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ARTICLE TYPE

Coupling interaction of cathodic reduction and microbial metabolism in aerobic biocathode of microbial fuel cell

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Certain mixed consortia colonized on the aerobic biocathode can improve 4-electron oxygen reduction of cathode, yet the coupling interaction of cathodic reaction and microbial metabolism remains unclear. To better understand the above-mentioned interaction evolved in cathode process, biocathodes were enriched using nitrifying sludge and operated at various NH_4Cl and $NaHCO_3$ concentrations in both open and closed external circuit conditions. Based on the variation of nitrification and cathodic oxygen reduction activity, it was shown that oxygen reduction process, to some extent, relied on the nitrification activity of biocathode; the external electrons from cathode in turn might benefit the nitrifying bacteria selected in MFC habitat by entering the electron transfer chains as energy source. Nitrifiers, including *Nitrosomonas* sp., *Nitrospira* sp. and *Nitrobacter* sp. were detected in all biocathodes that cultured in different conditions, even that were cultured without NH_4Cl in the medium. These findings provided valuable insights into possible working mechanism of biocathode.

Introduction

Microbial fuel cells (MFC) are promising devices that can directly harvest electricity from organic pollutants in wastewater.[1, 2] In the anode, electrochemical active bacteria take up organic substrates, gain energy for growth and maintenance and transfer electrons to anode electrode which travel to cathode through external circuit; in cathode, electrons are consumed by electron acceptors, such as oxygen for most cases, to complete electricity production. Platinum catalyst is widely applied to reduce the overpotential of cathode reactions, however suffers from high cost and short service life due to loss, agglomeration and poisoning. The advent of biocathodes has shed light on the lower-cost, efficient and sustainable construction and operation of MFCs. The electron acceptors of biocathode are also extended to a variety of substrates, e.g. oxygen[3], nitrate[4], sulfate[5], tetrachloroethene[6], CO_2[7], Cr (VI)[8], U (VI)[9] and dyes[10, 11] in wastewater.

Biocathode has attracted much attention for its superiorities, yet the working mechanism study still remains a big challenge for researchers. The working process of biocathode is complex combination of cathode reaction and metabolism and multiplication of bacteria. It is widely accepted that extracellular electrons are evolved in microbial metabolism of biocathode via direct or indirect electron transfer pathways.[12-14] Although the electrochemical active bacteria of biocathode can be selected in natural environment, the metabolism activity of bacteria that is stimulated by extracellular electrons in MFC habitat should be different from that in natural circumstance. Ferrous oxidizing bacteria (FeOB) have been utilized in biocathode. Originally, Fe (III) was considered as the only electron acceptors in cathode, while the role of FeOB was to recycle Fe (II) in the catholyte which was reduced to Fe (III) by external electrons.[15-18] Later, Carbajosa et al. found that *Acidithiobacillus ferrooxidans* exhibited redox characteristic without Fe (II) in the solution and could grow using external electrons as sole energy source.[19, 20] The report indicated that extracellular electrons may influence and support microbial activity and inspired us to further explore the working mechanism evolved in biocathode.

$$4H^+ + O_2 + 4e^- \xrightarrow{\text{nitrifying bacteria}} 2H_2O \quad (1)$$

$$2NH_4^+ + 4O_2 \xrightarrow{\text{nitrifying bacteria}} 2NO_3^- + 2H_2O + 4H^+ \quad (2)$$

We carried out the research taking nitrifying biocathode as example. In the nitrifying biocathode process, two main reactions occur during the microbial metabolism process simultaneously: cathodic reaction (Eq. 1) and nitrification (Eq. 2). Our aim was to explore the complex interaction between the two reactions in biocathode process. We firstly enriched biocathode using nitrifying sludge and operated the biocathode in different $NaHCO_3$ and NH_4Cl concentrations in open or closed circuit conditions, aiming to evaluate the variations of oxygen reduction and nitrification activities in the biocathode process. The nitrate formation kinetics was used to evaluate the nitrification activity of biocathode, while the voltage and power output of MFC was used to represent the activity of cathodic reaction. The microbial communities under different culture condition were also evaluated, providing further understanding of biocathode working mechanism.

