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ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · DECEMBER 2011

Impact Factor: 5.33 · DOI: 10.1021/es202356w · Source: PubMed

Environmental Science & Technology

ARTICLE

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Efficient Reduction of Nitrobenzene to Aniline with a Biocatalyzed Cathode

Ai-Jie Wang,*[,†] Hao-Yi Cheng,[†] Bin Liang,[†] Nan-Qi Ren,[†] Dan Cui,[†] Na Lin,[†] Byung Hong Kim,[†] and Korneel Rabaey[‡,§]

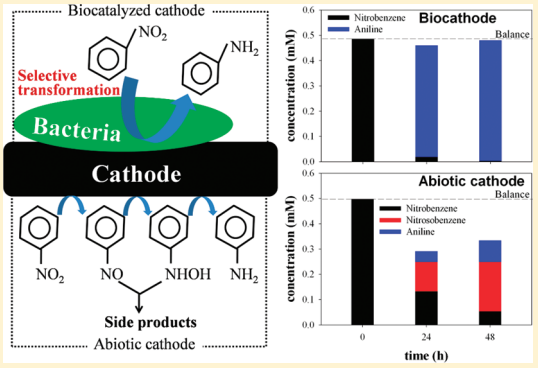
[†]State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150090, P. R. China

[‡]Advanced Water Management Centre, The University of Queensland, St. Lucia, QLD 4072, Australia

[§]Laboratory for Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

Supporting Information

ABSTRACT: Nitrobenzene (NB) is a toxic compound that is often found as a pollutant in the environment. The present removal strategies suffer from high cost or slow conversion rate. Here, we investigated the conversion of NB to aniline (AN), a less toxic endproduct that can easily be mineralized, using a fed-batch bioelectrochemical system with microbially catalyzed cathode. When a voltage of 0.5 V was applied in the presence of glucose, 88.2 ± 0.60% of the supplied NB (0.5 mM) was transformed to AN within 24 h, which was 10.25 and 2.90 times higher than an abiotic cathode and open circuit controlled experiment, respectively. AN was the only product detected during bioelectrochemical reduction of NB (maximum efficiency 98.70 ± 0.87%), whereas in abiotic conditions nitrosobenzene was observed as intermediate of NB reduction to AN (decreased efficiency to 73.75 ± 3.2%). When glucose was replaced by $NaHCO_3$, the rate of NB degradation decreased about 10%, selective transformation of NB to AN was still achieved (98.93 ± 0.77%). Upon autoclaving the cathode electrode, nitrosobenzene was formed as an intermediate, leading to a decreased AN formation efficiency of 71.6%. Cyclic voltammetry highlighted higher peak currents as well as decreased overpotentials for NB reduction at the biocathode. 16S rRNA based analysis of the biofilm on the cathode indicated that the cathode was dominated by an *Enterococcus* species closely related to *Enterococcus aquimarinus*.



INTRODUCTION

Nitrobenzene (NB) is widely used during chemical processes producing dyes, pharmaceuticals, aniline, and solvents. This nitro-compound is very toxic to human beings and other organisms. Many governments[1,2] introduced strict limitations for NB concentrations in the environment, e.g. 17 μg/L in the US and 20 μg/L in China. Moreover, due to the fact that the electrophilic effect of nitryl decreases the electron density of the benzene,[3] NB is only to a limited extent degraded in aerobic biological processes. An effective strategy is to transform NB to aniline (AN) first, for aromatic amines are a factor 500 less toxic and considerably easier mineralized than their corresponding nitroaromatics.[4] To date, a range of chemical and biological methods involved in transformation NB to AN have been developed, such as microelectrolysis,[5] electrochemical reduction,[3] and biological anaerobic process.[6] Generally, the chemical methods can convert NB at a higher rate than biological processes but consume more energy or require significant quantities of chemicals and expensive noble metal catalysts, which increase the cost and may cause secondary pollution. The biological methods are more cost-effective but suffer from lower degradation rates.

Recently, bioelectrochemical systems (BES) have been developed for the reductive removal or transformation of various contaminations including nitrate,[7] perchlorate,[8] Cr^{6+},[9] azo dye[10] etc. BESs decrease the energy needs as well as the reductant consumption (organic matter as electron donor) compared to the conventional biological methods. BESs with a bioanode and a chemical cathode have recently been shown to reduce NB to AN.[11] Compared to the conventional biological approach, a much higher NB reduction rate and a lower reductant usage ratio were obtained. The energy consumption of 0.05 KWh m^{-3} d^{-1} was about 10 times lower than that of the pure electrochemical reduction. However, nitrosobenzene was detected in effluent at higher current density conditions. Nitrosoaromatics are more toxic than parent nitroaromatics in many instances.[6] They also

