



# A membrane-free, continuously feeding, single chamber up-flow biocatalyzed electrolysis reactor for nitrobenzene reduction

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## ABSTRACT

A new bioelectrochemical system (BES), a membrane-free, continuous feeding up-flow biocatalyzed electrolysis reactor (UBER) was developed to reduce oxidative toxic chemicals to less- or non-toxic reduced form in cathode zone with oxidation of electron donor in anode zone. Influent was fed from the bottom of UBER and passed through cathode zone and then anode zone. External power source (0.5 V) was provided between anode and cathode to enhance electrochemical reactions. Granular graphite and carbon brush were used as cathode and anode, respectively. This system was tested for the reduction of nitrobenzene (NB) using acetate as electron donor and carbon source. The influent contained NB (50–200 mg L<sup>-1</sup>) and acetate (1000 mg L<sup>-1</sup>). NB was removed by up to 98% mainly in cathode zone. The anode potential maintained under -480 mV. The maximum NB removal rate was up to 3.5 mol m<sup>-3</sup> TV d<sup>-1</sup> (TV = total empty volume) and the maximum aniline (AN) formation rate was 3.06 mol m<sup>-3</sup> TV d<sup>-1</sup>. Additional energy required was less than 0.075 kWh mol<sup>-1</sup> NB. The molar ratio of NB removed vs acetate consumed varied from 4.3 ± 0.4 to 2.3 ± 0.1 mol mol<sup>-1</sup>. Higher influent phosphate or acetate concentration helped NB removal rate. NB could be efficiently reduced to AN as the power supplied of 0.3 V.

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## 1. Introduction

In recent years, bioelectrocatalysis using biological materials or microorganisms as catalysts for electrochemical processes [1] or bioelectrochemical system (BES) [2,3] become attractive for wastewater treatment and removal of various contaminants via electrical biochemical reactions. In a BES, oxidization of organic compounds such as glucose, acetate, etc. [2,3] and inorganic compounds such as sulfide [4] occurs at anode. At the cathode, oxygen [5] or other electron acceptors such as nitrate [6], nitrobenzene (NB) [7], perchlorate [8], and reduced metal ions can be reduced. To date, BES has been tested for reductive dehalogenation [9–12], decolorization of azo dyes [7,13] and copper (II) reduction to metallic copper [14,15] as well as for the generation of H<sub>2</sub> [16], CH<sub>4</sub> [17], H<sub>2</sub>O<sub>2</sub> [18], ethanol [19], and desalination [20].

NB is one of toxic contaminants in industrial wastewaters such as dying and printing wastewater [21]. NB is difficult to be oxidized due to the strong electron affinity of the nitro group [22] but it can be reduced to aniline (AN) via reductive pathway [23] and AN is then degraded in natural ecosystem or wastewater treatment system under aerobic or denitrifying condition [24]. Recently,

attempts to use BES for NB reduction were reported [7,25]. To ensure the reduction thermodynamically favorable, external power source was used to enhance the reduction of NB to AN in cathode chamber [7].

Except for sediment biofuel cells [3,26], most BES reactors are designed with ion exchange membrane (IEM) to separate anode and cathode chambers [2,27,28], including those tested for NB reduction [7,25,45]. In general, IEM is one of costly components of BESs for either operational maintenance or replacement during long-term operation for wastewater treatment [29]. In addition, the installation of membrane could cause pH gradient [29] and increase internal resistance. Development of membrane-free BES could be a cost-effective approach for potential application and further enhancement of power density [30] or decrease of potential loss by reducing the resistance [31]. Attempts to develop membrane-free, single chamber reactor have been reported on MFCs for power generation and MEC for hydrogen production [27,30–32]. All reported configurations were set up by installing anode and cathode vertically in one chamber. The electrogenic microorganisms on anode could be inhibited if influent contained inhibitory or toxic compounds [33,34]. Penetration of cathode content to anode chamber is a design and operational issue for cathodic reduction-type BES treating toxic metals and other compounds for reductive detoxification [14]. The inhibition of NB to bioanode was confirmed by introducing NB to anode chamber in a MFC, which caused decline

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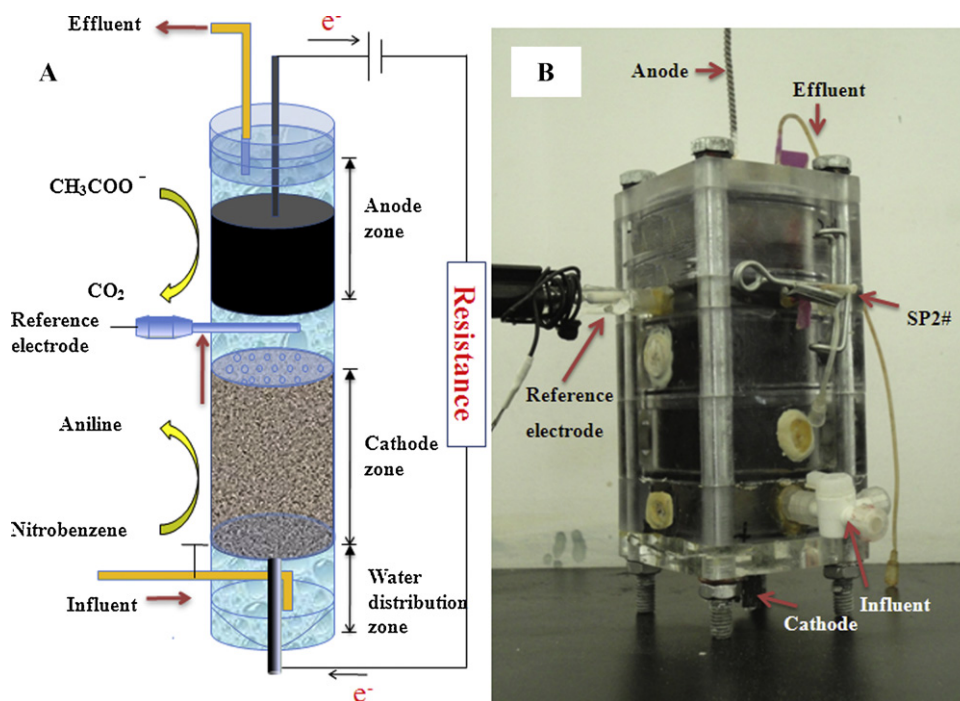


Fig. 1. Conceptual design of up-flow biocatalyzed electrolysis reactor (UBER). Left: schematic diagram of the system. Right: laboratory scale reactor for NB reduction.

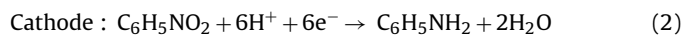
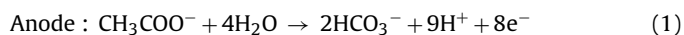
of power density as NB concentration increased [25]. After NB is reduced to AN, the toxicity is significantly reduced. Aromatic amines are 500 times averagely less inhibitory than their corresponding nitroaromatics [35]. Our previous data indicated that the influence on polarization curve by adding AN was much less than adding NB (unpublished data). Considering the difference of toxicity between NB and its reduced daughter products, the reactor design should set cathode zone prior to anode zone in order to reduce influent NB to less inhibitory AN and reduce or prevent negative impact on anode microorganisms.

In this study, we have developed a membrane-free, continuously feeding, single chamber up-flow biocatalyzed electrolysis reactor (UBER) by setting cathode below anode. This system has been tested for the reduction of NB to AN with acetate as an electron donor source. The results demonstrated the feasibility of NB reduction in the novel system at volumetric loading rate (LR) at  $3.5 \text{ mol m}^{-3} \text{ d}^{-1}$  with >98% NB removal efficiency.