Materials and Methods

Process setup and operation

Two-chambered reactors were constructed. Both anode and cathode chambers were cylinder rooms of the same sizes (4 cm long by 3 cm in diameter, 28 ml liquid volume). Carbon fibre brushes, heat treated in a muffle furnace at 450 ºC for 30 min, were used as both anode and cathode electrode.[21] The homogeneous anion exchange membrane (Qianqiu Co Ltd, China) was used to separate anode and cathode chamber.

The anode chambers were inoculated with the effluent of glucose-fed MFCs and the details were described previously.[22] The cathode chambers were inoculated with municipal wastewater (50%, v/v) collected on HIT campus, and the medium contained $NaHCO_3$ (1.0 g/L) and NH_4Cl (0.3 g/L) during the startup period. Phosphate buffered solution (KCl 0.13 g/L, $NaH_2PO_4 \cdot 2H_2O$ 3.32 g/L and $Na_2HPO_4 \cdot 12H_2O$ 10.32 g/L), tracer minerals and vitamins were added in the medium solution as described previously.[23] The anode was sealed with rubber plug to keep anaerobic circumstance except during the replacement of anolyte, while the cathode was continuously aerated with a pump during the experiment (DO 5.0±0.3 mg/L). The anode and cathode chambers were refilled with inoculation medium simultaneously every 24 h until a stable voltage was achieved. The external resistance was kept at 1000 Ω except where otherwise noted. All tests were conducted at a constant temperature of 30 ºC. To avoid the growth of algae and photosynthetic bacteria, the MFC reactors were wrapped with aluminium foil.

After successful enrichment, the anodes of all MFCs were operated with the same substrate (glucose 1.0 g/L and NH_4Cl 0.1 g/L), while the cathodes were operated under different culture conditions to investigate the performance of nitrification and oxygen reduction process. The cathode substrate for the first group (MFC-1) was composed of 0.3 g/L NH_4Cl and $NaHCO_3$ of different concentrations (1.0, 0.5 and 0 g/L). The second group (MFC-2) were cultured with fixed concentration of $NaHCO_3$ (1.0 g/L) but different NH_4Cl concentrations (0.3, 0.1 and 0 g/L), and the 3rd group (MFC-3) were operated with fixed substrate containing $NaHCO_3$ 1.0 g/L and NH_4Cl 0.3 g/L but with external circuit connected to 1000 Ω or open (Table 1). All the experiment was duplicated for repeatability.

Table 1. Culture conditions of biocathode MFC.

	$NaHCO_3$ concentration, g/L			NH_4Cl concentration, g/L			External circuit	
MFC-1	1.0	0.5	0	0.3			1000 Ω	
MFC-2	1.0			0.3	0.1	0	1000 Ω	
MFC-3	1.0			0.3			1000 Ω	Open-circuit

Calculations and analysis

The cell voltages (V) were recorded with a data acquisition board every 30 minutes. Power density, polarization curves, coulombic efficiency (CE) and total electric quantity (Q) were obtained as described previously.[24]

Samples obtained from the cathode and anode compartments were immediately centrifuged (10,000×g) to remove biomass. Chemical oxygen demand (COD), NH_4^+-N, NO_2^--N and NO_3^--N were measured according to the standard methods of American Public Health Association.[25] Total nitrogen (TN) was calculated as the sum of NH_4^+-N, NO_2^--N and NO_3-N.

Microbial community analysis

Biofilm samples of biocathodes were collected by removing a small amount of carbon brush fibre with sterile scissors from cathode materials that were operated stably at different culture conditions. Sample S1 was collected from MFC-1 fed with $NaHCO_3$ 1.0 g/L and NH_4Cl 0.3 g/L in closed circuit condition for ca. 15 cycles. Sample S2 was collected from MFC-2 fed with $NaHCO_3$ 1.0 g/L but no NH_4Cl in closed circuit condition after ca. 15 cycles. Sample S3 was collected from MFC-3 fed with $NaHCO_3$ 1.0 g/L and NH_4Cl 0.3 g/L in open circuit condition after ca. 21 cycles. The DNA extraction and PCR amplification, purification and clone were carried out as previously described.[26] 16S rRNA gene sequences were analyzed at National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/blast.cgi). A sequence similarity >97% was defined as an operational taxonomic unit (OTU).