Received: July 8, 2011
Accepted: October 10, 2011
Revised: October 3, 2011
Published: October 10, 2011

dx.doi.org/10.1021/es202356w | Environ. Sci. Technol. 2011, 45, 10186–10193

chemically condense with hydroxylamino aromatics, another intermediate usually detected in the reduction of nitroaromatics by abiotic cathodes, to produce azo compounds which are difficult to mineralize. The straight electrochemical reduction of NB has been the subject of several studies,[3,12] in which intermediates such as aminophenol, aniline, azobenzene, and azoxybenzene were found.[12] Low pH and noble metal modified electrodes[13−15] are usually required for selective transformation of NB to AN. Within the context of BESs, it may be attractive to use a biologically catalyzed cathode,[16] as a number of bacteria are known to completely convert nitroaromatics to their corresponding animo aromatic compounds.[17]

Therefore, we have used carbon cloth electrodes as electron donor to selectively reduce NB to AN catalyze by an NB reducing mixed culture. The performance of this reactor is discussed in terms of the NB removal efficiency, the efficiency of aniline transformation from NB, and the Coulombic efficiency.

MATERIALS AND METHODS

Reactor Design and Construction. The BES reactor was constructed by assembling four equal-size Lexan plates ($7 \times 7 \times 2$ cm^3) with a cylindrical cavity (5 cm in diameter, 2 cm in length) as shown in Figure S1 of the Supporting Information. The four plates were bolted together between two equal-size Lexan plates ($7 \times 7 \times 1$ cm^3) with a half excavated cylindrical cavity (5 cm in diameter, 0.5 cm in length) and separated by a cation exchange membrane (Ultrex CMI-7000, Membranes International, U.S.). The internal 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 the anode. Two different cathodes were used during this study. While the anodic microorganisms were enriched (MFC mode), carbon paper (5 cm in diameter, E-TEK, U.S.) coated with 0.5 mg Pt/cm^2 (20 wt % Pt/C, Johnson MaRhey, U.K.) was used as the cathode, and for the nitrobenzene reduction experiments (in power supply mode) carbon cloth (5 cm in diameter, nonwetproof, YB-20, YiBang Technology Co. Ltd. China) was used replacing the Pt-coated cathode that was used during the anodic enrichment phase. Both kinds of cathodes were pretreated by immersing them in 1 M hydrochloric acid for 24 h and then in deionized water for another 24 h.

Titanium wire (1 mm in diameter, Baoji LiXing Titanium Group Co., Ltd., China) was pressed onto the cathode as current collector. An Ag/AgCl reference electrode (0.195 V vs SHE, model-217, Shanghai Precise Sci. Instru. Co., Ltd. China) was inserted into the cathode chamber to measure the cathode potential. The anode and cathode were connected through a resistor of 1000 Ω when the reactor was operated in MFC mode. A high-precision resistor at 5 Ω with a power supply in series were used for the connection when the reactor was in power supply mode (see below).

Enrichment of the Anodic Microbial Community. Domestic wastewater was used as the inoculum, mixed (v:v = 50:50) with nutrient medium (0.31 g/L NH4Cl, 0.1 g/L KCl, 10 mL/L Wolf's vitamins, 10 mL/L Wolf's trace elements, 50 mM PBS, pH = 7), and subsequently added to the anode chamber with sodium acetate (1.5 g/L) as electron donor. The cathode chamber contained 50 mM phosphate buffer solution (PBS, pH = 7) and was aerated continuously. Prior to closing the circuit, the anodic solution was flushed with nitrogen from a nitrogen generator (purity >99.9%). Voltages were recorded using a data acquisition system (Model 2700, Keithley Instruments Inc., U.S.). The reactors was operated in a fed-batch mode, completely replacing the liquid solution after each cycle when voltage dropped below 20 mV. When the voltage output was stable, anode polarization experiments were conducted, and those anodes with similar polarization characteristics were chosen for the NB reduction experiment.

Enrichment of NB Reducing Consortium. The NB reducing consortium was enriched using serum bottles under anaerobic conditions. 10 mL of activated sludge (collected from a local sewage treatment plant) and 80 mL of nutrient medium containing 0.5 mM NB and 500 mg/L glucose were filled into twelve identical serum bottles and then incubated for 7 days at 30 °C. The culture with the highest efficiency and rate of aniline production was transferred to the same medium 4 times before inoculation. The performance of nitrobenzene reduction and aniline formation of the inoculum is shown in Figure S2 of the Supporting Information. 92.3% nitrobenzene can be transformed to aniline in 168 h.