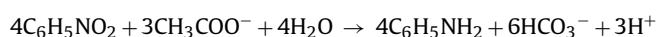
## 2. Materials and methods

### 2.1. Concept of UBER

The conceptual diagram of the UBER is presented in Fig. 1A. This reactor is composed of a cathode zone followed by an anode zone. Influent containing target compound (NB in this study) and electron donor source (acetate) is continuously fed from the reactor bottom. The target compound is reduced as it passes through the cathode zone and the electron donor was mainly oxidized as it passes anode zone. Acetate oxidation occurs in anode zone and NB is reduced to AN in cathode zone:



The overall reaction is



(3)

Based on the stoichiometry, the reduction of one mole of NB to AN requires 0.75 mol of acetate (or  $0.365 \text{ g acetic acid g}^{-1} \text{ NB}$ ). The actual ratio in BES could be more than 0.75 because of microbial cell synthesis using acetate as carbon source and oxidation of acetate near anode due to non-electrogenic reactions such as methanogenesis.

A laboratory scale UBER in this study was constructed with a pile of parallel plexiglas plates with a thickness of 3.0 cm (Fig. 1B). The outside shape of the reactor appeared to be rectangular (H 13 cm  $\times$  6 cm  $\times$  6 cm) and the internal reaction zone was in cylinder shaped (ID 5.0 cm  $\times$  H 13.0 cm) with total empty volume (TV) of 250 mL and an effective liquid volume of 180 mL after installation of cathode and anode. Carbon brush (ID 4.5 cm  $\times$  L 4.0 cm, TOHOTENAX Co. Ltd., USA) was used as anode, which was fixed on the upper portion of the reactor (1.0 cm below the outlet). Graphite granules with diameter from 3.0 to 5.0 mm (Harbin Northern Electrical Carbon Co. Ltd., China) were filled in the down portion as cathode with an inserted graphite rod (Harbin Northern Electrical Carbon Co. Ltd., China) for electricity conductance. Two plates with even distribution holes were installed at 2.0 cm above the bottom of the reactor and the top of cathode zone (8.0 cm above the bottom) to ensure even distribution of up-flow fluid. The distance between the anode zone and cathode zone was 2.0 cm. Three sample ports (SP) were stilled at 1.0, 6.0 and 12.0 cm above the bottom (named as SP1#, SP2# and SP3#) to monitor aqueous chemical composition inside the reactor.

The pH of the influent solution was maintained at 7.0 with conductivity of  $2.77\text{--}7.53 \text{ mS cm}^{-1}$  depends on influent phosphate concentration (Supplementary data, Table S1). An Ag/AgCl reference electrode (+0.197V vs Standard hydrogen electrode) (Shanghai Precision Scientific Instruments Co., Ltd., China) was placed between anode and cathode for the measurement of half potentials. A DC power device was connected to the UBER to supply constant external power between the anode and cathode. To record the current by a data acquisition recorder (Keithley 2700,

Keithley Co., Ltd., USA), an external resistor of  $20\ \Omega$  was connected in series with UBER. The current was calculated according to Ohm's law ( $I = V/R$ , where  $V$  is the voltage between the external resistor  $\Omega$ ). To avoid excess power loss on the external resistor, the low resistance of  $20\ \Omega$  was chosen. Also recorded by the data acquisition was the half-cell voltage vs Ag/AgCl reference electrode. All tests were performed at  $25\ ^\circ\text{C}$ .

## 2.2. Inoculation and operational conditions

The bioanode was carbon brush which was pre-incubated in a MFC with acetate as carbon resource in our laboratory [36]. The original inoculum source was active sludge collected from a local municipal wastewater treatment plant in Harbin, China. After biofilm growth on it, the bioanode and cathode (without inoculation) were installed in the UBER. Sodium acetate and NB were used as electron donor and acceptor, respectively. The influent (pH 7.0, conductivity  $7.53\ \text{mS cm}^{-1}$ ) contained potassium phosphate,  $50\ \text{mM}$ ; sodium acetate,  $1.0\ \text{g L}^{-1}$ ; NB,  $15\text{--}200\ \text{mg L}^{-1}$ ; KCl,  $0.13\ \text{g L}^{-1}$ ;  $\text{NH}_4\text{Cl}$ ,  $0.31\ \text{g L}^{-1}$ ; trace element solution,  $1.0\ \text{mL L}^{-1}$ ; and vitamin solution,  $1.0\ \text{mL L}^{-1}$ . It was continuously fed into the UBER with a peristaltic pump (BT100-1L, Longer Pump Co., Ltd., China) at a rate of  $600\ \text{mL d}^{-1}$ , resulting in a hydraulic retention time (HRT) of 7.2 h.

The experimental period can be divided into five phases. In Phase 1, the UBER was started with an external resistor of  $20\ \Omega$  and  $0.5\ \text{V}$  external power source at a HRT of 7.2 h. The UBER was fed continuously and the volumetric NB LR was maintained at  $0.49\ \text{mol NB m}^{-3}\ \text{TV d}^{-1}$  for 40 days. In this study, the volumetric LR was calculated based on the NB mass fed per day and divided by TV. Although most NB was removed in the cathode zone, a small fraction of NB was reduced by non-bioelectrochemical reactions in the membrane-less UBER. Thus it could reflect a whole result of UBER when TV was used as the dividend. Phase 2 was to test the reactor performance with step-up increased NB LR from 0.49 to  $3.9\ \text{mol NB m}^{-3}\ \text{TV d}^{-1}$ . Nine different rates were employed with respective duration of 5 days for each rate. Phase 3 was to examine the impact of different influent phosphate concentrations on effluent pH and NB reduction performance. Phase 4 was to determine the ratio between NB and acetate by using different influent acetate concentrations. Finally, in Phase 5, the influence of applied voltages was examined.

## 2.3. Analytical methods

### 2.3.1. Chemical analysis

Liquid samples taken from the reactor were immediately filtered through a  $0.22\ \mu\text{m}$  filter. NB and AN were measured by using high performance liquid chromatography (HPLC, model e2695, Waters Co., USA) equipped with C18 column ( $5\ \mu\text{m}$ ;  $5\ \text{mm} \times 150\ \text{mm}$ , Waters Co., USA). The mobile phase was methanol/ $\text{H}_2\text{O}$  (6:4) at  $1\ \text{mL min}^{-1}$ . The detection was performed at 254 nm. Under our analytical condition, the retention time of AN, NB and nitrosobenzene was 2.48, 4.50 and 5.35 min, respectively. For acetate analysis, formic acid ( $0.1\ \text{mL}$ , 100% purity) was added to sample ( $0.9\ \text{mL}$ ). Analysis was performed with a gas chromatography equipped with a polar capillary column (HP-INNOWAX 19095N  $30\ \text{m} \times 0.53\ \text{mm} \times 1\ \mu\text{m}$ , Agilent Co., Ltd., USA) at oven temperature of  $250\ ^\circ\text{C}$  with a flame ionization detector at  $300\ ^\circ\text{C}$ .

NB removal efficiency (NE) as %, volumetric NB removal rate (NR) as  $\text{mol m}^{-3}\ \text{TV d}^{-1}$ , AN formation efficiency (AE) as %, and volumetric AN formation rate (AR) as  $\text{mol m}^{-3}\ \text{TV d}^{-1}$  were calculated based on the difference between influent and effluent NB and AN concentrations and divided by TV.

