for the dechlorination of AMCl₂ to AMCl in abiotic cathode BES.

Figure 5. Cyclic voltammograms of biocathode and abiotic cathode in the presence of CAP. Solid line: abiotic cathode; dashed line: biocathode; dashed-dotted line: biocathode without glucose. Insert: enlarging for comparison of biocathode and abiotic cathode in the potential window between 0.6 V and -0.2 V (A). Cyclic voltammograms of biocathode in the absence of CAP. Dashed line: without CAP only; dashed-dotted line: without CAP and glucose; solid line: blank, which represented the case without bacteria in despite of the presence of glucose, as CVs of abiotic cathode with or without glucose did not have obvious difference here (B). Concentration of CAP and glucose was 32 mg/L and 500 mg/L, respectively, if used. Scan rate for all tests was 5 mV/s.

Table 1. Phylogenetic Identification of Prominent DGGE Bands Based on the V3 Region of the 16S rRNA Gene

band (accession no.)	closest type strain in GenBank Database (accession no.)	phylum/class	similarity (%)
1 (JQ319508)	<i>Elizabethkingia miricola</i> GTC862 ^T (AB071953)	bacteroidetes/flavobacteriia	97.88
2 (JQ319509)	<i>Elizabethkingia miricola</i> GTC862 ^T (AB071953)	bacteroidetes/flavobacteriia	97.87
3-1 (JQ319510)	<i>Fluviicola taffensis</i> DSM 16823 ^T (CP002542)	bacteroidetes/flavobacteriia	96.70
3-2 (JQ319511)	<i>Alcaligenes faecalis</i> subsp. parafaecalis G ^T (AJ242986)	proteobacteria/ β -proteobacteria	98.45
4 (JQ319512)	<i>Tepidimicrobium ferrophilum</i> SB91 ^T (AY656718) or <i>Peptoniphilus methionivorax</i> RRL B-23883 ^T (GU440754)	firmicutes/clostridia	94.68
5 (JQ319513)	<i>Salmonella bongori</i> NCTC 12419 ^T (FR877557) or <i>Enterobacter cloacae</i> subsp. dissolvens LMG 2683 ^T (Z96079)	proteobacteria/ γ -proteobacteria	97.41 (or 97.40)
6 (JQ319514)	<i>Stenotrophomonas nitritireducens</i> L2 ^T (AJ012229)	proteobacteria/ γ -proteobacteria	100.00
7 (JQ319515)	<i>Ferribacterium limneticum</i> cda-1 ^T (Y17060) or <i>Dechloromonas denitrificans</i> ED1 ^T (AJ318917)	proteobacteria/ β -proteobacteria	95.88
8 (JQ319516)	<i>Devosia psychrophila</i> Cr7-05 ^T (GU441678)	proteobacteria/ α -proteobacteria	100.00
9 (JQ319517)	<i>Clavibacter michiganensis</i> subsp. insidiosus LMG 3663 ^T (U09761)	actinobacteria/actinobacteridae	100.00
10 (JQ319518)	<i>Pseudomonas beteli</i> ATCC 19861 ^T (AB021406)	proteobacteria/ γ -proteobacteria	96.30
11 (JQ319519)	<i>Ochrobactrum lupini</i> LUP21 ^T (AY457038)	proteobacteria/ α -proteobacteria	98.12
12 (JQ319520)	<i>Myroides odoratus</i> ATCC 4651 ^T (M58777)	bacteroidetes/flavobacteriia	97.01
13 (JQ319521)	<i>Pseudomonas putida</i> DSM 291 ^T (Z76667)	proteobacteria/ γ -proteobacteria	100.00
14 (JQ319522)	<i>Flavobacterium lindanitolerans</i> IP-10 ^T (EF424395)	bacteroidetes/flavobacteriia	100.00

