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Efficient removal of nitrobenzene and concomitant electricity production by single-chamber microbial fuel cells with activated carbon air-cathode

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Abstract Removal of inhibitory nitrobenzene (NB) was investigated in single-chamber microbial fuel cells (MFCs) assembled with pre-enriched bioanodes and active carbon (AC) air-cathodes. High NB tolerance and NB removal were obtained in the present single-chamber MFCs with high electricity production. The maximum power over 25 W m^{-3} could be obtained when the present S-MFCs were operated in the NB loading range of 1.2 to $6.2 \text{ mol m}^{-3} \text{ d}^{-1}$, and stable electricity production over 13.7 W m^{-3} could be produced in a NB loading range of 1.2 to $14.7 \text{ mol m}^{-3} \text{ d}^{-1}$. The present single-chamber MFCs with AC air-cathodes exhibited high NB removal performance with NB removal efficiency over 97% even when the NB loading rate was increased to $17.2 \text{ mol m}^{-3} \text{ d}^{-1}$. The potential NB reduced product (i.e. aniline) could also be effectively removed from influents. The findings in this study means that single-chamber MFCs assembled with pre-enriched bioanodes and AC air-cathodes could be developed as effective bioelectrochemical systems to remove NB from wastewaters and to harvest energy instead of consuming energy.

Key words Nitrobenzene removal; microbial fuel cells; active carbon air-cathode; bioelectrochemical system

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Introduction

Nitrobenzene (NB) is widely used as an important chemical for the production of aniline (AN), dyes, pharmaceuticals, and pesticides (Couto et al., 2016; Ren et al., 2015), and as a solvent in products like paints, shoes and floor metal polishes. Due to its recalcitrance and mutagenicity, NB has been listed as a priority pollutant, with strict limitations (e.g. 17 µg/L in the US and 20 µg/L in China).

To remove NB from wastewater, methods based on different mechanisms such as adsorption, ozonation, advanced oxidation processes and reduction have been developed over recent decades. However, it is still a challenging task to improve the efficiency of these methods. For example, novel adsorption material with high adsorption performance should be developed to overcome the low adsorption of NB caused by low water solubility (Rauthula & Srivastava, 2011; Wen et al., 2012), and catalysts with high performance are required to improve the NB oxidation (Ayati et al., 2016; Chen et al., 2014; Rabaaoui et al., 2013; Shen et al., 2014). Moreover, most methods based on chemical processes generally need to consume significant amounts of energy and/or require significant quantities of chemicals, which are cost-intensive and may cause secondary pollution (ElShafei et al., 2010; Rabaaoui et al., 2013; Xie et al., 2014). Comparatively, biological methods are more cost-effective, but generally suffer from lower degradation rates and toxicity of NB to microorganisms at high concentrations. Bacteria could degrade NB by oxidation mechanisms coupled to reducing oxygen or sulfate (Kirui et al., 2016; Zheng et al., 2009), or by reduction mechanisms coupled to aniline production (Spain, 1995; Wang et al., 2011).

Microbial fuel cells (MFCs) aimed at the generation of electricity or removal of waste compounds have received significant attention over the past decade (Logan et al., 2015). Up to now, in addition to effective removal of complex organic wastes by oxidization processes in the anode chamber, MFCs have also been shown to successfully remove various contaminations including nitrate (Clauwaert et al., 2007), hexavalent chromium (Scialdone et al., 2014; Wang et al., 2008), by reduction processes in the cathode chamber. However, there are few studies of NB removal by MFC operation (Li et al., 2010). Recently, Mu et al. indicated that NB can be converted to aniline in the cathode chambers in bio-electrochemical systems with a bio-anode and a chemical cathode (Mu et al., 2009). Subsequently, other researchers found that the efficiency of NB transformation in the cathode chamber of bio-electrochemical system can be enhanced by using a bio-cathode instead of a chemical cathode (Liang et al., 2014; Wang et al., 2011; Wang et al., 2012; Zhang et al., 2015). However, instead of operating the systems in MFC mode, power input was needed to operate the bio-electrochemical systems, i.e. microbial electrolysis cells, to effectively convert NB to other less toxic products (e.g. aniline), although the energy consumption was much lower than required in pure electrochemical systems. For instance, a power input for NB removal was reported to be *ca.* 0.075 kWh mol⁻¹ NB in a membrane-free, continuous feeding up-flow biocatalyzed electrolysis reactor (Wang et al., 2012). Thus, it is attractive proposition to develop MFCs for efficient removal of nitrobenzene and concomitant electricity (Li et al., 2010).

