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COMMUNICATION

Azide as an oxidant in cathodic reaction of bioelectrochemical systems (BESs)

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Azide could be used as an oxidant in cathodic reaction in BESs, which produced a peak current of 0.37 mA. An estimation based on electron balance showed that azide reduction at abiotic cathode was an eight-electron reaction, with NH₃ as the only reduction product.

Azide has been used in many industrial products such as explosive detonators and automobile air bags but also known to have toxic effects on many species from bacteria and fungi to mammals (Hamuda et al. 1996). Azide toxicity is widely assumed that azide acts by inhibiting cytochrome *c* oxidase of these living organisms (Wilson 1967). To eliminate the risk of exposure to azide, chemical oxidation involving nitrous acid (Stedma 1959) or hypochlorite (Betterton et al. 2010) is proposed as a available method for azide wastewater disposal, where N₃⁻ is converted into N₂. Electrochemistry also offers an approach for azide disposal, whereas the resulting products appear to depend on electrode material, electrode potential as well as acid-base properties of azide solutions. For example, with platinum (Pt) electrode, azide is oxidized to N₂, NO, NO₂ and N₂O when potential is set above 0.76 V vs. Ag/AgCl, while it is reduced to N₂, N₂H₂ and possibly NH₃ when potentials below -0.44 V vs. Ag/AgCl (Dalmia et al. 1995).

Biological treatment may be more attractive due to lack of chemical addition or energy consumption, but much is still unknown about biodegradability of azide except that azide can be

reduced by nitrogenase from specific anaerobes such as *Azotobacter vinelandii* (Iwahashi and Someya 1992; Rubinson et al. 1985). Recently, an uncharacterized *Geobacter* species enriched from single-chamber air-cathode MFCs was found to respire with azide (Zhou et al. 2014), causing decrease of current generation. These demonstrated that there were some enzymes on electron transport chain serving as azide reductase.

Given that azide had a higher redox potential than anode, it was hypothesized that azide could be as an oxidant in cathode reaction of BESs. The possibility of current generation, potential reduction products and electrochemical properties of azide were investigated in this work. In addition, the possible pathways for N₃⁻ reduction at cathode were also estimated according to electron balance.

Two-chamber aqueous cathode MFCs were used in the studies. Anode was made of carbon fibers, wound into a core consisting of two twisted titanium wires (Feng et al. 2011). Cathode was made of carbon cloth by applying Pt (0.5 mg/cm²) on one side and four diffusion layers (4 polytetrafluoroethylene layers) on the other side (Logan et al. 2007). The two-chamber, aqueous cathode MFC, with the electrodes separated by a proton exchange membrane (Nafion 117, Dupont), had liquid volumes of 28 mL for the anode chamber and 14 mL for the cathode chamber. The anode was connected to the cathode through an external circuit containing a resistor of 1000 Ω.

To reduce enrichment period, well-developed anodes from single-chamber air-cathode MFCs were directly transferred to two-chamber MFCs as anodes. The acetate (20 mM) medium contained the following (per liter): KCl, 0.13 g; NaH₂PO₄·2H₂O, 3.32 g; Na₂HPO₄·12H₂O, 10.32 g; NH₄Cl, 0.31 g; vitamin (5 mL); trace mineral (12.5 mL) (Lovley and Phillips 1988). Before operation, reactors had to be divided into two groups, one (MFC-A) with 50 mM phosphate buffer solution (PBS, pH 7.0) as catholytes (containing 20 mM azide), the others as controls (MFC-C) just containing 50 mM PBS. All anode chambers were fed with acetate medium.

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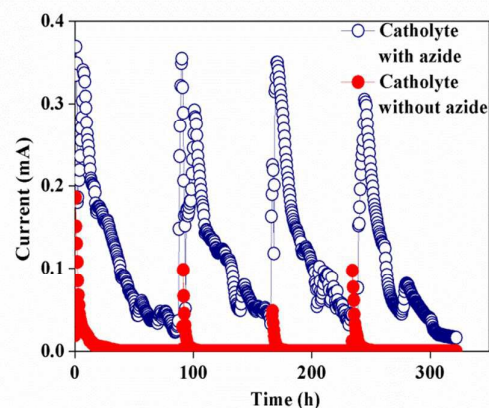
† Footnotes relating to the title and/or authors should appear here. Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

To avoid oxygen entering the systems, these reactors were each contained in a clear self adhesive seal plastic bag (11 × 21 cm), leaving an open “mouth” with a width of 1.5 cm as the passage for circuits and gas streams. A nitrogen purging system (NPS) was opened to continuously purge the bags with nitrogen gas (purity, 99.999 %) throughout the experiment. Three 100-mL bottles containing anolytes and catholytes (with and without azide) were used to feed the anode and cathode chambers with the help of the NPS. Before feeding, two custom-made needles were first inserted through the rubber stopper into each bottle, one connected with the NPS for removing oxygen from the solution, the other connected with a silicon tube for letting gas out of the bottle. After purging for 30 min, the end of the silicon tube was moved through the “mouth” into the bag and reached the port of the anode or cathode chamber, which was quickly filled up with nitrogen-purged solution by adjusting the needle depth in the bottle. Subsequently, the tube was slowly pulled out of the bag, and the port of the reactor was tightly sealed with a stand-by rubber stopper inside the bag. When cell voltage was lower than 50 mV, solutions in the reactors were completely replaced by emptying the chamber and refilling it with fresh one. All MFC reactors were operated in fed-batch mode and at room temperature (30 ± 2 °C).