Data deposition

All sequences in this study have been deposited in the NCBI database. 16S rRNA gene sequences were deposited in GenBank with accession numbers KJ627743~ KJ627767.

Results

Interactions of nitrification and O_2 reduction reaction depending on $NaHCO_3$ concentrations

Nitrifying bacteria and the most biocathode bacteria is autotrophic microorganism, utilizing inorganic carbon, such as HCO_3^-, CO_3^{2-} and CO_2, as carbon source for growth.[27] The results demonstrated that both nitrification and cathodic activity decreased as the initial $NaHCO_3$ concentrations in cathode chamber declined from 1.0 g/L to 0 g/L (Fig.1). The average nitrate formation rates were ca. 6.08, 4.91 and 3.02 mg/(L·h) and maximum power output were 1191, 1070 and 1013 mW/m^2 for initial $NaHCO_3$ concentrations 1.0, 0.5 and 0 g/L, respectively. The polarization curves showed that the effect of $NaHCO_3$ concentrations was more obvious at current density higher than 2.0 A/m^2. The maximal current density (3.988 A/m^2) was obtained with in bicarbonate concentration 1.0 g/L and followed by 0.5 g/L (2.898 A/m^2) and 0 g/L (2.735 A/m^2). Since the anode potential showed slightly difference with variation of $NaHCO_3$ concentrations, the difference in power generation was mainly caused by cathode performance (Fig. 1c).

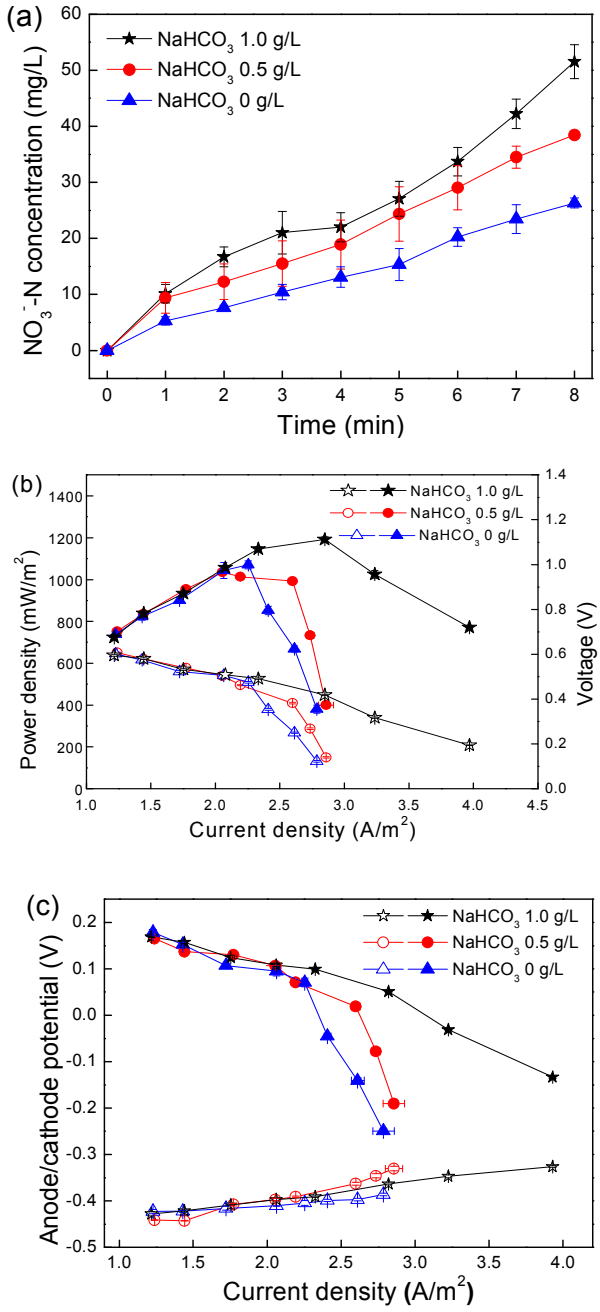

Fig. 1. (a) Nitrate concentrations in the cathode chamber during the initial 8 hrs of a cycle; (b) power density (filled symbol) and polarization (open symbol) curves; (c) anode potential (open symbol) and cathode potential (filled symbol) (vs Ag/AgCl) of nitrifying biocathode MFC cultured with different bicarbonate concentrations.