For wastewater treatment, single-chamber MFCs with an air-cathode are generally accepted to be one of most suitable configurations for practical application due to the economic benefits of a simplified design, a reduced total volume, and improved power output (Liu & Logan, 2004; Liu et al., 2004). Additionally cost-effective catalysts for oxygen reduction at air-cathodes have been attracting interest for constructing single-chamber MFCs. Activated carbon (AC), due to its high surface area, was considered to be an attractive cost-effective catalyst alternative to noble metal

catalysts for oxygen reduction for single-chamber MFC configuration (Zhang et al., 2009). The performance of the AC cathode can be maintained at high levels over long-term MFC operation (Zhang et al., 2011), and under variable pH conditions (Zhang et al., 2016). In the present study, we found that NB can be effectively removed by single-chamber MFCs with pre-enriched bioanodes and AC air-cathodes, with concomitant electricity.

2. Materials and Methods

2.1. MFC configuration and operation

For comparison, two types of MFCs were employed in this study; single-chamber MFCs (S-MFCs) with microbial anodes and AC air-cathodes and double-chamber MFCs (D-MFCs) with microbial anodes and ferricyanide-cathodes. MFC chambers were made of plexiglass with a total empty volume (TV) of 118 mL (cylinder with 5.0 cm diameter and 6.0 cm length) and effective solution volume of ~60 mL. Electrodes assembled with three pieces of graphite felt (radius 2.3 cm, thickness 1.0 cm) and stainless steel (316L) bolt and nuts were used as anodes in S-MFCs and as anodes and cathodes in D-MFCs. Air-cathodes in S-MFCs was prepared using AC (Xinshen Carbon, Fujian), PTFE microporous filtering film (0.45 μm) and stainless steel mesh (316L) as catalyst, air-diffusion layer and current collector, respectively, as described in the previous study (Zhang et al., 2016). To operate D-MFCs with ferricyanide-cathodes, a cation exchange membrane (Shenzhen) was installed to separate two identical chambers. Details of the MFC setup are provided in **Fig. 1S**.

Bioanodes were first pre-enriched for S-MFCs. A constant external resistance of 100 Ω was connected between the anode and cathode. To activate the MFCs, S-MFCs were initially operated by feeding NB-free artificial growth medium (AGM) in batch mode. The original inoculum source was taken from a separately operated (long-term running) MFC anode chamber which was inoculated with anaerobic activated sludge (sampled from the waste treatment plant in Yangzhou brewery) and was operated using acetate as electron donor over one year. The AGM, of pH 7.0, was prepared with the following constituents (in grams per liter of deionized water): NaAc, 1.6; NaHCO_3 , 2.5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1; KCl, 0.1; NH_4Cl , 1.5; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.6; NaCl, 0.1; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.005; $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.001; yeast extract 0.05. The initial pH of AGM was adjusted to 7.0 using HCl and NaOH solution.

After the S-MFCs were operated in batch mode over one month to obtain mature anodic biofilms, MFCs were run by feeding AGM containing NB of 0.5 mM for subsequent batch cycles over one month, enabling MFCs to acclimate to the presence of NB. Following NB acclimatization, the MFC operation was transferred from batch mode to flow mode by continuously feeding AGM containing NB and increasing the concentration of NB in steps (0.5 mM ~7.0 mM), to investigate the effect of NB loading rate on electricity production and NB removal by S-MFCs. The flow rate was controlled at 0.2 ml min^{-1} with a peristaltic pump (S1.5-2B, Signal Liquid Co., China), resulting in a hydraulic retention time (HRT) of 5 h. To compare the effect of NB on MFCs with different configuration, mature bioanodes developed in S-MFCs acclimating to 0.5 mM NB over one month, were employed to install D-MFCs. To run

D-MFCs, the NB-containing AGM and the ferricyanide solution (50 mM buffered by 50 mM phosphate), were continuously fed into the anode chamber and the cathode chamber, respectively, with peristaltic pumps at a rate identical to that in the operation for S-MFCs.