### 2.3.2. Electrochemical monitoring and calculation

The current was calculated from the voltage on the external resistance using Ohm's law. The Coulombic efficiency for NB removal ( $\text{CE}_{\text{NB}}$ ) was calculated as follows:

$$\text{CE}_{\text{NB}} = \frac{n \times (C_{\text{in-NB}} - C_{\text{ef-NB}}) \times (Q_A \times 10^3 / 24 \times 3600) \times F}{I} \quad (4)$$

where  $C_{\text{in-NB}}$  is the influent NB concentration (mM);  $C_{\text{ef-NB}}$  is the effluent NB concentration (mM);  $n=6$ , the theoretical number of electrons for the reduction of NB to AN ( $\text{mol mol}^{-1}$ ) as shown in Eq. (2);  $Q_A$  is the influent flow rate of the reactor ( $\text{m}^3\ \text{d}^{-1}$ );  $F$  is Faraday's constant ( $96,485\ \text{C mol}^{-1}\ \text{e}^-$ );  $I$  is the current (mA).

The Coulombic efficiency for AN formation ( $\text{CE}_{\text{AN}}$ ) was estimated as:

$$\text{CE}_{\text{AN}} = \frac{n \times C_{\text{ef-AN}} \times (Q_A \times 10^3 / 24 \times 3600) \times F}{I} \quad (5)$$

where  $C_{\text{ef-AN}}$  is the effluent AN concentration (mM) and no AN was in the influent.

The Coulombic efficiency based on acetate oxidation was estimated as:

$$\text{CE}_{\text{Ac}} = \frac{I}{8 \times (C_{\text{in-Ac}} - C_{\text{ef-Ac}}) \times (Q_A \times 10^3 / 24 \times 3600) \times F} \quad (6)$$

where  $C_{\text{in-Ac}}$  is the influent acetate concentration (mM),  $C_{\text{ef-Ac}}$  is the effluent acetate concentration (mM). 8 is the molar number of electrons produced by complete oxidation of 1 mol of acetate as shown in Eq. (1).

The energy required ( $P_{\text{UBER}}$ ,  $\text{kWh mol}^{-1}\ \text{NB}$ ) for UBER was evaluated as follows:

$$P = \frac{UI \times 24}{10^6 \times Q_A \times (C_{\text{in-NB}} - C_{\text{ef-NB}})} \quad (7)$$

where  $U$  is the power supplied (V).

## 3. Results and discussion

### 3.1. Startup of UBER

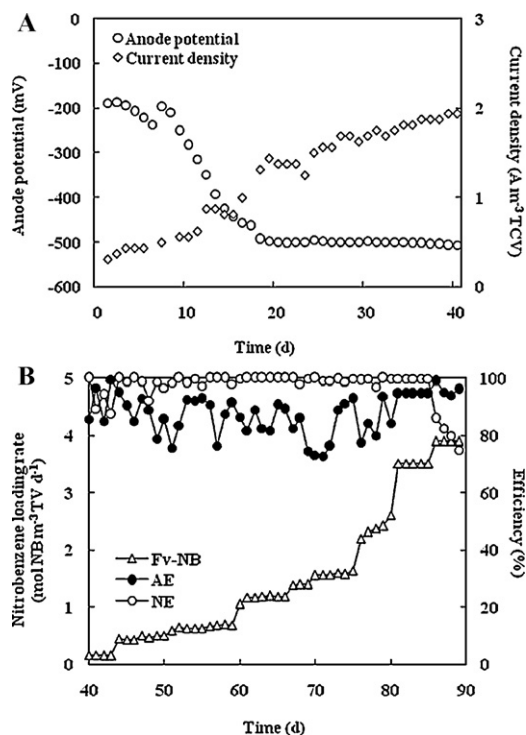
During the startup period (Phase 1), the influent NB concentration was maintained at  $15\ \text{mg L}^{-1}$  with a LR of  $0.49\ \text{mol NB m}^{-3}\ \text{TV d}^{-1}$  for 40 days. With the external resistor of  $20\ \Omega$  and power supplied of  $0.5\ \text{V}$ , the anode potential at open circuit was decreased from  $-200$  to  $-500\ \text{mV}$  after 18 days and the current density was increased gradually from  $0.3$  to  $2\ \text{A m}^{-2}\ \text{TCV}$  (total cathode zone volume, TCV) (Fig. 2A). This indicates that the microbial community for acetate oxidation on anode was developed and generated electrons for NB reduction on cathode. The oxidation reactions on anode reached stable state based on anode potential (Fig. 2A). The startup of UBER was successful.

### 3.2. Effect of NB LR on NB removal

#### 3.2.1. Efficiency of NB reduction and AN formation in the UBER

After 40 days, NB LR was step-up increased. The adsorption of NB onto the carbon electrodes was likely limited based on the observation of NB removal efficiency (NE) and AN formation efficiency (AE) as shown in Fig. 2(B). The adsorption of both compounds could have reached an equilibrium state during most test period. Therefore, the NE and AE become relatively close as NB LR was increased step-up in the UBER. As NB LR increased from  $0.49$  to  $3.51\ \text{mol NB m}^{-3}\ \text{TV d}^{-1}$ , NE increased from  $95 \pm 5\%$  to  $98 \pm 3\%$ , indicating that NB was efficiently reduced. Further increase in NB LR to  $3.9\ \text{mol NB m}^{-3}\ \text{TV d}^{-1}$  after day 80 caused decline of NE from  $98\%$  to  $75\%$  on day 90, implying that the NB LR had been beyond the reduction capacity of the

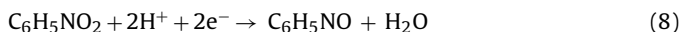




**Fig. 2.** Reactor operation in Phase 1 and 2. (A) Change in anode potential and current density during Phase 1 startup period. (B) NB removal efficiency (NE) and AN formation efficiency (AE) at different NB loading rates in Phase 2.

reactor. At that time, the influent NB concentration was 200 mg L<sup>-1</sup>, which was much higher than NB levels reported in most NB containing wastewaters [37].

In the effluent, AN was a dominant product as more than 98% NB was removed. AN formation efficiency varied from 92.0 ± 0.3% to 81.0 ± 0.2%. At higher NB LR (>3.51 mol NB m<sup>-3</sup> TV d<sup>-1</sup>), low concentration of nitrosobenzene was observed, indicating production of less reduced intermediate. The formation efficiency of nitrosobenzene was 2.2–3.6%. This reaction likely occurred in the cathode zone as



The volumetric NB removal rate and AN formation rate increased linearly with the increase of NB LR when NB LR was below 3.51 mol NB m<sup>-3</sup> TV d<sup>-1</sup> (Fig. 3A). The maximum NB removal rate and AN formation rate were 3.50 and 3.06 mol m<sup>-3</sup> TV d<sup>-1</sup>, respectively. Based on the linear regression of the data of these two rates vs NB LR (Fig. 3A), the slope for NB removal rate is slightly higher than AN formation rate, i.e. 0.992 vs 0.892 (Supplementary data). The difference was likely due to the formation of another reduced intermediate, such as nitrosobenzene, especially at high NB loading rates.