Interactions of nitrification and O_2 reduction reaction influenced by NH_4Cl concentrations

Nitrifiers, including ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), obtain energy for growth from the oxidation of inorganic nitrogen, such as ammonia and nitrite. The amounts of ammonia in the medium were thus certain to affect the activity and quantity of nitrifiers in the cathode. In this work, cathode substrate was composed of different NH_4Cl concentrations (0.3, 0.1 and 0 g/L) with fixed concentration of $NaHCO_3$ (1.0 g/L). The power generation decreased with the decline of the initial NH_4Cl concentrations (Fig. 2a). As the NH_4Cl concentration was 0.3 g/L, the MFC delivered maximal power output 1098 mW/m^2 and current density 3.26 A/m^2. As the NH_4Cl concentration dropped to 0.1 g/L, the power generation declined to 989 mW/m^2. When no NH_4Cl was supplied to cathode microorganism, the maximal power generation was 797 mW/m^2. The variation in power generation was mainly caused by cathode performance, which was confirmed by anode and cathode potential during the polarization test (Fig. 2b).

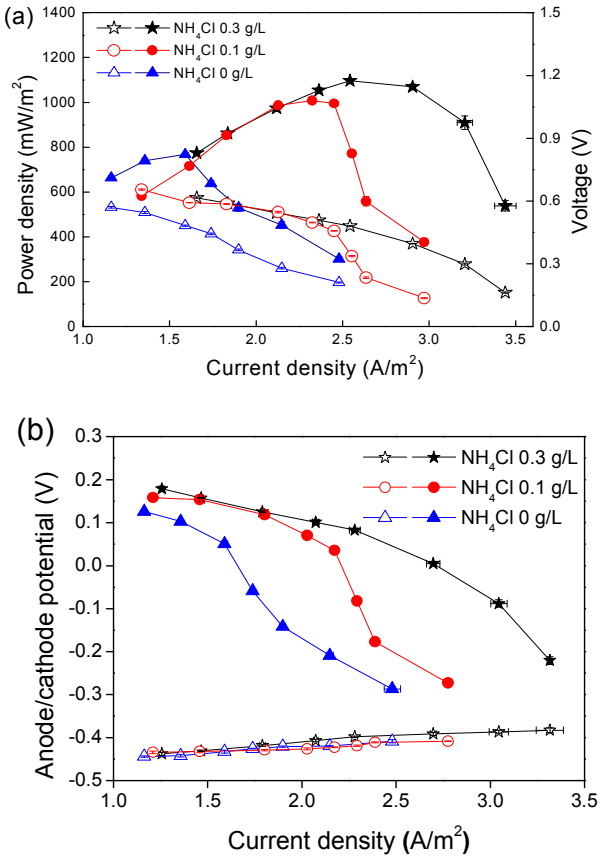

Fig. 2. (a) Power density (filled symbol) and polarization (open symbol) curves; (b) anode potential (open symbol) and cathode potential (filled symbol) (vs Ag/AgCl) of nitrifying biocathode MFC cultured with different NH4Cl concentrations.

Nitrification activity influenced by cathodic reaction

As nitrification activity greatly affected the cathode catalysis activity, to address the influence of cathodic reaction in turn towards nitrification activity, nitrate formation kinetics was studied with the external circuit of biocathode MFC connected to 1000 Ω or disconnected (Fig. 3). As the external circuit connected to 1000 Ω, the nitrate concentration in the early 8 hrs of a cycle was 25.7 mg/L and the nitrification rate 2.838 mg/(L·h), respectively. As the external was turned to open, the nitrification dynamics showed two different tendencies: first, slightly declined to 1.614 mg/(L·h) in the first 7 cycles and increased dramatically to 5.322 mg/(L·h) after circuit open for more than 21 cycles.