2.2 Electrochemical measurements

The voltage produced by MFCs with an external load of 100 Ω , and the electrode potentials relative to a double salt bridge electrode saturated calomel electrode (SCE, Leici 217) was continuously measured using a multiple-channel high impedance voltmeter (Keithley 2700). Polarization curves of MFCs were measured using a battery testing system (Neware CT-3008W, Shenzhen, China) in the mode of constant current discharge. Cyclic voltammetry (CV) for bioanodes was performed using a potentiostat (CHI 660c, Shanghai, China) with SCE and the cathodes as reference electrode and counter electrode, respectively. For polarization and CV measurements, MFCs were first disconnected from the circuit until the open circuit voltage plateaued before measurements. Current density and power density were normalized to the total volume of MFC chamber for comparison with literature.

2.3 Chemical analysis and calculations

Liquid samples taken from the anode chamber in MFCs were immediately filtered through a 0.22 μm filter. Aniline, NB and nitrosobenzene were measured by using high performance liquid chromatography (HPLC, Agilent 1200) equipped with Unison UK-C18 column (3 μm ; 150 mm $\times$ 4.6 mm, Imtakt Co., Japan). The mobile phase was methanol/H₂O (4:6) at 0.35 mL min⁻¹. The detection wave length for aniline, NB and nitrosobenzene was 230 nm, 268 nm and 280 nm, respectively. Column temperature was 28 $^{\circ}\text{C}$. Under the analytical condition, the retention time of aniline, NB and nitrosobenzene was 6.57, 7.75 and 7.34 min, respectively. NB loading rate (LR_{NB} , mol m⁻³TV d⁻¹) was calculated based on the initial NB concentrations in influents ($c_{\text{NB-inf.}}$ /mM),

AGM flow rate (r_{flow} /mL min⁻¹) and the total empty volume of the chamber (V_{TV} /m³) by the formula (1):

$$\text{LR}_{\text{NB}} = \frac{r_{\text{flow}} \times 60 \times 24 \times 0.001 \times c_{\text{NB-inf.}} \times 0.001}{V_{\text{TV}}} \quad (1)$$

NB removal efficiency (%) was calculated based on the NB initial concentrations in influents ($c_{\text{NB inf.}}$) and the NB concentrations in effluents ($c_{\text{NB eff.}}$) from the MFCs by the formula (2):

$$\text{NB removal efficiency (\%)} = \frac{c_{\text{NB inf.}} - c_{\text{NB eff.}}}{c_{\text{NB inf.}}} \times 100 \quad (2)$$

3 Results and Discussion

3.1. MFC activation and NB acclimatization

MFCs acclimated to the presence of NB were developed in S-MFCs with AC air-cathodes by batch operation. In the first stage, the S-MFCs were activated by continuously replacing NB-free AGM at the end of each cycle. After 3-5 operation cycles, repeatable cycles of electricity production was achieved, resulting in stable voltage output over 0.45 V with the external load of 100 Ω . To obtain mature electroactive biofilms on anodes, the activated MFCs were run with NB-free AGM over one month. Typical voltage production was shown in **Fig. 1A**. At the end of the first operation stage, maximum power production was $28.7 \pm 2.4 \text{ W m}^{-3}\text{TV}$ (six duplicates). With initial acetate concentration of 20 mM in AGM, periods of stable voltage production within operation cycles were 27 ± 4 hours.