Experiments of NB Reduction in BESs. The reactors with the enriched anode were changed from MFC mode to power supply mode for NB bioelectrochemical reduction. This involved replacing the Pt-coated cathode with a carbon cloth cathode, insertion of a high-precision resistor (5 Ω) in series with DC power supply (0.5 V), and use of 50 mM PBS cathodic solution with nutrient medium containing 0.5 mM NB and 500 mg/L glucose. 20 mL of NB reducing culture was centrifuged, and the pellet was mixed with the catholyte and added to the cathode chamber in the initial five cycles. Then the cathodic solution was only replaced by filter-sterilized catholyte (0.22 μm). All of the solution replacements were performed in an anaerobic glovebox. To avoid acetate depletion in the anode chamber anolyte was refreshed every time when the catholyte was renewed. In parallel, several control batch experiments were also performed under identical conditions, e.g. absence of the microbial culture (abiotic control tests) or absence of current (open circuit control test).

To further understand the mechanism of the bioelectrochemical reduction of NB, the following experiments were performed: (i) replacing the carbon source from glucose to $NaHCO_3$ (10 mM) and (ii) decreasing the applied voltage from 0.5 V to 0.15 V. All of the experiments mentioned above were carried out at room temperature (~25 °C) and repeated at least 3 cycles.

Cyclic Voltammetry. Cyclic voltammetry (CV) was performed using an electrochemical workstation (model-660D, CH Instruments Inc. U.S.) equipped with three-electrode system. Ag/AgCl electrode was used as the reference electrode, the BES anode as the counter electrode, and the abiotic cathode/biocathode as the working electrode. Cyclic voltammograms were recorded at 25 °C at a low scanning rate of 2 mV/s.

Chemical Analyses and Calculations. Samples taken from the cathode chamber were filtered through a 0.45 μm filter. The concentrations of nitrobenzene, nitrosobenzene, and aniline were measured using a high performance liquid chromatography system (model-2695, Waters, US) equipped with a Waters Symmetry C18 column (5 μm 4.6 × 250 mm) for separation at 35 °C and a UV/visible detector (model-2489 Waters, US) for measurement at 254 nm. The mobile phase consisted of 50% methanol and 50% deionized water and was used at a flow rate of 1 mL/min.

The current (I) was calculated with I = V/R according to the Ohm's law, where V was the voltage obtained from the high-precision resistor through the data acquisition system.

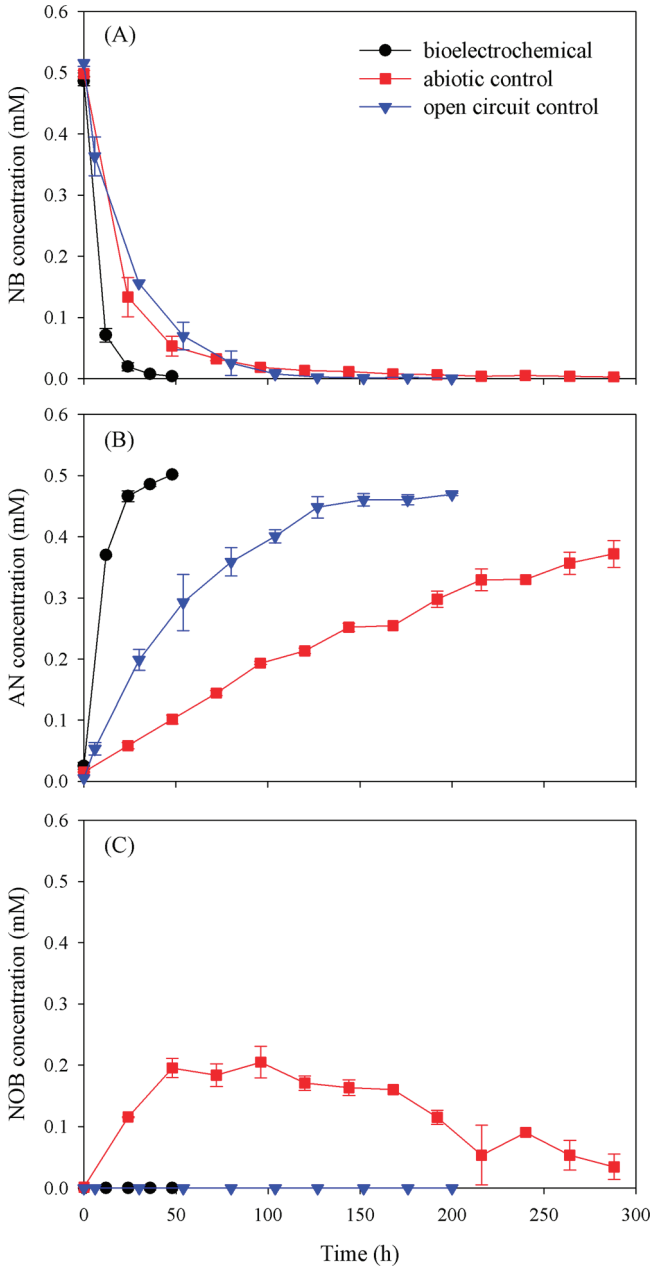


Figure 1. Reduction of NB and product formation at an applied voltage of 0.5 V. A: NB removal; B: AN formation; C: NOB production. Circle: bioelectrochemical; Square: abiotic control; Triangle: open circuit control (without current).