Voltage (U) across an external resistor (R) was recorded at 30-min intervals using a data acquisition system (PISO-813, ICP DAS Co., Ltd). Current was calculated from $I=U/R$. In one batch cycle, when water samples were expected to be analyzed, NH_4Cl was not allowed to be contained in prepared anolytes and catholytes. Acetate concentration in water samples was measured as previously described (Zhou et al. 2014). Total nitrogen (TN) in the catholytes was determined using a TOC/TN₀ analyzer (multi N/C^o 2100 S, Germany) and used to indirectly calculate N_3^- content. Total ammonia nitrogen (TAN, sum of $\text{NH}_4^+\text{-N}$ and $\text{NH}_3\text{-N}$) was measured by salicylate method using a Test N' Tube kit (HACH, Loveland, CO, USA). Electrochemical behavior of sodium azide at Pt electrode in NaOH (1 M) electrolyte was characterized in a three-electrode system (Zhou et al. 2015). All potentials were presented versus Ag/AgCl.

From the first cycle of operation, the MFC-A reactors produced consistent and reproducible currents over feeding cycles (Fig. 1) with a peak current of 0.37 mA, while the MFC-C reactors produced no current except at the beginning of each cycle. It was possibly due to residual oxygen when refilling cathode chambers. Results of this study demonstrated for the first time that azide could serve as an oxidant in cathode chamber of a BES.

Variations of major parameters at the end of each feeding cycle were presented in Table 1. As can be seen, acetate loss was detected in the MFC-A reactors, while that in the controls was inappreciable. Although anolytes and catholytes were prepared by adding equimolar amounts of acetate and azide, acetate concentration only decreased by about 18.5 % in the MFC-A reactors. This low removal efficiency should be ascribed to azide properties as well as difference of liquid volumes between anode and cathode chambers. Azide in aqueous solutions can be readily protonated even at modest pH, with great parts of N_3^- anions



converted into volatile hydrazoic acid (HN_3) (Betterton 2003).

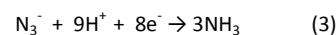
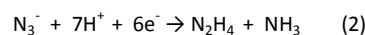
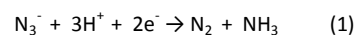
Fig.1 Current generation over feeding cycles in two-chamber aqueous cathode MFCs (1000 Ω) with catholytes added with and without azide

According to Henry's law constant, K_H , at pH 7 (3.78×10^{-5} kPa $\text{m}^3 \text{mol}^{-1}$), HN_3 is considered to be a relatively insoluble gas, implying that a great part of HN_3 will further partition to gas phase (Betterton and Robinson 1997). This may explain why there was only 3.1 ± 0.1 mM azide remaining in the prepared catholytes. As a reaction product, $\text{NH}_3\text{-N}$ (5.0 ± 0.5 mM) was significantly detected in effluents from cathode chamber of the MFC-A reactors, but not in the controls. Furthermore, a coulombic efficiency of 42.1 ± 0.8 % was obtained for the MFC-A reactors, which contrasted with that (< 30 %) of typically used single-chamber air-cathode MFCs (Cheng et al. 2006).

Table 1 Basic parameters obtained at the end of each feeding cycle

Parameter	Catholytes	Acetate	$\text{NH}_3\text{-N}$	TN loss	CE
		loss			
Unit		mM	mM	mM	%
MFC	PBS+Azide	3.70 ± 0.50	5.0 ± 0.5	3.3 ± 0.3	42.1 ± 0.8
	PBS only	0.05	-	-	

Generally, there exists only three pathways for azide reduction (Dalmia et al. 1995):



For the MFC-A reactors, the number of electrons consumed per N_3^-

over feeding cycles was calculated to be 8.3 ± 0.5 on the assumption that N_3^- anions in influents (3.1 ± 0.1 mM) were completely converted into NH_3 (Eq.(3)). Since eight electrons consumed per N_3^- is the upper limit for azide reduction, the mean value (8.3) seems implausible from the perspective of electron balance. Given the current generation from the controls at the beginning of each cycle (Fig. 1), the slight difference between the two numbers was very likely due to residual oxygen in the chamber, which consumed the extra electrons. Hence, azide reduction at the abiotic cathode was an eight-electron reaction, with ammonia as the only reduction products (Fig. 2).