Following the first stage, S-MFCs were operated by replacing AGM containing 0.5 mM NB at the end of each cycle. The data showed that NB addition has no observable effect on the stable voltage output (**Fig. 1A**). During continuously replacing AGM with 0.5 mM NB over one month, S-MFCs were observed to produce stable voltage over 0.45 V, nearly identical to the voltage production during the first stage without NB addition. In a previous study, NB was observed to reduce the voltage production at concentrations over 0.4 mM in the anode-chamber in double-chamber MFCs due to its potential toxicity to microorganisms and competitive electron consumption with anodic processes (Li et al., 2010). By comparison, the present single-chamber MFCs with AC air-cathodes exhibited higher NB tolerance in terms of voltage production. However, the observed NB tolerance in the present S-MFCs was found to depend on the formation of biofilms on anodes. In separated experiments, the present S-MFCs were operated by initially feeding AGM containing 0.5 mM NB with the inoculum, and the voltage output was observed to be less than 0.05 V over consecutive cycles, much lower than S-MFCs initially fed with NB-free AGM (**Fig. 1B**). The data in **Fig. 1** indicate that NB, even at low concentration, would largely inhibit the development of electroactive biofilms on anodes in the present S-MFCs. It is generally accepted that biofilm-growing microorganisms are self-regenerating, spatially and metabolically well organized, and less affected by toxic substrate and/or products (Borole et al., 2011; Halan et al.). So, the power production by S-MFCs in the presence of NB could be attributed to the mature anodic biofilms pre-enriched over long-term operation under NB-free conditions (**Fig. 1A**).

3.2. Effect of NB addition on electricity production

Following the NB acclimatization, S-MFCs were transferred to flow operation by continuously feeding AGM containing NB (with step increases in concentration) in order to investigate the effect of NB. During flow operation, the acetate loading rate was maintained at $48.2 \text{ mol m}^{-3} \text{TV d}^{-3}$, and the NB loading rate was step-up increased from $1.2 \text{ mol m}^{-3}\text{TV d}^{-1}$ to $17.2 \text{ mol m}^{-3}\text{TV d}^{-1}$ (initial NB concentrations in AGW: 0.5 mM to 7.0 mM). As a whole, the voltage decreased slightly prior to increasing NB loading rate to $14.8 \text{ mol m}^{-3} \text{TV d}^{-1}$ (**Fig. 2A**). However, voltage was always higher than 0.4 V in S-MFCs over 20 day running, resulting in stable power output over $13.7 \text{ W m}^{-3} \text{TV}$. Further increasing NB to $17.2 \text{ mol m}^{-3} \text{TV d}^{-1}$ quickly

reduced the voltage output below 0.3 V, but the voltage could recover to previous high levels by re-feeding NB-free AGW following short-term (< 2 days) large NB loading (Fig. 2A). While the cathode potential was relatively stable during the operation by step-up increasing NB loading, the anode potential was found to synchronously change with the NB-induced variation in voltage (Fig. 2B), indicating that increased NB has a greater inhibiting effect on bioanodes than AC air-cathodes in the present S-MFCs. Polarization measurements (Fig. 3) showed that S-MFCs produced the maximum power between $27.7 \pm 3.5 \text{ W m}^{-3}$ and $25.9 \pm 1.3 \text{ W m}^{-3} \text{ TV}$ (3 duplicates) when the systems were operated with NB loading rate less than $6.2 \text{ mol m}^{-3} \text{ TV d}^{-1}$ (initial NB concentration: 2.5 mM). When the NB loading rate was increased to $12.3 \text{ mol m}^{-3} \text{ TV d}^{-1}$ (initial NB concentration: 5.0 mM), the maximum power output decreased to $14.3 \pm 2.4 \text{ W m}^{-3} \text{ TV}$ (3 duplicates). However, the systems were observed to still generate stable voltage over 0.4 V at this NB loading rate (Fig. 2A). When the bioanode was largely inhibited by NB input as high as $17.2 \text{ mol m}^{-3} \text{ TV d}^{-1}$ (initial NB concentration: 7.0 mM), the maximum power was $7.7 \pm 0.9 \text{ W m}^{-3} \text{ TV}$.