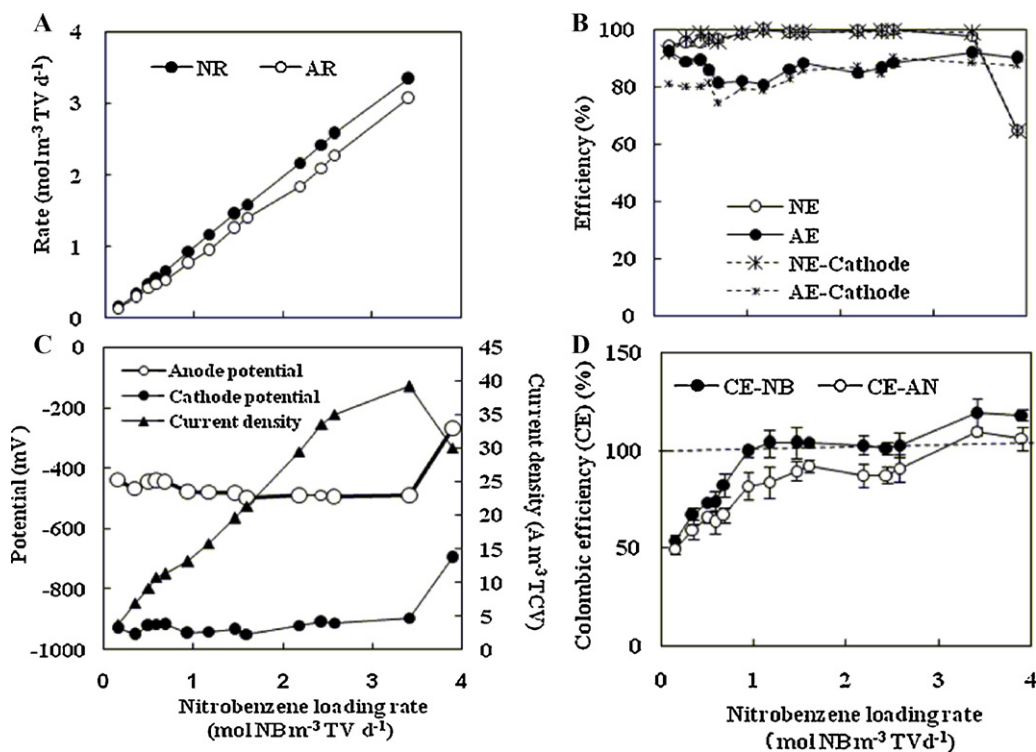
The samples taken from SP2# (sample pot above cathode zone and below anode zone in Fig. 1B) were analyzed to determine the efficiency of NB reduction in cathode zone. The efficiencies of NB removal and AN formation determined in SP2# were compared with the data from the effluent (Fig. 3B). The both efficiencies were nearly the same, indicating that NB was mainly converted to AN in the cathode zone. Although AN could be used as electron donor for electrochemical oxidation, but it was not oxidized further when cathode effluent flowed through anode zone. This was presumably due to the anaerobic condition in the reactor and the presence of acetate.

### 3.2.2. Cell potential and current density

The relationship between anode and cathode potentials and NB LR is illustrated in Fig. 3C. As NB LR was step-up increased from 0.49 to 3.51 mol NB m<sup>-3</sup> TV d<sup>-1</sup>, anode potential dropped slightly but still maintained at about -500 mV. Correspondingly, cathode potentials were stable at about -950 mV. Current density increased linearly from 3.8 to 39.2 A m<sup>-3</sup> TCV (As current was generated by electrogenic reactions on the electrodes. The electrons given on the anode should be equal to those accepted on the cathode. Current density could be expressed based on the volume of anode or cathode zone. In this study, NB was mainly reduced by electrochemical reactions on the cathode. So the current density was evaluated based on the TCV). After NB LR reached 3.9 mol NB m<sup>-3</sup> TV d<sup>-1</sup>, which was likely beyond the reduction capability of cathode zone, the NB concentration at SP2# increased from 0.37 to 50 mg L<sup>-1</sup> accompanied with the anode and cathode potentials increased rapidly to nearly -250 and -700 mV, respectively. At the same time, the current density dropped from 39.2 to 30 A m<sup>-3</sup> TCV. This observation indicated that the high NB LR caused the failure of NB reduction in the cathode zone. When cathode effluent with NB concentration >50 mg L<sup>-1</sup> entered anode zone, anodic bacteria were inhibited gradually and lost electrogenic activity, resulting in the increase of anode potential. It is because AN is less inhibitory to microorganisms, a high AN concentration in cathode zone effluent would not cause negative effect on anodic microorganisms [30] if the conversion of NB to AN was efficient in cathode zone. On the other hand, high NB concentration from cathode effluent could be inhibitory to anode microorganisms if the efficiency of reduction of NB to AN became poor as observed. To confirm this, the variation of anode potential and current at different nitrobenzene concentrations was tested (Fig. S1 in Supplementary data). When NB concentration reached higher than 50 mg L<sup>-1</sup>, anode gradually lost its function. Based on the above observation, application of a proper NB LR to ensure high extent of NB reduction in cathode zone is essential for the maintenance of active anode microbial community and stable operation of the UBER.

### 3.2.3. Coulombic efficiency (CE)

The Coulombic efficiency of NB removal at the cathode was evaluated as the ratio of the current calculated based on complete NB removal or formation of AN vs the current measured across circuit of the UBER. As shown in Fig. 3D, Coulombic efficiency of NB removal and AN formation rose with the step-up increase in NB LR from 0.15 to 1.17 mol NB m<sup>-3</sup> TV d<sup>-1</sup>. Coulombic efficiency of NB removal reached to 99% with 82% of Coulombic efficiency of AN formation at NB LR of 2.5 mol NB m<sup>-3</sup> TV d<sup>-1</sup>, indicating that majority of electrons from cathode electrode was used for NB reduction. At the beginning, the increase in Coulombic efficiency was accompanied with the increase in NB LR (<1.17 mol NB m<sup>-3</sup> TV d<sup>-1</sup>). This suggested that a part of electrons was consumed for other reactions in cathode zone. Hydrogen generation could be a competitive reaction for electrons because at cathode potentials ranging between -980 and -890 mV (Fig. 3C), generation of hydrogen gas was energetically favorable. Besides, when NB LR reached above 2.5 mol NB m<sup>-3</sup> TV d<sup>-1</sup>, the calculated Coulombic efficiencies for NB reduction appeared slightly higher than 100%. This deviation was likely due to that a part of NB was removed or reduced by non-electrochemical processes in cathode zone. Even though the cathode was not inoculated previously, there was no membrane to separate anode and cathode zones and the cathode zone could not be operated under sterile condition, microbial growth was observed in cathode zone although it was limited (Fig. S2 in Supplementary data). Therefore, oxidation of acetate could also occur and released electrons for NB reduction in cathode zone. This hypothesis was further confirmed by an experiment with open circuit,



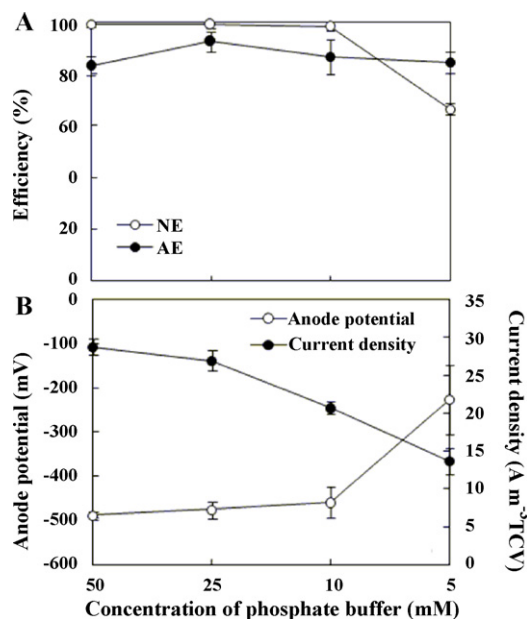
**Fig. 3.** Reactor performance at different NB loading rates in Phase 2. (A) NB removal rate and AN formation rate vs NB loading rate. (B) Comparison of the NB removal (NE) and AN formation (AE) in the effluent vs samples from SP2# (NE-Cathode and AE-Cathode). (C) Anode potential, cathode potential and current density. (D) Coulombic efficiency base on NB reduction (CE-NB) and AN formation (CE-AN).

i.e. a small fraction of NB (<15%) was reduced to AN. Considering this effect, the calculated Coulombic efficiency based on total NB reduction could be overestimated by at least 15%.