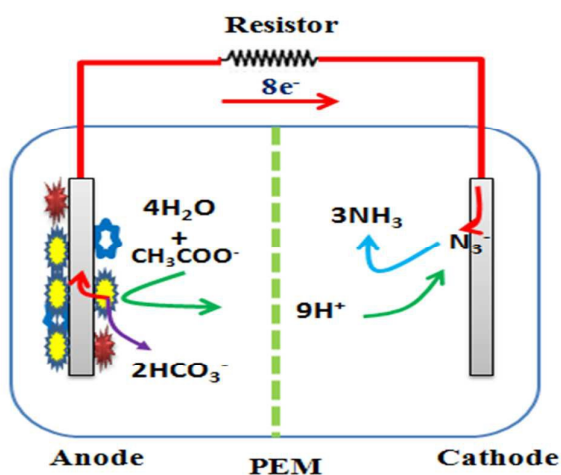


Fig.2 Schematic of azide reduction at abiotic cathode in two-chamber aqueous cathode MFCs

In our early research, azide was found to be reduced in anode chamber of a MFC, where electrochemically active bacteria reduced azide instead of transferring electrons to anode. This means that azide has a higher redox potential than anode (biofilms) (Zhou et al. 2014). In this case, the potential difference should also exist on the condition that anode biofilm and azide were present in anode and cathode chambers, making azide reduction thermodynamically possible. Obviously, the results, as noted above, support this assumption.

Given that azide reduction at abiotic cathode in a BES was a Pt-catalyzed chemical reaction, the electrochemical behavior of azide was investigated at Pt electrode in a three-electrode system. When the potential was scanned in the region of -0.8 V \sim -0.37 V, negative (or cathodic) current was readily produced, reflecting flow of electrons from electrode to azide, but positive (or anodic) current was not evident at potentials above -0.37 V (Fig. 3A). Correspondingly, the first derivative analysis of sweeps produced an evident peak at -0.53 V at which the maximum rate of azide reduction was reached (Fig. 3B). This was in good agreement with previous report that at potentials < -0.48 V azide could be reduced at Pt electrode (Dalmia et al. 1995). For the two-chamber aqueous cathode MFCs, the normal potential of the anode stabilized at about -0.52 V under open circuit mode, and about -0.47 V under closed circuit mode (1000Ω). Like the electrochemical system, the contact between both electrodes in two-chamber aqueous cathode

MFCs made the anode potential developed as an applied potential to the cathode, allowing for azide reduction thermodynamically favorable.

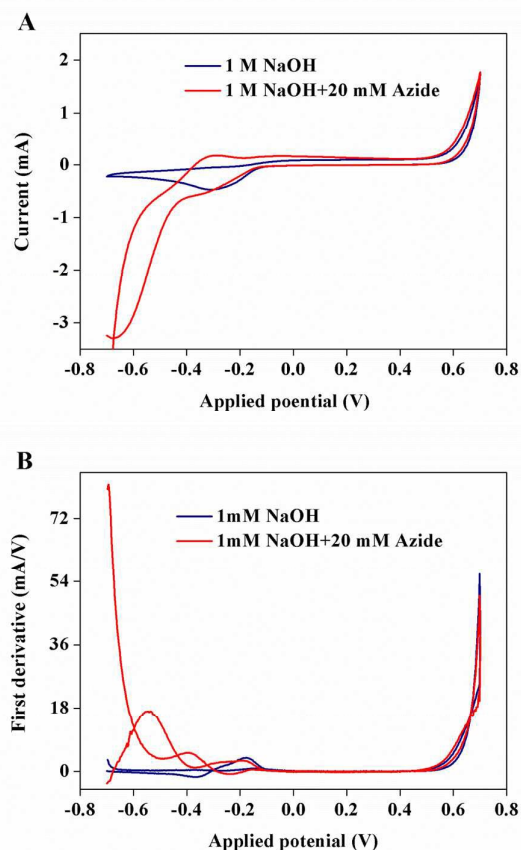


Fig.3 Cyclic voltammograms (A) and first derivative cyclic voltammograms of azide (B) on Pt electrode in NaOH electrolyte.

Reproducible current generation was observed with a peak value of 0.37 mA in azide containing reactors, while there was almost no current generation in azide free reactors. $\text{NH}_3\text{-N}$ was significantly detected in effluents of the MFC-A reactors, but not in the MFC-C reactors. The number of electrons consumed per N_3^- (8.3 , mean value) was very close to the number of 8 , at which NH_3 is assumed to be the only reduction product. The bacteria-coated anode in BESs offered the favorable redox potential allowing for azide reduction by eight electrons at an abiotic cathode with NH_3 as the only reduction product.

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