NB is toxic to microorganisms at high concentrations. However, the results above show that MFCs with pre-enriched bioanodes and AC air-cathodes exhibited high electricity production in the presence of NB. The findings presented in this study are in contrast to previous studies in which power was required to run the NB-fed bioelectrochemical systems (Table 1). Li et al. investigated the feasibility of electricity production in double-chamber MFCs in the presence of NB, but obtained lower power production and lower NB tolerance (Li et al., 2010). To understand the high performance of the present S-MFCs in terms of electricity production and NB tolerance, separate experiments in D-MFCs with NB-acclimated bioanodes and ferricyanide-cathodes were carried by feeding NB at an identical rate to S-MFCs. Unlike S-MFCs, bioanodes in D-MFCs were inhibited at NB loading rate of $3.7 \text{ mol m}^{-3} \text{ TV d}^{-1}$ (1.5 mM NB in influent) (Supplementary data, Fig.S2), indicating that D-MFCs had much lower NB tolerance than S-MFCs. The difference between S-MFCs and D-MFCs suggested that high NB tolerance in S-MFCs might be attributed to the use of AC air-cathodes. AC air-cathodes have high NB adsorption capacity and have been demonstrated to have high electrocatalytic activity to oxygen reduction reaction in single-chamber MFCs (Dong et al., 2013; Zhang et al., 2016; Zhang et al., 2011). Chemical analysis showed that the present S-MFCs also have high NB removal (see the following section). The excellent performance of AC in adsorption and electrochemical catalysis may be one of the main reasons for high NB tolerance of electricity production and high NB removal efficiency in the present S-MFCs with AC air-cathodes.

Table 1 Operating conditions in some reported bioelectrochemical systems with bioanodes.

bioelectrochemical system		nitrobenzene feeding conditions		power I/O** ($\text{W m}^{-3} \text{ TV}$)	Ref.
cathode	structure	initial concn. (mM)	loading rate ($\text{mol m}^{-3} \text{ TV d}^{-1}$)		
biocathode with NB	double-chamber	0.5		-0.15 ~ -0.5 ^b	(Wang et al., 2011)
biocathode with NB ^a	single-chamber	1.6 ~ 3.2		-0.5 ~ -1.2 ^b	(Zhang et al., 2015)
biocathode with NB	single-chamber	0.1~1.6	0.49~3.90	-1.7 ~ -17.6	(Wang et al., 2012)
liquid with NB	double-chamber	1.5	4.6~20	+0.25 ~ -17.6	(Mu et al., 2009)
ferricyanide-cathode	double-chamber	0.4 ~ 2.0		+8.5 ~ +20.2	(Li et al., 2010)

liquid with NB	double-chamber	2.0		+6.3	(Li et al., 2010)
AC air cathode	single-chamber	0.5 ~ 7.0	1.2 ~ 17.2	+7.7 ~ +27.7	This study

** In this column, the negative indication means the power input, and the positive sign means the power output.

a In this system, the anode was zero valent iron

b These were applied voltage values (V).

3.3. NB removal from S-MFCs with AC air-cathodes

Samples were periodically taken from effluents in S-MFCs for the analysis of NB and aniline when the NB loading rate was step increased. Removal efficiency and the concentration of NB and aniline in effluents are shown in [Fig. 4](#). High NB removal efficiencies (over 97%) were observed even when NB loading rates increased to $17.2 \text{ mol m}^{-3} \text{ TV d}^{-1}$ in the present S-MFCs. In flow operation of the S-MFCs, the initial concentrations of NB in influents were in the range of 0.5~7.0 mM, much higher than NB concentrations (*ca.* 0.1~4.8 mM) in most industrial wastewaters (Kuşçu & Sponza, 2009), implying that the present S-MFCs with AC air-cathodes might have a potential application in treating industrial wastewaters with high NB concentrations. To test the limiting NB removal capacity of the present MFCs, the NB loading rate was increased to $36.9 \text{ mol m}^{-3} \text{ TV d}^{-1}$ by feeding AGW containing 5.0 mM NB at an enhanced rate of 0.6 ml min^{-1} over 20 hours. It was found that NB could still be removed by over 68% at that high NB loading rate. However, stable power output was only $\sim 3.4 \text{ W m}^{-3}$ because the bioanodes were largely inhibited by that high NB loading. NB concentrations in effluents were detected between 0.002~0.12 mM when NB loading rate were controlled in the range of $1.2\sim 17.2 \text{ mol m}^{-3} \text{ TV d}^{-1}$ (corresponding initial NB concentrations in influents: 0.5~7.0 mM). However, aniline, the dominant product of NB reduction reaction in the cathode chamber of bioelectrochemical systems (Mu et al., 2009; Wang et al., 2011; Wang et al., 2012; Zhang et al., 2015), was also found to be much lower in effluents in the present S-MFCs ([Fig. 4](#)). Another potential intermediate product of NB reduction reaction, nitrosobenzene, was not detected in all effluent samples. These observations illustrated that NB, as well as its reactions products, could be effectively removed by the present S-MFCs. NB could be effectively converted to less toxic aniline through electrochemical reduction at chemical/biological cathodes at lower cathode potentials (Mu et al., 2009; Wang et al., 2011; Wang et al., 2012; Zhang et al., 2015). However the fate of NB in the present S-MFCs could not be fully understood only by considering the NB reduction reaction at AC air-cathodes because the detected aniline was much lower in effluents. Apart from the electrocatalytic reduction of NB, high adsorption capacity of AC might contribute to the high removal of NB and its reaction products in S-MFCs with AC air-cathodes. To fully elucidate the fate of NB in the present S-MFCs further studies will be investigated in future.