The efficiency of aniline production from NB ($E_{NB\text{-}AN}$, %), the NB removal efficiency (E_{rNB}, %), and the Coulombic efficiency (*CE*, %) were calculated as the factors evaluating the performance of NB reduction according to Mu et al.[11]

Constructing 16S rRNA Gene Clone Libraries. At the end of the biocathode experiment, a piece of the cathode was cut for total genomic DNA extraction using a UNIQ-10 DNA Isolation Kit (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.) according to the manufacturer's instructions. The bacterial 16S rRNA gene clone libraries were constructed by using universal primer sets 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification was performed following the conditions below:

Table 1. NB Removal and AN Formation over 24 h under the Different Applied Conditions

	applied voltage/ V	carbon source	$C_{NB\text{-removed}}$/ mM	$C_{AN\text{-formation}}$/ mM
biocathode	0.5	glucose	0.467 ± 0.001	0.441 ± 0.003
	0.5	$NaHCO_3$	0.424 ± 0.002	0.394 ± 0.002
	0.15	$NaHCO_3$	0.382 ± 0.002	0.233 ± 0.021
abiotic cathode	0.5	-	0.365 ± 0.027	0.043 ± 0.006
	0.15	-	0.278 ± 0.022	0.038 ± 0.008
open circuit	-	glucose	0.316 ± 0.011	0.152 ± 0.009

5 min of denaturation at 98 °C, followed by 35 cycles at 95 °C for 35 s, 55 °C for 35 s, and 72 °C for 90 s, with a final extension at 72 °C for 8 min. The PCR products were purified on a 1% agarose gel, extracted with a UNIQ-10 gel-extraction kit (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.), then ligated to vector pUCm-T, and cloned into *Escherichia coli* DH5α competent cells following the manufacturer's protocol. Ninety-five plasmids containing positive insert from this sample were sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster, CA) with 27F primer by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). GenBank accession numbers for the sequences are JF911621 to JF911664. 16S rRNA gene sequences were analyzed using the BLASTN search tools (http://www.ncbi.nlm.nih.gov/blast) and EzTaxon server.[18] Alignments with different 16S rRNA gene sequences from GenBank were performed using Clustal X 1.8.3 with default settings. Phylogenesis was analyzed with MEGA version 4.0 software, and distances were calculated using the Kimura 2 parameter distance model. A phylogenetic tree was built using the neighbor-joining method. Each data set was bootstrapped 1000 times.[19]

RESULTS AND DISCUSSION

Bioelectrochemical Reduction of NB. The bioelectrochemical reduction of NB and the formation of AN occurred considerably faster than in the control experiments (Figure 1A and 1B). Over 24 h, 0.467 ± 0.001 mM NB was removed, and 0.441 ± 0.003 mM AN was produced in the BES (current and microorganisms) at an applied voltage of 0.5 V. This was 1.30 and 10.25 times higher than the abiotic cathode control experiment (current, no microorganisms) and 1.48 and 2.90 times higher than the open circuit control experiment (no current, microorganisms) (Table 1), respectively. AN was the only product detected during bioelectrochemical reduction of NB, while NOB was also detected in the abiotic cathode mode (Figure 1C). This corroborates earlier reports of NB reduction in a BES with an abiotic cathode.[11] Under abiotic cathode conditions (Figure 1B and 1C), the production of NOB was considerably faster than the AN production. NOB only began to decrease with a concomitant AN increase when NB was almost exhausted (96 h). This indicates that NOB was the intermediate of NB transformation to AN by an abiotic cathode.

Regardless of whether the BES was operated with a biotic or abiotic cathode, over 99%, efficiency of NB reduction ($E_{rNB\text{-max}}$) was achieved. However, the maximum efficiencies of AN production ($E_{NB\text{-AN-max}}$) were notably different. As shown in Table 2, $E_{NB\text{-AN-max}}$ for the abiotic cathode was 73.75 ± 3.2%, whereas $E_{NB\text{-AN-max}}$ increased to 98.70 ± 0.87% under

Table 2. Efficiencies of NB Reduction by BES under Different Conditions

	applied voltage/V	carbon source	$E_{rNB\text{-}max}$/%	$E_{NB\text{-}AN\text{-}max}$/%	CE/%
biocathode	0.5	glucose	99.29 ± 0.01	98.70 ± 0.87	64.28 ± 7.70
	0.5	$NaHCO_3$	99.13 ± 0.06	98.93 ± 0.77	41.25 ± 2.17
	0.15	$NaHCO_3$	96.16 ± 0.22	88.26 ± 0.42	97.86 ± 0.42
abiotic cathode	0.5	-	99.62 ± 0.08	73.75 ± 3.20	25.64 ± 1.59
	0.15	-	94.33 ± 0.18	75.64 ± 1.12	74.15 ± 2.20
open circuit	-	glucose	99.93 ± 0.02	91.99 ± 1.67	-