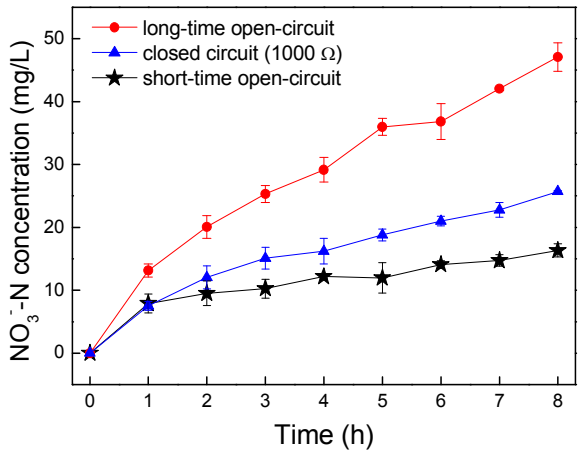

Fig. 3. Nitrate formation dynamics during the initial 8 h of a cycle with external circuit connected to 1000 Ω or open (★ for MFC with circuit connected to 1000 Ω; ● for MFC with circuit open for less than 7 cycles; ▲ for MFC with circuit open for more than 21 cycles).

Analysis of microbial community

To investigate the phylogenetic differentiation depending on NH_4Cl as energy source for nitrifiers and external circuit conditions, the biofilm attached on the biocathode were analyzed by gene libraries. For S1, S2 and S3, the positive clones were 99, 99 and 98, grouped into operating taxonomic units (OTUs) 17, 29 and 18, respectively, on the basis of more than 97% sequence similarity within an OTU. Coverage for the samples are 90.8%, 83.8% and 87.8%, respectively, indicating that the three clone libraries could reflect the microbial community composition in the samples (Fig. 4).

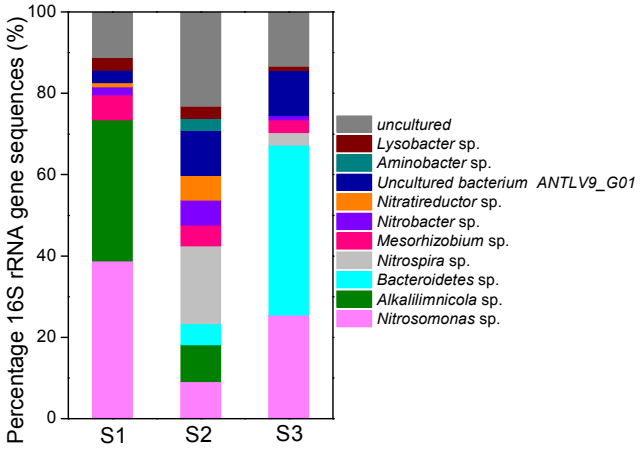

Fig. 4. Bacterial community composition and relative abundance of nitrifying biocathodes determined from 16S rRNA gene libraries (S1 for the biocathode cultured with NH4Cl in closed circuit condition; S2 for the biocathode cultured without NH4Cl in closed circuit condition and S3 for the biocathode cultured with NH4Cl in open circuit condition).

The most abundant sequence in the three samples all belonged to *Proteobacteria* which was found colonized on both anode and cathode electrodes and generally called "*exoelectrogens*".[14, 28] The ratios of *Proteobacteria* were S1 89.8%, S2 44.4% and S3 34.7%, respectively. The nitrifiers, including *Nitrosomonas* sp., *Nitrospira* sp. and *Nitrobacter* sp., were detected in the three samples. The ratios of total nitrifiers were S1 40.8%, S2 34.3% and S3 29.6%. The highest abundance of *Nitrosomonas* sp. was 38.8% (S1), followed by 25.5% (S3) and 9.1% (S2). Nitrite-oxidizing bacteria (NOB), including *Nitrospira* sp. and *Nitrobacter* sp. accounted for a small fraction (S1 2.0% and S3 4.1%); S2 that had the lowest abundance of AOB (9.1%) showed highest ratio of NOB (25.3%). Even though the abundance of NOB was much lower than that of AOB in S1 and S3, accumulation of NO_2^- was not observed in catholyte. At the end of each cycle, ca. 99% of ammonia was converted to nitrate. This was probably because ammonia oxidation was often the rate-limiting step of the total nitrification process and nitrite rarely accumulated in most environments.[29] *Alkalilimnicola* sp. was also detected in S1 (34.7%) and S2 (9.1%), but not detected in S3.