(SI Figure S7). Stoichiometrically complete reduction of 200 mg/L (0.62 mM) CAP to AMCl should produce 21.94 mg/L (0.62 mM) Cl^- . Hence, the Cl^- recovery efficiency in abiotic cathode BES were 107.8% and 15.7% at -0.7 V and -0.4 V cathodic potential, respectively (SI Figure S7). This observation indicated that electrochemical hydrodechlorination mechanism⁴⁹ was incorporated in the abiotic dechlorination reaction. Complete dechlorination of AMCl to AM may be possible but requires further study such as via decreasing cathode potential to below -0.7 V or enriching specific dechlorinating microbial consortium. This is the first report for microbial or pure

electrochemical dechlorination of amine product of CAP. We proposed the pathway of CAP reduction in the cathodic BES based on the intermediates and products identified (Figure 4). In the abiotic cathode BES, CAP was reduced to AMCl₂ with the HOAM and NO as intermediates, and the formed AMCl₂ can be further dechlorinated to AMCl via electrochemical hydrodechlorination. In the biocathode BES, CAP was transformed to AMCl₂ without toxic intermediate accumulation and then AMCl₂ was converted to AMCl likely by dehalogenase of microorganisms. In the biocathode BES, CAP-acetyl was found as an intermediate and eventually disappeared.

The dechlorination pathway of CAP in abiotic cathode and biocathode BES is significantly different, and biocathode BES shows higher dechlorination rate from AMCl₂ to AMCl than abiotic cathode BES.

Microbial Role in CAP Reduction on Biocathode. The CV tests with CAP in abiotic BES was started at around -0.3 V. The peak potential for CAP reduction was observed at about -0.6 V (peak a). An oxidation peak (peak b) appeared in the return half-cycle, accompanying with a new reduction peak (peak c') coming out in the second cycle (SI Figure S9). Similar CV behavior was observed during CAP reduction on the boron-doped diamond electrode⁵⁰ and that of the other nitro compounds on carbon based electrode,⁵¹ whose pair redox peaks was proposed as the reversible reaction of the hydroxylamine and nitroso derivatives. We proposed that the intermediates HOAM and NO in abiotic BES were contributing to the peak b' and peak c', respectively, in the CV measurements.

Compared to the CV profile of abiotic cathode (Figure 5A, curve C), the CV of biocathode, despite of the presence of glucose or not (Figure 5A, curves A and B), revealed much higher positive onset potential (0 V vs -0.3 V) with the increase in cathodic current. No increase in cathodic current was observed before -0.6 V in the biocathode CV in the nutrient medium (Figure 5B, curve E). Therefore, the onset potential of 0 V in CV noted for biocathode should be at least partially contributed by the CAP reduction. Based on the further negative scanning on cathode potential, much higher cathodic current was generated in biocathode than that in abiotic cathode, indicating the higher electrochemical activity for CAP reduction on biocathode to be revealed below -0.6 V (Figure 5A). The enhancement of cathodic current and the positive shift of onset potential for the cathodic current indicated that the cathodic biofilm catalyzed the bioelectrochemical CAP reduction. The hydrogen evolution is theoretically inactive at the cathode potential higher than -0.41 V at neutral pH.⁵² The noted onset potential of CAP reduction by biocathode (0 V) suggested that the electron transfer from cathode to microbial cells was not via cathodic produced hydrogen.³⁰ Additionally, the pair peak involved in the redox reaction of HOAM and NO intermediates only found in the CV profile of abiotic cathode were not observed in the CV of biocathode (Figure 5A, curve A). This observation is consistent with the no-detection of HOAM and NO intermediates in the biocathode BES. In the absence of CAP, addition of glucose caused the pronounced increase of cathodic current of biocathode at a more positive potential (Figure 5B, curve D compared to curve E), indicating certain cathodic reactions to occur in the presence of glucose.

Microbial Communities of Cathode Biofilm. Succession of the bacterial community from inoculum to cathode biofilm was revealed by 16S rRNA gene analysis (Table 1 and SI Figure S10). The DGGE bands were most closely affiliated with the 16S rRNA genes of type species from Bacteroidetes (*Flavobacteriia*) and Proteobacteria (α , β , and γ -Proteobacteria), both were dominated in the inoculum and cathode biofilm. The microbial community in the cathode biofilm was different from the inoculated culture. Two major bands, affiliated to *Salmonella bongori* NCTC 12419^T (or *Enterobacter cloacae* subsp. *dissolvens* LMG 2683^T) and *Clavibacter michiganensis* subsp. *insidiosus* LMG 3663^T, respectively, were noted in the cathode biofilm but were not observed in the inoculum. Both genus were capable of reducing nitroaromatic compounds with