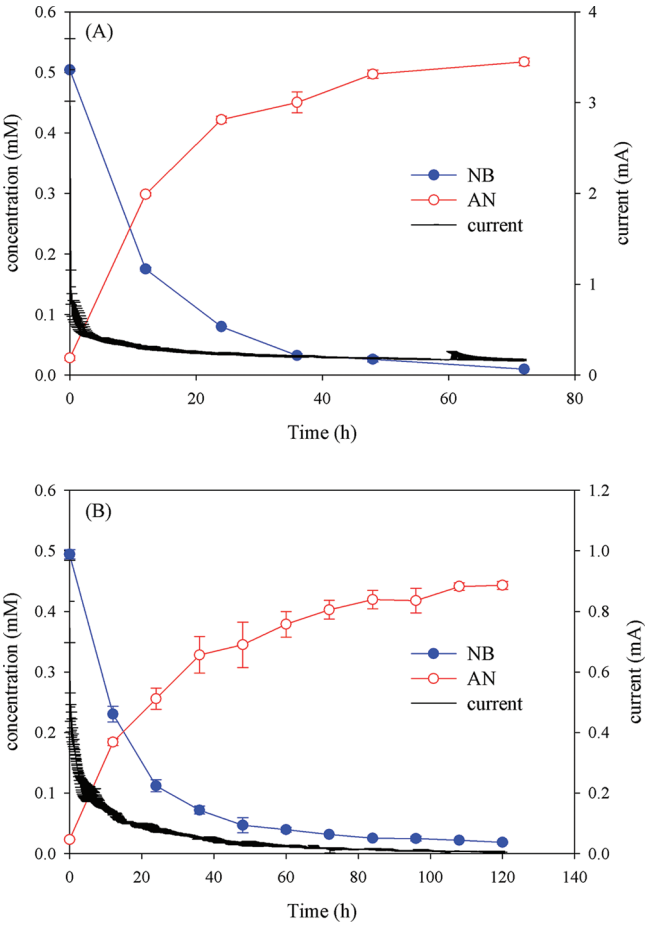


Figure 2. Current generation during NB reduction to AN when the cathode served as the sole electron donor. A: applied voltage 0.5 V; B: applied voltage 0.15 V.

bioelectrochemical mode. It has been documented that NB is reduced electrochemically to aniline in three steps through nitrosobenzene and phenylhydroxylamine.[3,12] These intermediates are converted not only to aniline but also to side products, such as azoxybenzene, azobenzene, p-aminophenol, and benzidine. Since NOB was detected during NB transformation to AN by the abiotic cathode, this production of reasonably recalcitrant side products is likely the reason of the low efficiency of AN formation. Compared to the study where NB was reduced on a carbon cloth cathode coated with Pt catalyst, bioelectrochemical NB removal rate was lower in this study (86% NB was removed compared to 96% in the first 12 h).[20] Besides noble mental catalyst used at the cathode, much lower catholyte pH (pH = 3) could be another reason for the higher NB removal rate in that study. However, strongly acidic catholyte might result in the production of polyaniline during NB reduction as suggested by authors, which might be more difficult to be mineralized completely in a natural environment or an aerobic biological process.

Evidence for Microbial Role in Cathode Catalysis. In a second phase, glucose was replaced with $NaHCO_3$ to ensure that electrons for the reduction of NB can solely come from the cathode or from lysic/cryptic growth. As shown in Table 1, 0.424 ± 0.002 mM NB was removed leading to the production of 0.394 ± 0.002 mM AN, indicating that the role of glucose was not of major importance to the transformation of NB to AN in the system evaluated here. The NB removal and AN formation were 16% and 816% higher than the abiotic cathode control. AN was also the only product detected during the experiments, and the $E_{NB\text{-}AN\text{-}max}$ was 98.93 ± 0.77%. NOB accumulation was found after autoclaving the cathode, and the $E_{NB\text{-}AN\text{-}max}$ decreased to 71.6%. These results indicate higher efficiency and selectivity of bioelectrochemical reduction of NB.