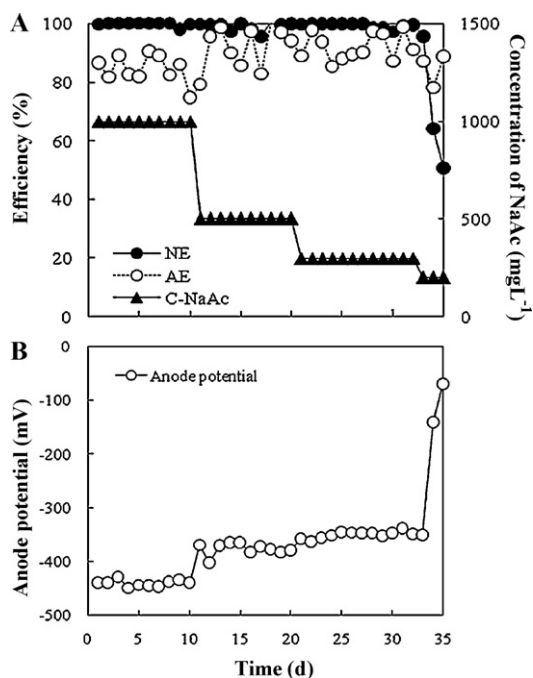
### 3.3. Effect of phosphate buffer concentration

The pH is an important parameter that can greatly influence the BES performance. When acetate is oxidized on anode (Eq. (1)), acidity is generated. In order to maintain a neutral pH and a low anodic over-potential and thereby a high microbial activity, the supplementation of enough buffer capacity in BES should be considered [38–40]. High buffer capacity can maintain proper pH in anode chamber [40]. In Phase 3, the effect of the influent phosphate concentration on UBER was examined. The influent NB LR was kept at 2.0 mol NB m<sup>-3</sup> TV d<sup>-1</sup> with influent NB of 100 mg L<sup>-1</sup> and HRT of 7.2 h. During the test, influent phosphate concentration was gradually decreased from 50 to 25, 10 and 5 mM at the end. Influent pH was kept at 7.0 but respective conductivity was 7.53, 5.01, 3.34 and 2.73 mS cm<sup>-1</sup>. The change in phosphate buffer concentration resulted in only slight decline in effluent pH from 7.0 to 6.7 (Table S1 in Supplementary data). When influent phosphate concentration was higher than 5 mM, NB removal efficiency was higher than 90% but rapidly dropped to below 70% with influent phosphate of 5 mM. But AN formation efficiency was less influenced and remained above 80% throughout the test (Fig. 4A). Correspondingly, the anode potential was kept about -500 mV and sharply increased to -250 mV as phosphate concentration decreased from 10 to 5 mM (Fig. 4B). The current density was correlated to influent phosphate concentration and declined from 28.7 ± 0.8 to 13.7 ± 1.3 A m<sup>-3</sup> TCV as the phosphate concentration decreased from 50 to 5.0 mM (Fig. 4B). In general, microbial activity in anode cannot change dramatically as pH drops from 7.0 to 6.7. The gradual decline in current density and then NE could be caused by the decline of ionic

strength of the electrolyte and electrolyte conductivity, resulting in decreased reaction rate. Our data showed that the UBER was favorable to overcome protons transfer limitation for NB reduction at cathode without affecting the function of anode in lower phosphate concentration, although an optimal hydraulic residence time should be adjusted because of the lower conductivity.



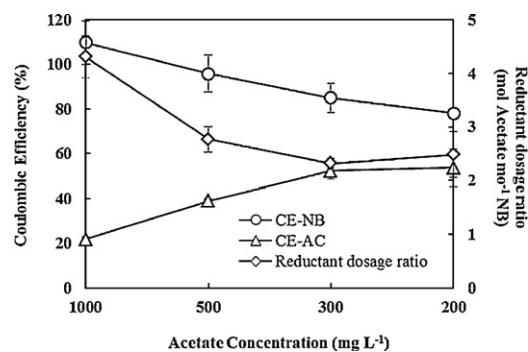
**Fig. 4.** Effect of different influent phosphate concentrations on reactor performance. (A) NE and AE at different influent phosphate concentrations. (B) Anode potential and current density at different phosphate concentrations.



**Fig. 5.** Effect of influent acetate concentration on NB reduction performance (A) NE and AE with different acetate concentrations. (B) Anode potential and current density at different acetate concentrations.

### 3.4. Influence of acetate concentration

In this study, acetate was used as the electron donor and carbon source for anodic bacteria growth. Phase 4 test was designed to investigate the influence of acetate concentration on NB reduction. The influent contained phosphate concentration of 50 mM and NB of 100 mg L<sup>-1</sup>. The influent acetate concentration was gradually decreased from 1000 to 500, 300 and 200 mg L<sup>-1</sup> with respective influent ratio of acetate vs NB of 15.0, 7.5, 4.5 and 3.0 mol mol<sup>-1</sup>. The experiment lasted 35 days. NB was efficiently removed (>95%) when acetate concentration was between 300 and 1000 mg L<sup>-1</sup> (Fig. 5A). The NE dropped rapidly to 55% when influent acetate concentration was 200 mg L<sup>-1</sup>. AN formation efficiency was higher than 80%, indicating that NB was mostly reduced to AN. Correspondingly, the anode potential gradually increased from -450 to -350 mV when acetate concentration was decreased from 1000 to 300 mg L<sup>-1</sup> (Fig. 5B). The anode potential quickly increased above -100 mV as the acetate concentration was further decreased to 200 mg L<sup>-1</sup>, indicating the anodic microbial electrogenic activity become poor. Coulombic efficiency on NB removal decreased from 105 ± 1% to 78 ± 1% when influent acetate