3.4. Electroactivity of the bioanode in MFCs

The present S-MFCs with AC air-cathodes exhibited effective NB removals with concomitant

electricity. In microbial fuel cells, the power output is generally determined by the electroactivity of bioanodes. CV measurements were employed to evaluate the effect of NB addition on the electroactivity of bioanodes in MFCs under different operating conditions. All CV curves measured at different S-MFC operating stages had similar current-potential relationship characterizing a peak current in the potential range of $-0.35\text{ V} \sim -0.2\text{ V}$ (vs SCE), but differ greatly in the peak currents (**Fig. 5**). Bioanodes in S-MFCs that had been operated at lower NB loading rate ($< 6.2\text{ mol m}^{-3}\text{ TV d}^{-1}$, and initial NB concentration in influent: 2.5 mM) over ten days (**curve II in Fig. 5**), had similar peak current (over $150\text{ A m}^{-3}\text{ TV}$) to that of bioanodes never contacting NB (**curve I in Fig. 5**). However, the catalytic peak current of bioanodes largely decreased to about $50\text{ A m}^{-3}\text{ TV}$ (**curve V in Fig. 5**) when S-MFCs were operated by feeding NB over $17.2\text{ mol m}^{-3}\text{ TV d}^{-1}$. Re-feeding NB-free AGW could only partially restore the electroactivity of bioanodes experiencing heavy NB inhibition (**curves IV and V in Fig. 5**).

3.5. Significance of S-MFCs for treating NB-contaminated wastewater treatments

By operating single-chamber MFCs with AC air-cathodes in flow mode, the present study demonstrates high NB removal and concomitant electricity in S-MFCs. This provides an attractive method for treating wastewater containing inhibitory chemicals in a cost-effective manner. Inhibitory chemicals, such as NB, have significant toxicity to microorganisms. Most strains described in the literature cannot effectively degrade more than 2 mM NB (Zheng et al., 2009), and NB was found to largely inhibit the electroactivity of bioanodes in double-chamber MFCs at concentrations over 1.5 mM in previous study (Li et al., 2010) and in the present study (**Supplementary data, Fig. S2**). Thus, the observed high NB-tolerant capacity of the present S-MFCs (**Fig. 2**), demonstrates that single-MFCs with pre-enriched bioanodes and AC air-cathode have an interesting advantage in the treatment of wastewaters with heavy NB contamination. Compared with other published laboratory-scale reactor configurations including trickling filter (Dickel et al., 1993), fixed bed reactor (Gerlach et al., 1999) and anaerobic migrating blanket reactor (Kuşçu & Sponza, 2009), bioelectrochemical systems generally showed higher NB removal performance in terms the maximum NB removal rate and NB removal efficiency (Wang et al., 2012). However, effective NB conversion by electrochemical and/or bioelectrochemical reduction at liquid cathodes were only achieved by applying an external voltage to drive cathode potentials to more negative ranges, resulting in a net energy consumption of the system in previous studies (Mu et al., 2009; Wang et al., 2011; Wang et al., 2012; Zhang et al., 2015). In the present S-MFCs, AC air-cathodes exhibited remarkable electrocatalytic activity under high NB loading conditions, and were shown to effectively alleviate inhibitory effect of NB on bioanodes. Consequently, the present simple MFC construction, in which the AC air-cathode and the bioanode were assembled into a single chamber, allowed the bioelectrochemical system to generate high power even in the case of heavy NB loading. The maximum power up to $25\text{ W m}^{-3}\text{ TV}$ was obtained at high NB loading rate up to $6.2\text{ mol m}^{-3}\text{ TV d}^{-1}$ (**Fig. 3**), and no obvious decays of stable power generation were observed when the S-MFC was operated for a long time by flowing AGW containing 2.5 mM NB, the typical NB concentrations in most industrial wastewaters. Compared with previously reported bioelectrochemical systems exhibiting high NB removal performance (i.e. $6.5\text{ mol m}^{-3}\text{ TV d}^{-1}$ and $7.0\text{ mol m}^{-3}\text{ TV d}^{-1}$) (Mu et al., 2009; Wang et al.,