As shown in Figure 2A, after the circuit was closed, the current decreased with decreasing NB concentrations, leveling out at 0.16 ± 0.03 mA. A Coulombic efficiency of 41.25 ± 2.17% was obtained finally, implying parallel electrochemical reaction also occurred on the cathode. The most possible side-reaction was hydrogen evolution, as the cathode potentials of biocatalyzed and abiotic cathode were about −0.74 V and −0.79 V, respectively (Supporting Information Figure S3),[21] both of which were more electronegative than the theoretical potential for hydrogen evolution (−0.41 V vs SHE). The putative hydrogen production was consistent with the results obtained from the cyclic voltammetry (see below). It is well documented that hydrogen can serve as electron donor for NB reduction.[6,20] To understand whether the electron transfer between cathode and the attached microbe was direct or mediated by hydrogen, additional control experiments were conducted. After the removal of NB from the catholye, the current remained around 0.15 mA (Supporting Information Figure S4). Assuming the current was converted to hydrogen completely, 0.067 mmol hydrogen would be produced in 24 h. If microorganisms could capture all of this hydrogen and transform NB to AN, 0.022 mmol AN would be produced. Even adding the AN of 0.004 mmol produced abiotically by the cathode (Table 1, 0.043 mM × 0.085 L = 0.004 mmol, where 0.085 is the volume of the cathode chamber), the theoretial maximum yield of AN produced by BES would only be 0.026 mmol. This cannot account for the observed 0.033 mmol (Table 1, 0.0395 mM × 0.085 L = 0.033 mmol, where 0.085 is the volume of the cathode chamber) obtained in the original experiment. A second control involved operating the BES at 0.15 V applied voltage instead of 0.5 V, leading to a cathode potential increase from −0.74 ± 0.02 to −0.39 ± 0.01 V during NB reduction (Figure S3 of the Supporting Information). As a result,

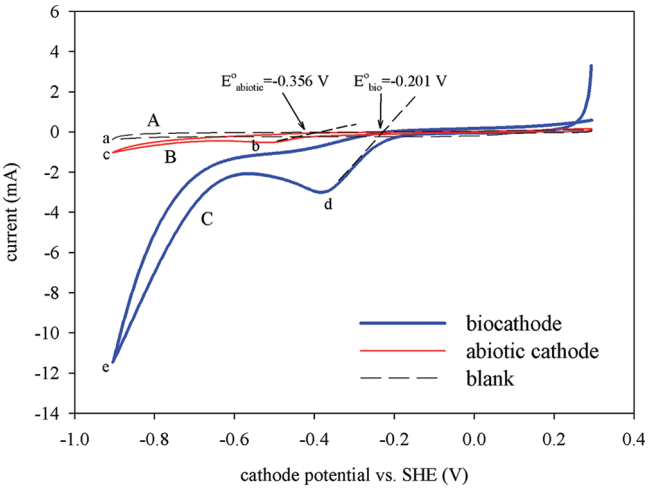


Figure 3. Cyclic voltammetry for NB reduction. Curve A: abiotic cathode without NB; B: abiotic cathode with 0.5 mM NB; C: biocathode with 0.5 mM NB. a, c, e: peak of hydrogen evolution; b, d: peak of NB reduction.

the likelihood for hydrogen formation was considerably decreased as earlier studies observed only negligible hydrogen formation at cathode potentials above −0.6 mV vs SHE.[21] During this experiment, the pH of catholyte was increased slightly from 7.05 to 7.43. As shown in Figure 2B, current decreased with the reduction of NB and finally reached almost zero when the concentration of NB and AN were not further changed. Almost all of the electrons were recovered by the transformation of NB to AN (Coulombic efficiency 97.86 ± 0.42%, Table 2). The result indicated that the hydrogen evolution was indeed unlikely to account for the electron transfer toward the NB reducing organisms. The bioelectrochemical reduction of NB still showed higher efficiencies and selectivity than the abiotic reduction, indicating that microbial NB reduction was not interrupted in the absence of hydrogen production and self-mediation of the organisms (direct or indirect via shuttles) was likely.

Further evidence to support the formation of a microbially catalyzed cathode was gained from cyclic voltammetry. The results of CV were compared with those for controlled experiments. In the absence of microorganisms and NB, only one peak (Curve A, peak a) related to hydrogen evolution was observed (Figure 3). Upon NB addition (Curve B in Figure 3), a new peak (peak b) appeared in addition to the hydrogen evolution peak (peak c). A similar CV curve (Curve C) was observed when using a biocathode; however, the peak current (peak d) related to NB reduction was about 6 times higher than that obtained in the abiotic cathode (peak b). The observed highest reduction potential for NB was −0.356 V in the abiotic cathode, which was lower than the −0.15∼−0.25 V reported by Mu et al. in a BES using graphite granules with higher surface area.[11] After the formation of biocathode, highest reduction potential was positively shifted from −0.356 V to −0.201 V. The increase of peak current and the positive shift of reduction potential are typical electrochemical indications for improved catalysis[22,23] and were consistent with the electroenzymatic system for nitro-compounds reduction.[24] The reduction of nitroaromatics is usually catalyzed by a nitroreductase in bacteria.[6] Flavin mononucleotide (FMN) was found as the functional group in many bacterial nitroreductases.[25] The measured midpoint potential of the FMN cofactor of *Enterobacter cloacae* nitroreductase was −0.19 V vs SHE,[25] which is higher than most published data obtained in cathodic reductions.[3] This is a further indication that this enzyme could be involved in bioelectrochemcial reduction of NB. As the potential for NB reduction in our biocathode was −0.201 V, electron transfer from the putative redox compound (membrane-bound or free) to nitroreductase is thermodynamically possible. However, the understanding of the electron transfer mechanisms toward cells as well as the mechanisms for downstream nitrobenzene reduction are as yet unclear.[6] Clearly, further studies are warranted to better understand the mechanism of nitrobenzene reduction in biocathodes.