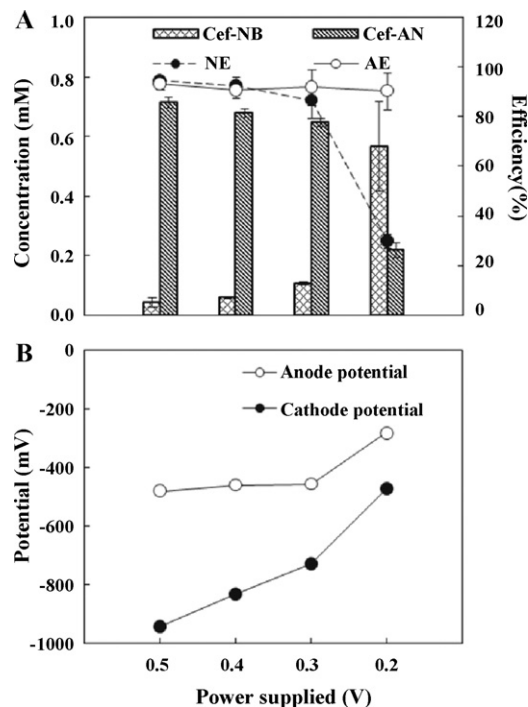


**Fig. 6.** Coulombic efficiency and reductant dosage ratio at various acetate concentrations.

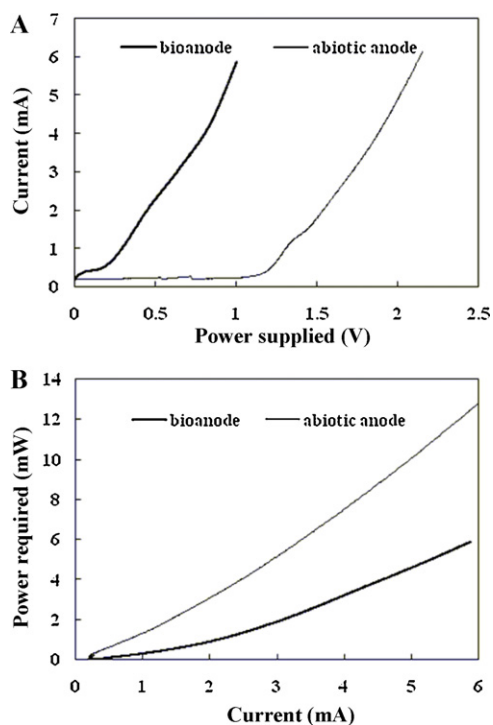
concentration decreased from 1000 to 200 mg L<sup>-1</sup> (Fig. 6). Correspondingly, Coulombic efficiency on acetate oxidation increased from 21.8 ± 0.8% to 53.6 ± 3%, which was lower than that on NB removal. Theoretically, 0.75 mol acetate is required per mol of NB converted to AN. The reductant dosage ratio in the UBER varied from 4.3 ± 0.4 to 2.3 ± 0.1 mol acetate mol<sup>-1</sup> NB and the lowest value was at influent acetate concentration of 300 mg L<sup>-1</sup>. The lower Coulombic efficiency on acetate oxidation and higher reductant dosage may be due to that the acetate was converted to methane by acetoclastic methanogenesis and used for cell synthesis [42]. These results indicated that influent acetate of 300 mg L<sup>-1</sup> was minimum level for the reduction of NB at 100 mg L<sup>-1</sup> with removal >95% in the UBER.

### 3.5. Effect of power supplied

NB was efficiently reduced to AN when the applied voltage was 0.5 V in the UBER. As the reductive potentials of many nitroaromatic compounds are often more negative than -700 mV [43], the cathode potential in the UBER varied from -800 to -900 mV as the external power 0.5 V was supplied, which could ensure complete NB reduction to AN. However, the less power was supplied, the less energy was required. Thus, Phase 5 work was to test the effect of the different power levels supplied. The volumetric NB LR was kept at 2.0 mol NB m<sup>-3</sup> TV d<sup>-1</sup> (influent NB of 100 mg L<sup>-1</sup>) with HRT of 7.2 h. The external power supplied was gradually decreased from 0.5 to 0.2 V. As shown in Fig. 7A, when the power was reduced from 0.5 to 0.3 V, effluent NB concentration increased from 5.5 ± 0.2 to 13.3 ± 4.3 mg L<sup>-1</sup>, companied with the NE decreased from 94.0 ± 0.2% to 86.6 ± 4.3%. Correspondingly, effluent AN concentration decreased from 66.5 ± 1.3 to 60.2 ± 1.0 mg L<sup>-1</sup>. This demonstrated that NB was removed effectively even when 0.3 V was supplied. When power supplied was further reduced to 0.2 V, effluent NB concentration quickly reached to 70 ± 4.7 mg L<sup>-1</sup> with a low NE of 30.0 ± 4.7%, indicating the reaction rate of NB reduction was greatly decreased. As shown in



**Fig. 7.** (A) Concentrations of effluent NB and AN and NB removal efficiency (NE) and AN formation efficiency (AE) at different power supplied. (B) Anode and cathode potential at different power supplied.



**Fig. 8.** Comparison of bioanode with abiotic anode electron output (A) Linear volt-ampere (VA) of bioanode and abiotic anode with external power supply. (B) Comparison of power required by bioanode vs abiotic anode.

Fig. 7B, both anode and cathode potentials increased as power supplied decreased. When power supplied varied from 0.5 to 0.3 V, anode and cathode potentials were still below  $-400$  and  $-700$  mV, respectively. These levels were suitable for anodic microbial growth on acetate and NB reduction on the cathode. When power supplied reduced to 0.2 V, the anode and cathode potential respectively increased to  $-279$  and  $-469$  mV, indicating the redox potentials become less favorable for anodic microorganisms and reduction of NB at cathode. During Phase 5 test, NE was maintained at above 90% (Fig. 7A), suggesting that the removal NB was mainly reduced to AN. Thus, high NB removal efficiency can achieve with at least 0.3 V power supplied in the UBER.

### 3.6. Bioanode vs abiotic anode

In this study, the NB removal rate greatly increased in the presence of small power was supplied, implying that the combination of biological and electrochemical reactions in UBER was successful. The external power needed (0.3–0.5 V) was small with limited cost and much lower than electrochemical reduction. Based on our estimation (Table S3 in Supplementary data), when influent NB concentration increases from 0.41 to 1.46 mM, power required would be less than  $0.075 \text{ kWh mol}^{-1}$  NB, which was just 10% of that required for electrochemical methods [36]. The low external energy requirement by UBER was mainly contributed by the electrogenic function of bioanode with added electron donor (acetate). One experiment was designed for the comparison of linear volt-ampere (VA) for bioanode with abiotic anode. The results showed that the current quickly increased as power supplied reached to 0.17 V for bioanode, while 1.2 V for abiotic anode (Fig. 8A). Bioanode needed less power than abiotic anode at the same current (Fig. 8B). This demonstrated that NB could be reduced at an external power source of 0.17 V when bioanode was employed, which is much lower than that required for electrochemical reduction with

abiotic anode. This was mainly due to the lower overpotential of bioanode in UBER [28,44].

### 3.7. Significance of UBER concept and potential implementation

The results in this study demonstrated the feasibility of the concept of membrane-free, continuously feeding UBER. This system is especially favorable to treat wastewater containing inhibitory chemicals which can be electrochemically reduced to less inhibitory products (such as NB to AN). In this study, we used synthetic wastewater containing acetate as electron donor and NB as target organic pollutant. Other substrates or municipal wastewater will be tested as electron donor in further, as well as other toxic organic chemicals as electron acceptor, to enlarge the application of UBER.

Although no inoculum was introduced to the UBER except for installation of pre-enriched bioanode, the microbial growth in cathode zone could still occur by slow bacterial migration from anode zone since no membrane was used to separate both zones. The number of bacteria on cathode was limited based on SEM observation (Supplementary data, Fig. S2). The bacteria attached on cathode could partially contribute to NB reduction with organic carbon as electron donor as observed in other study with biocathode which enhanced NB reduction significantly in a fed-batch BES [45]. However, in this study, the transformation rate of NB to AN was likely limited due to almost no or limited attached bacteria on cathode and, the transformation of NB to AN, therefore, was mainly due to abiotic cathodic reduction. Further research will be conducted to examine whether biocathode could enhance NB reduction in the UBER.