nitroreductase.^{53,54} Species belonged to *Enterobacter* are capable of reducing nitrobenzene or O₂ in the cathode.^{36,55,56} Some strains of *Pseudomonas* spp. appeared in both the inoculum and cathode biofilm, which were also reported to reduce nitroaromatic compounds with nitroreductase⁵⁴ and could directly uptake electron from the electrode for nitrate and sulfate reduction.^{57,58} This suggested that *Pseudomonas* spp. on biocathode may involve in the bioelectrochemical reduction of CAP. Metabolites (e.g., pyocyanin, phenazine-1-carboxamide, and biosurfactants)-based mutualism between *Pseudomonas* and *Enterobacter* species or Gram-positive species formed coculture to enhance current generation in BES fed with glucose or acetate.^{59,60} One species in the cathode biofilm has the highest 16S rRNA gene sequence similarity with type strain *Dechloromonas denitrificans* ED1^T (or *Ferribacterium limneticum* cda-1^T). *Dechloromonas agitate* CKB formed biocathode was reported to be capable of reducing perchlorate with 2,6-anthraquinone disulfonate as an electron shuttle mediator in a BES.³⁵ The cathodophilic functional microbes could reduce CAP with nitroreductase and dehalogenase as terminal reductases and obtain electrons directly from electrode or metabolite-based mutualism between these cathodophilic microbes. Further study is needed to understand the mechanism of electron transfer from electrode surface to cathodophilic functional microbes in order to enhance the efficiency of the CAP-reducing biocathode in BES. Additionally, several species in the inoculum disappeared or nearly disappeared after 4 months enrichment but they were found on the biocathode (SI Figure S10). The microbial community structure in the BES was changed with continuous electrochemical stimulation.

■ OUTLOOK

Factually, wastewater treatment plants are important point sources of antibiotic entry into the environment.^{5,61} In this study, we demonstrated the feasibility of using bioelectrochemical transformation to convert CAP to AMCl₂, which was further dechlorinated to AMCl in biocathode BES. Along with this reduction, the antibacterial activity was eliminated in the biocathode BES, which also enhanced biodegradability of the CAP-containing wastewaters. Reduction of nitro group of CAP and AMCl₂ dechlorination with biocathode BES showed higher efficiency than abiotic cathode BES. Bacterial dehalogenases catalyze the cleavage of carbon-halogen bonds and play key roles in the detoxification of chlorinated compounds and even in the energy catabolism of strictly anaerobic bacteria^{22,48,62} such as cathodophilic bacteria using AMCl₂ as electron acceptor. Nitro group reduction combined with dechlorination reaction enhanced the detoxication efficiency of CAP in BES. The results of this study demonstrated the feasibility of use of electrochemical bioreduction in biocathode BES to detoxify chlorinated nitro-group compounds such as CAP. The biocathode BES could have potential in advanced wastewater treatment for nitroaromatic antibiotic, explosives, pesticides, and herbicides. Potential target nitroaromatic antibiotic may also include nitrofurans, nitroimidazoles, phenylpyrrole, aureothin, neo-aureothin, thaxtomin, and azomycin. Such a process has its advantage over conventional wastewater treatment process on the minimization of the evolution of novel antibiotic resistant bacteria/genes in the environments.

■ ASSOCIATED CONTENT

Supporting Information

Identification of CAP reductive intermediates and products and Figures S1–S10. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +86-451-86282195. E-mail: djlee@ntu.edu.tw (D. J. L.); waj0578@hit.edu.cn (A. J. W.).

Author Contributions

[†]B. Liang and H. Y. Cheng contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the financial support by the National Natural Science Foundation of China (Grant No.51078100), by National Science Foundation for Distinguished Young Scholars (Grant No.51225802), by National Creative Research Groups Project (Grant No.51121062), by the National High-tech R&D Program of China (863 Program, Grant No. 2011AA060904), by The Ph.D. Programs Foundation of Ministry of Education of China (20102302110055), by “Hundred Talents Program” of the Chinese Academy of Sciences, and by Heilongjiang Science Foundation for Distinguished Young Scholars (Grant No. JC201003). Dr. Wei-Min Wu was a guest professor at Harbin Institute of Technology during this study.

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