Community Analysis of the Biofilm on the Cathode. 101 clones were randomly selected for sequencing, 95 of them were successfully sequenced. Phylogenetic analysis indicated the clone library sequences belonged to 4 phyla including *Firmicutes*, *δ-proteobacteria*, *γ-Proteobacteria*, and *Bacteroidetes* (Figure 4). Relative abundances (Table S1) indicated that the dominant populations in the biofilm of cathode related to *Firmicutes* (72 of 95 clones), and the most abundant sequence type (71 of 95 clones) was closely related (96.8−97.5% Similarity) to *Enterococcus aquimarinus* LMG 16607.

As mentioned above, nitroreductase is considered a key enzyme in the catalytic reduction of NB. Its presence has been confirmed in several *Enterococcus* species and other detected populations in the clone library of this study.[6,26,27] Normally, nitroaromatics are reduced to their corresponding amines by nitroreductase via nitroso and hydroxylamino derivatives.[28] The nitroso intermediate is usually hard to detect, for its reduction rate is much faster than the formation rate.[29] This could explain why NOB was not observed and AN formation was much quicker using a biocathode to reduce NB. Since nitroreductase is the core of NB reduction in the cell, it is very interesting to know how electrons are transferred from cathode to bacteria. *Enterococcus* have been found as dominant members in anode communities and have demonstrated some electrochemical activity.[30−32] To our best knowledge, electron transfer to *Enterococcus* from cathodes has not yet been reported. Earlier studies at the anode suggested that *Enterococcus* might excrete redox mediators[30,32] or cooperate with other bacteria[33] to transfer electrons to the anode. Whether *Enterococcus*-like bacteria get electrons from a cathode via similar mechanisms should be further investigated. *Enterococcus* may have had a strong role in the initial glucose-fed stage; however, the fact that AN formation efficiency was retained after omission of the glucose and that the community analysis was performed one month after glucose was omitted from the medium suggests that *Enterococcus*-like bacteria on the cathode are actively catalyzing NB reduction. *Enterococcus faecalis* (known as *Streptococcus faecalis* previously[34]) has been described as having the ability to fix CO_2,[35] indicating CO_2 fixation might support the growth of *Enterococcus*-like bacteria on cathode after the replacement of glucose with bicarbonate. However, the maintenance of the microbial catalyzed activity might be independent of growth once the electrochemical biofilm is formed, similar to a recent study on *Sporomusa ovata*.[36]

OUTLOOK

Here, we demonstrated for the first time a clear effect of cathode biocatalysis on the transformation of NB. Particularly important is the fact that NB was selectively transformed to