Discussion

Cathodic reaction is promoted by electrochemical active bacteria in biocathode where metabolism and multiplication of microorganism occurs simultaneously. The competitive and synergetic relations exist between the two reactions. Take nitrifying biocathode for example, there is competition for oxygen which is essential for both nitrification and 4-electron oxygen reduction of cathode;[30, 31] proton consumption by cathodic reaction (Eq.1) and proton reproduction by biological nitrification (Eq.2) will benefit each other by maintaining pH balance in cathode chamber.[32] In our work, the interactions of cathodic reaction and nitrification were carried out in terms of substrate concentration and external circuit conditions. Nitrifying bacteria are affiliated with chemoautotrophic microorganisms[33, 34], thus availability of inorganic carbon source and inorganic energy source affects the metabolism and multiplication of bacteria.[35] In our work, the variations of MFC performance and nitrification rate towards substrate changes demonstrated that the cathodic activity was strongly correlated to the microbial activity of bacteria. With higher nitrification activity, the cathodic reaction rate was also faster.

Besides, cathodic reaction is not passively relied on the microbial activity of nitrifying bacteria, but influencing the nitrification activity by entering the electron transfer chains of bacteria[36, 37] Evidence in our work was the differences of nitrification rate with the external circuit closed and open (Fig. 3). With the external circuit changing from close to open, the rapid increase of nitrification activity was expected to happen, since the oxygen competition from cathodic reaction was terminated. However, the obvious decrease of nitrification rate was observed in the early stage of open-circuit condition. One possible explanation was that external electrons may benefit the microorganisms as energy source by enter the electron transport chains and once the electron transfer was cut off, the activity of nitrifying bacteria declined. Liang et al. also reported accelerated reduction of chlorinated nitroaromatic antibiotic chloramphenicol (CAP) by biocathode bioelectrochemical system (BES) connected to external circuit compared with that in open circuit condition.[38] The dramatic increase of nitrification rate after long-term open-circuit condition in our work may be due to the microbial community changes caused by external circuit; the nitrifying bacteria that were suppressed in MFC condition multiplied quickly once the external circuit turned to open and caused the

dramatic increase in nitrification rate.

Microbial community study offered another deep insight into working mechanism of biocathodes. *Proteobacteria* are considered possible bacteria responsible for electron transfer in biocathode.[14, 28] The voltage and power output was correlated with the abundances of *Proteobacteria* in S1, S2 and S3. The highest voltage output was achieved with the same culture condition as S1, followed by S2 and the MFC operated in open circuit condition as S3 showed lowest voltage output (Fig. S1, †ESI and Fig. 2b).

Bacteria of biocathode can be selected in natural environment, but the living habitat in MFC is quite different. Nitrifying bacteria obtain energy from oxidation of ammonia and nitrite in natural environment, however, in MFC habitat the abundance of nitrifying bacteria were influenced by both substrate concentration and external electron transfer. The nitrifying bacteria were detected in S2 which was cultured without NH_4Cl in closed circuit condition for more than 15 cycles. Nitrifying bacteria in S2 accounted for 34.3%, even higher than S3 (29.6%) that cultured with NH_4Cl in open circuit condition in case of other parallel conditions, such as $NaHCO_3$, DO and pH. The results suggested that nitrifying bacteria may survive using external electrons as energy source in MFC habitat, except for the energy from ammonia and nitrite oxidation. This hypothesis was also supported by changes of the nitrification rate in close and open circuit conditions (Fig. 3). Carbajosa et al also reported growth of the acidophile bacterium *At. ferrooxidans* directly on a graphite electrode using an applied voltage of 0V as the only energy source, without adding redox mediators to the solution.[19] Sorokin et al reported versatile metabolism of *Alkalilimnicola* sp. with nitrate as electron acceptors and H_2 or polysulfide as electron donors.[39] But in our work, no organic or inorganic matters that *Alkalilimnicola* sp. could utilize existed in the cathode. We assumed that *Alkalilimnicola* sp. might be also responsible for cathodic reaction and utilize the electrons from cathode as energy source. This may explain why *Alkalilimnicola* sp. disappeared in S3 when external circuit was cut off. Isolation and electrochemical studies about *Alkalilimnicola* sp.are ongoing in our lab.