Higher NB removal performance was achieved by UBER than other published laboratory-scale reactor configurations including trickling filter [46], fixed bed reactor [47] and anaerobic migrating blanket reactor [37] in term of the maximum NB removal rate ( $3.5$  in this study vs  $0.044$ ,  $1.93$  and  $0.44 \text{ mol m}^{-3} \text{ TV d}^{-1}$ , respectively) and NB removal efficiency (Supplementary data, Table S2). Li et al. [25] showed that NB could be reduced on the cathode for power generation in a batch feeding two-chamber MFC. The NE was 98% in 24 h, which was much slower than UBER and Pt was used as catalyst resulting in a high cost. Compared with the published results using continuously feeding mode BES with IEM [7], NB removal rate in UBER based on the cathode volume was slightly higher at similar current density (i.e.  $7.0 \text{ mol m}^{-3} \text{ TCV d}^{-1}$  in UBER at current density of  $39.2 \text{ A m}^{-3} \text{ TCV}$  and  $6.5 \text{ mol m}^{-3} \text{ TCV d}^{-1}$  in ref. [7] at current density of  $44.6 \text{ A m}^{-3} \text{ TCV}$ ) (Supporting data, Table S2), but lower than their maximum of  $8.57 \text{ mol m}^{-3} \text{ TCV d}^{-1}$  at current density of  $59.5 \text{ A m}^{-3} \text{ TCV}$ , indicating optimal condition, such as higher applied voltage to enhance current, would further improve the performance of UBER. However, the energy requirement by our UBER was slightly higher than the two-chamber BES by Mu et al. at similar current density and NB removal rate [7]. This suggests that further research is needed for optimization of energy consumption by UBER for scaling-up.

Acetate was used as electron donor source in this study. The ratio of NB reduced vs acetate consumed ( $\text{mol mol}^{-1}$ ) was 2.3, which was about 3 times of the theoretical value ( $0.75 \text{ mol mol}^{-1}$ ) for reduction of NB to AN. This suggests that a part of acetate was not directly used for the reduction and the performance of the UBER could be improved further considering low microbial cell synthesis for acetate consumption ( $<0.1$ ) under anaerobic condition. Other electron donor source such as ethanol, glucose or complicated organics in municipal wastewater could also work for NB reduction since these substrates work in other BESs [32,41].

The ion strength or conductivity of wastewater influences electron transfer and internal resistance of BES. In this study, we found the higher influent phosphate concentration resulted in higher



NB removal performance and 10 mM was minimum level without greatly affecting the NE. Except for enhancement of buffer capacity favorable for kinetics, proper ion strength or conductivity could benefit to the electron transfer and thus the reduction performance. Further research is needed to examine the mechanism of the enhancement and optimization of UBER operation.

In addition, the lower power needed (0.3 V) resulted in a lower energy required and the absence of membrane made a great contribution to reduce the cost of UBER.

#### 4. Conclusion

A membrane-free, continuously feeding up-flow biocatalyzed electrolysis reactor (UBER) was developed for the wastewater treatment with electrochemical reduction. NB was selected as a target chemical and acetate was used as electron donor source. The results indicate that NB can be continuously and efficiently removed (>99%) with AN as a major product (>80%). The ratio of acetate consumed vs NB removed varied from  $4.3 \pm 0.4$  to  $2.3 \pm 0.1$  mol acetate mol<sup>-1</sup> NB, being 6–3 times of theoretical value. The reduction reaction occurred mainly in cathode zone. High Coulombic efficiencies for NB reduction (99%) and A formation (>80%) were observed. This demonstrated the feasibility of the UBER as single reactor with anodic biological and cathodic electrochemical functions.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.11.034.