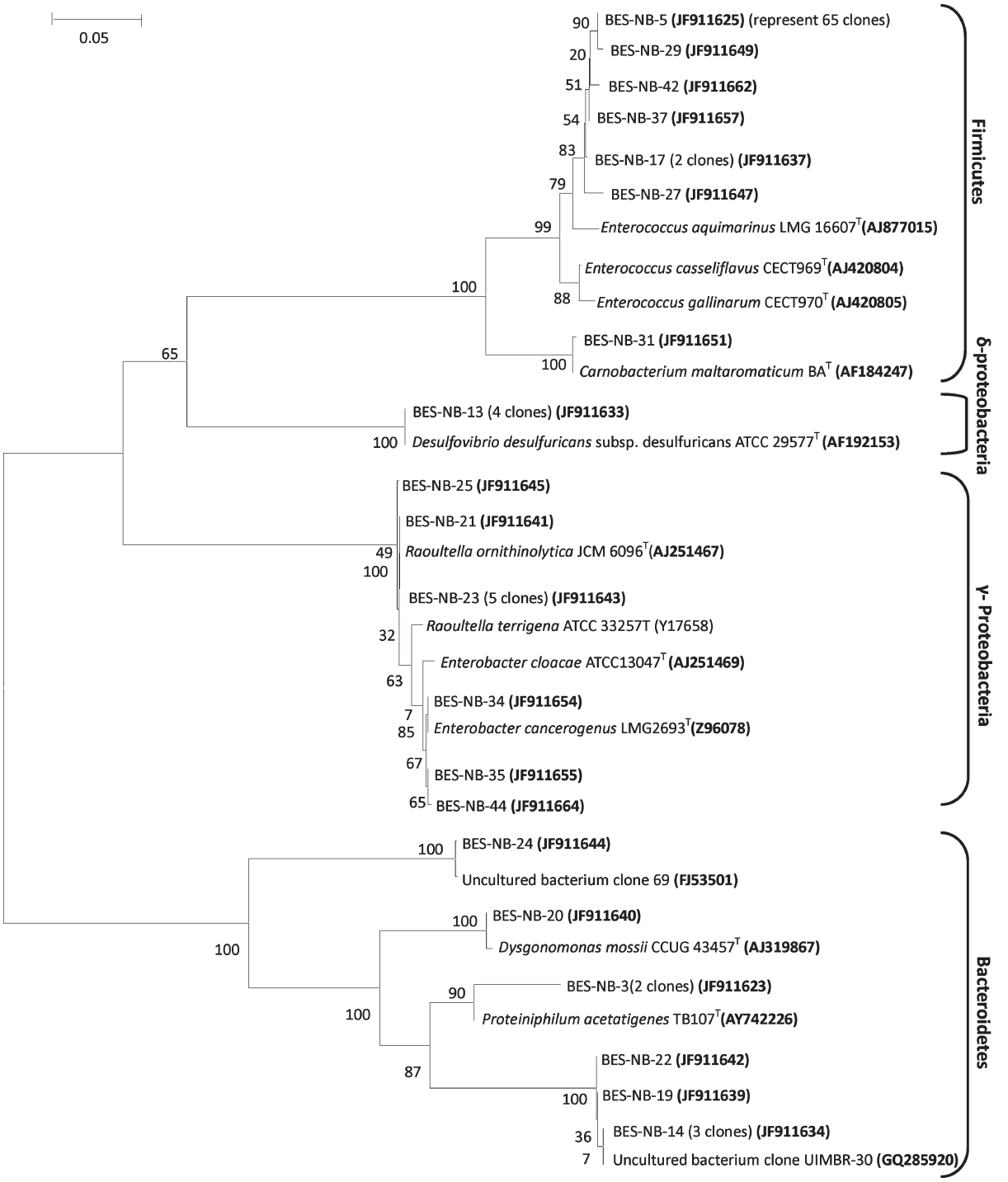


Figure 4. Phylogenetic tree of 16S rRNA gene sequences recovered from the biocathode of the BES. BES-NB-5 represents the 65 clones (with highly similarity from 98.7 to 100% in each other, accession numbers are JF911621, JF911622, JF911624 to JF911632, JF911635, JF911636, JF911638, JF911646, JF911648, JF911650, JF911652, JF911653, JF911656, JF911658 to JF911661 and JF911663, as shown in Table S1 of the Supporting Information), since the phylogenetic tree obtained from all the sequences is redundant. Bootstrap values (%) bare indicated at the nodes. The scale bar represents 0.05 substitutions per site, and GenBank accession numbers are in parentheses.

AN in the biological systems, as opposed to the straight electrochemical approach where nitroso compounds were detected at considerable concentration. In an earlier study, Mu et al. showed that by introducing a microbially catalyzed anode to support NB reduction at an abiotic cathode the energy consumption could be decreased about 1 order of magnitude compared to the conventional electrochemical system.[11] Here, with the microbially catalyzed biocathode, the overpotential of NB reduction was further decreased with about 0.155 V, indicating further energy savings. In a MFC study NB was reduced with electricity generation on a Pt catalyzed cathode at pH 3.[20] The NB reduction rate in our study was lower and energy input was needed;

however, we avoided the use of a platinum catalyst and used self-regenerating microorganisms instead.[37] Moreover, the MFC study applied a pH of 3 in the cathode, which artificially boosts up possible power production and will in practice almost certainly entail high chemicals cost. Finally, it is important to highlight that we were able to develop a biocathode in the presence of an organic carbon source (glucose). This implies that autotrophic conditions, typically used to develop a biocathode,[23,38,39] may not necessarily be essential. In real environments, organic carbon is generally present, and our observations thus improve the applicability of this process.

■ ASSOCIATED CONTENT

Supporting Information. Table S1 and Figures S1−S4. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: waj0578@hit.edu.cn.

■ ACKNOWLEDGMENT

We gratefully acknowledge the financial support by National Natural Science Foundation of China (Grant No. 50876024 and No. 51078100), by National Creative Research Groups Project (Grant No. 50821002), by Heilongjiang Science Foundation for Distinguished Young Scholars (Grant No. JC201003), and by State Key Laboratory of Urban Water Resource and Environment (Grant No. 2010DX11 and No. 2011TS09).

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