2012), higher NB removal rate up to $17.2 \text{ mol m}^{-3}\text{TV d}^{-1}$ and NB removal efficiency over 97% could be achieved in the present flow operation S-MFCs (Fig. 4). Moreover, one of the striking results was that the present S-MFCs could simultaneously remove NB as well as its reduced products (i.e. aniline) (Fig. 4). Although some experimental conditions, such as the inoculum, the loading rate of consumable substrate (i.e. acetate) and the preparation of AC air-cathodes, have not yet been optimized, the findings presented in this study undoubtedly suggest that single-chamber MFCs assembled with bioanodes and AC air-cathodes could be developed as suitable bioelectrochemical systems to efficiently treat high NB-contaminated wastewater and to obtain electrical energy, thereby reducing the cost of wastewater treatment.

4. Conclusion

Single-chamber MFCs assembled with pre-enriched bioanodes and AC air-cathodes were demonstrated to have high NB removal capacity and concomitant electricity. The maximum power over $25 \text{ W m}^{-3} \text{ TV}$ could be obtained when the present S-MFCs were continuously fed with NB-containing AGM even at high loading rates up to $6.2 \text{ mol m}^{-3}\text{TV d}^{-1}$. Stable electricity production over $13.7 \text{ W m}^{-3} \text{ TV}$ could be produced in the NB loading range of $1.2 \text{ mol m}^{-3}\text{TV d}^{-1}$ to $14.7 \text{ mol m}^{-3}\text{TV d}^{-1}$. High NB removal rates and NB removal efficiency over 97% could be achieved in the NB loading range of $1.2 \text{ mol m}^{-3}\text{TV d}^{-1}$ to $17.2 \text{ mol m}^{-3}\text{TV d}^{-1}$. Moreover, the potential NB reduced product (i.e. aniline) could also be effectively removed from influents by the present S-MFCs. These findings suggest that single-chamber MFCs assembled with pre-enriched bioanodes and AC air-cathodes could be developed as effective bioelectrochemical systems to treat NB-contaminated wastewater in a cost-effective manner.

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Figure 1

Fig. 1 Voltage of S-MFCs activated with NB-free AGW and operated with AGW containing 0.5 mM NB (A), and voltage of S-MFCs activated with 0.5 mM NB-containing AGW (B).

Figure 2

Fig. 2 Voltage (A) and electrode potentials (B) produced by NB-acclimated S-MFCs continuously feeding AGW with step-up creased NB concentrations (mM): 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 5.0, 6.0, 7.0.

Figure 3

Fig. 3 Polarization measurements of S-MFCs (3 duplicates) at stages with different NB loading rate ($mol\ m^{-3}TV\ d^{-1}$)

Figure 4

Fig. 4 NB removal efficiency and the concentration of NB and aniline in effluents from S-MFCs.

Figure 5

Fig. 5 CV measurements of bioanodes under different NB loading conditions. Curve I: anodes never experiencing NB addition; curve II: anodes experiencing lower NB loading rate ($<6.2 \text{ mol m}^{-3}\text{TV d}^{-1}$); curve III: anodes experiencing high NB loading rate ($>17.2 \text{ mol m}^{-3}\text{TV d}^{-1}$); curve IV: anodes restored with NB-free AGW for 5 days; curve V: anodes restored with NB-free AGW for 10 days.

Figure 15. Schematic diagram (A) and the photographs viewed from the side face (B), the air-cathode (C) and the anode (D).

Figure S2 Typical voltage and anode potential generated by D-MFCs with NB-acclimated bioanodes and ferricyanide-cathode when feeding step-up increased NB loading.