#### References

- [1] H.A.O. Hill, I.J. Higgins, Bioelectrocatalysis, *Philos. Tans. R. Soc. Lond. A* 302 (1981) 267–273.
- [2] B.E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: methodology and technology, *Environ. Sci. Technol.* 40 (2006) 5181–5192.
- [3] D.R. Lovley, Microbial fuel cells: novel microbial physiologies and engineering approaches, *Curr. Opin. Biotechnol.* 17 (2006) 327–332.
- [4] K. Rabaey, K. Van De Sompel, L. Maignien, N. Boon, P. Aelterman, P. Clauwaert, L. De Schampelaire, T.H. Pham, J. Vermeulen, M. Verhaege, P. Lens, W. Verstraete, Microbial fuel cells for sulfide removal, *Environ. Sci. Technol.* 40 (2006) 5218–5224.
- [5] A. Bergel, D. Feron, A. Mollica, Catalysis of oxygen reduction in PEM fuel cell by seawater biofilm, *Electrochem. Commun.* 7 (2005) 900–904.
- [6] P. Clauwaert, K. Rabaey, P. Aelterman, L.D. Schampelaire, H.T. Pham, P. Boeckx, N. Boo, W. Verstraete, Biological denitrification in microbial fuel cells, *Environ. Sci. Technol.* 41 (2007) 3354–3360.
- [7] Y. Mu, A.R. René, R. Korneel, K. Jürg, Nitrobenzene removal in bioelectrochemical systems, *Environ. Sci. Technol.* 43 (2009) 8690–8695.
- [8] C. Butler, P. Clauwaert, S. Green, W. Verstraete, R. Nerenber, Bioelectrochemical perchlorate reduction in a microbial fuel cell, *Environ. Sci. Technol.* 44 (2010) 4685–4691.
- [9] F. Aulenta, A. Catervi, M. Majone, S. Panero, P. Reale, S. Rossetti, Electron transfer from a solid-state electrode assisted by methyl viologen sustains efficient microbial reductive dechlorination of TCE, *Environ. Sci. Technol.* 41 (2007) 2554–2559.
- [10] S.M. Strycharz, T.L. Woodard, J.P. Johnson, K.P. Nevin, P.A. Sanford, F.E. Löffler, D.R. Lovley, Graphite electrode as a sole electron donor for reductive dechlorination of tetrachlorethene by geobacter lovleyi, *Appl. Environ. Microbiol.* 74 (2008) 5943–5947.
- [11] F. Aulenta, A. Canosa, P. Reale, S. Rossetti, S. Panero, M. Majone, Microbial Reductive Dechlorination of trichloroethene to ethene with electrodes serving as electron donors without the external addition of redox mediators, *Biotechnol. Bioeng.* 101 (2010) 85–91.
- [12] S.M. Strycharz, S.M. Gannon, A.R. Boles, A.E. Franks, K.P. Nevin, D.R. Lovley, Reductive dechlorination of 2-chlorophenol by *Anaeromyxobacter dehalogenans* with an electrode serving as the electron donor, *Environ. Microbiol. Rep.* 2 (2010) 289–294.
- [13] J. Sun, Z. Bi, B. Hou, Y.Q. Cao, Y.Y. Hua, Further treatment of decolorization liquid of azo dye coupled with increased power production using microbial fuel cell equipped with an aerobic biocathode, *Water Res.* 45 (2010) 1–9.
- [14] H.C. Tao, W. Li, M. Liang, N. Xu, J.R. Ni, W.M. Wu, A membrane-free baffled microbial fuel cell for cathodic reduction of Cu(II) with electricity generation, *Bioresour. Technol.* 102 (2011) 4774–4778.
- [15] H.C. Tao, M. Liang, W. Li, L.J. Zhang, J.R. Ni, W.M. Wu, Removal of copper from aqueous solution by electrodeposition in cathode chamber of microbial fuel cell, *J. Hazard. Mater.* 189 (2011) 186–192.
- [16] R.A. Rozendal, H.V.M. Hamelers, G.J.W. Euverink, S.J. Metz, C.J.N. Buisman, Principle and perspectives of hydrogen production through biocatalyzed electrolysis, *Int. J. Hydrogen Energy* 31 (2006) 1632–1640.
- [17] S. Cheng, D. King, D.F. Call, B.E. Logan, Direct biological conversion of electrical current into methane by electromethanogenesis, *Environ. Sci. Technol.* 43 (2009) 3953–3958.
- [18] R.A. Rozendal, E. Leone, J. Keller, K. Rabaey, Efficient hydrogen peroxide generation from organic matter in a bioelectrochemical system, *Electrochem. Commun.* 11 (2009) 1752–1755.
- [19] K.J.J. Steinbusch, H.V.M. Hamelers, J.D. Schaap, C. Kampman, C.J.N. Buisman, Bio-electrochemical ethanol production through mediated acetate reduction by mixed cultures, *Environ. Sci. Technol.* 24 (2009) 513–517.
- [20] X.X. Cao, X. Huang, P. Liang, K. Xiao, Y.G. Zhou, X.Y. Zhang, B.E. Logan, A new method for water desalination using microbial desalination cells, *Environ. Sci. Technol.* 43 (2009) 7148–7152.
- [21] M. Rodriguez, V. Timokhin, F. Michl, S. Contreras, J. Gimenez, S. Esplugas, The influence of different irradiation sources on the treatment of nitrobenzene, *Catal. Today* 76 (2002) 291–300.
- [22] P. Gurevich, A. Oren, S. Sarig, Y. Henis, Reduction of aromatic nitrocompounds in anaerobic ecosystems, *Water Sci. Technol.* 27 (1993) 89–96.
- [23] Ö.S. Kuscü, D.T. Sponza, Effects of nitrobenzene concentration and hydraulic retention time on the treatment of nitrobenzene in sequential anaerobic baffled reactor (ABR)/continuously stirred tank reactor (CSTR) system, *Bioresour. Technol.* 100 (2009) 2162–2170.
- [24] M.A. De, O.A. O'Connor, D.S. Kosson, Metabolism of aniline under different anaerobic electron-accepting and nutritional conditions, *Environ. Toxicol. Chem.* 13 (1994) 233–239.
- [25] J. Li, G. Liu, R. Zhang, Y. Luo, C. Zhang, M. Li, Electricity generation by two types of microbial fuel cells using nitrobenzene as the anodic or cathodic reactants, *Bioresour. Technol.* 101 (2010) 4013–4020.
- [26] A.J. Wang, H.Y. Cheng, N.Q. Ren, D. Cui, N. Lin, W.M. Wu, Sediment microbial fuel cell with floating biocathode for organic removal and energy recovery, *Front. Environ. Sci. Eng. China* (2011), Published online, July 11.
- [27] J. Heilmann, B.E. Logan, Production of electricity from proteins using a single chamber microbial fuel cell, *Water Environ. Res.* 78 (2006) 531–537.
- [28] V.M.H. Hubertus, T.H. Annemiek, H.J.A.S. Tom, W.J. Adriaan, P.B.T.B.S. David, J.N.B. Ceess, New applications and performance of bioelectrochemical systems, *Appl. Microbiol. Biotechnol.* 85 (2010) 1673–1685.
- [29] R.A. Rozendal, H.V.M. Hamelers, C.J.N. Buisman, Effects of membrane cation transport on pH and microbial fuel cell performance, *Environ. Sci. Technol.* 40 (2006) 5206–5211.
- [30] H. Liu, B.E. Logan, Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, *Environ. Sci. Technol.* 38 (2004) 4040–4046.
- [31] H. Liu, H.Q. Hu, Y.Z. Fan, Hydrogen production using single-chamber membrane-free microbial electrolysis cells, *Water Res.* 42 (2008) 4172–4178.
- [32] J.R. Kim, S.H. Jung, J.M. Regan, B.E. Logan, Electricity generation and microbial community analysis of ethanol powered microbial fuel cells, *Bioresour. Technol.* 98 (2007) 2568–2577.
- [33] D. Davila, J.P. Esquivel, N. Sabate, J. Mas, Silicon-based microfabricated microbial fuel cell toxicity sensor, *Biosens. Bioelectron.* 26 (2011) 2426–2430.
- [34] H.J. Kim, M. Kim, M.S. Hyun, G.M. Gadd, A novel biomonitoring system using microbial fuel cells, *J. Environ. Monit.* 9 (2007) 1323–1328.
- [35] B.A. Donlon, E. RazoFlores, G. Lettinga, J.A. Field, Continuous detoxification, transformation, and degradation of nitrophenols in upflow anaerobic sludge blanket (UASB) reactors, *Biotechnol. Bioeng.* 51 (1996) 439–449.
- [36] A.J. Wang, D. Sun, G.L. Cao, H.Y. Wang, N.Q. Ren, W.M. Wu, B.E. Logan, Integrated hydrogen production process from cellulose by combining dark fermentation, microbial fuel cells, and a microbial electrolysis cell, *Bioresour. Technol.* 102 (2011) 4137–4143.
- [37] Ö.S. Kuscü, D.T. Sponza, Effect of increasing nitrobenzene loading rates on the performance of anaerobic migrating blanket reactor and sequential anaerobic migrating blanket reactor completely stirred tank reactor system, *J. Hazard. Mater.* 168 (2009) 390–399.
- [38] C.I. Torres, A.K. Marcus, B.E. Rittmann, Proton transport inside the biofilm limits electrical current generation by anode-respiring bacteria, *Biotechnol. Bioeng.* 100 (2008) 872–881.
- [39] T.H.J.A. Sleutel, R. Lodder, H.V.M. Hamelers, C.J.N. Buisman, Improved performance of porous bio-anodes in microbial electrolysis cells by enhancing mass and charge transport, *Int. J. Hydrogen Energy* 34 (2009) 9655–9661.



- [40] T.H.J.A. Sleutels, H.V.M. Hamelers, C.J.N. Buisman, Reduction of pH buffer requirement in bioelectrochemical systems, *Environ. Sci. Technol.* 44 (2010) 8259–8263.
- [41] S. Freguia, Sequential anode–cathode configuration improves cathodic oxygen reduction and effluent quality of microbial fuel cells, *Water Res.* 42 (2008) 1387–1396.
- [42] B. Virdis, K. Rabaey, Z. Yuan, R. Rozendal, J. Keller, Electron fluxes in a microbial fuel cell performing carbon and nitrogen removal, *Environ. Sci. Technol.* 43 (2009) 5144–5149.
- [43] M.C.F. Oliveira, Study of the hypophosphite effect on the electrochemical reduction of nitrobenzene on Ni, *Electrochim. Acta* 48 (2003) 1829–1835.
- [44] A.K. Marcus, Conduction-based modeling of the biofilm anode of a microbial fuel cell, *Biotechnol. Bioeng.* 98 (2007) 1171–1182.
- [45] A.J. Wang, H.Y. Cheng, B. Liang, N.Q. Ren, D. Cui, N. Lin, B.H. Kim, K. Rabaey, Efficient reduction of nitrobenzene to aniline with a biocatalyzed cathode, *Environ. Sci. Technol.* (2011), Published online, October 28.
- [46] O. Dickel, W. Haug, H.J. Knackmuss, Biodegradation of nitrobenzene by a sequential anaerobic-aerobic process, *Biodegradation* 4 (1993) 187–194.
- [47] R. Gerlach, M. Steiof, C.L. Zhang, J.B. Hughes, Low aqueous solubility electron donors for the reduction of nitroaromatics in anaerobic sediments, *J. Contam. Hydrol.* 36 (1999) 91–104.