For nitrifying biocathode, the activity of oxygen reduction reaction was, to some content, dependent on the nitrification activity: the activity of oxygen-reducing reaction increased as the nitrification activity went up. The external electrons derived from cathode in turn may benefit the nitrifying bacteria selected in MFC habitat by entering the electron transport chain as another energy source besides the oxidation of ammonia and nitrite. Microbial community analysis confirmed the hypothesis about interaction of nitrification and cathodic reaction in biocathode.

Conclusions

For nitrifying biocathode, the activity of oxygen reduction reaction was, to some content, dependent on the nitrification activity: the activity of oxygen reduction increased as the nitrification activity went up. The external electrons derived from cathode in turn may benefit the nitrifying bacteria selected in MFC habitat by entering the electron transport chain as another energy source besides the oxidation of ammonia and nitrite. Microbial community analysis indicated that the nitrifiers might be able to catalyze the oxygen reduction and utilize the external electrons as energy source.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Fig. S1. Voltage generation of nitrifying MFC before open circuit experiment and after open circuit for 21 days.]. See DOI: 10.1039/b000000x/

1. Y. J. Feng, X. Wang, S. A. Cheng, M. D. Merrill, T. Saito and B. E. Logan, *Environ Sci Technol*, 2009, **43**, 6870-6874.
2. J. A. Sun, Z. Bi, B. Hou, Y. Q. Cao and Y. Y. Hu, *Water Res*, 2011, **45**, 283-291.
3. A. Ter Heijne, O. Schaetzle, S. Gimenez, F. Fabregat-Santiago, J. Bisquert, D. P. B. T. B. Strik, F. Barriere, C. J. N. Buisman and H. V. M. Hamelers, *Energ Environ Sci*, 2011, **4**, 5035-5043.
4. K. Y. Cheng, M. P. Ginige and A. H. Kaksonen, *Environ Sci Technol*, 2012, **46**, 10372-10378.
5. L. Pons, M. L. Delia and A. Bergel, *Bioresour Technol*, 2011, **102**, 2678-2683.
6. F. Aulenta, P. Reale, A. Canosa, S. Rossetti, S. Panero and M. Majone, *Biosens Bioelectron*, 2010, **25**, 1796-1802.
7. X. X. Cao, X. Huang, P. Liang, N. Boon, M. Z. Fan, L. Zhang and X. Y. Zhang, *Energ Environ Sci*, 2009, **2**, 498-501.
8. L. P. Huang, X. L. Chai, G. H. Chen and B. E. Logan, *Environ Sci Technol*, 2011, **45**, 5025-5031.
9. K. B. Gregory and D. R. Lovley, *Environ Sci Technol*, 2005, **39**, 8943-8947.
10. S. Kalathil, J. Lee and M. H. Cho, *New Biotechnol*, 2011, **29**, 32-37.
11. S. Kalathil, J. Lee and M. H. Cho, *Bioresour Technol*, 2012, **119**, 22-27.
12. M. Rosenbaum, F. Aulenta, M. Villano and L. T. Angenent, *Bioresource Technology*, 2011, **102**, 324-333.
13. L. Huang, J. M. Regan and X. Quan, *Bioresour Technol*, 2011, **102**, 316-323.
14. D. R. Lovley, *Curr Opin Biotech*, 2008, **19**, 564-571.
15. Y. Mao, L. Zhang, D. Li, H. Shi, Y. Liu and L. Cai, *Electrochim Acta*, 2010, **55**, 7804-7808.

16. A. Ter Heijne, H. V. M. Hamelers and C. J. N. Buisman, *Environ Sci Technol*, 2007, **41**, 4130-4134.
17. A. Lopez-Lopez, E. Exposito, J. Anton, F. Rodriguez-Valera and A. Aldaz, *Biotechnol Bioeng*, 1999, **63**, 79-86.
18. Z. Lewandowski, A. Rhoads and H. Beyenal, *Environ Sci Technol*, 2005, **39**, 4666-4671.
19. S. Carbajosa, M. Malki, R. Caillard, M. F. Lopez, F. J. Palomares, J. A. Martin-Gago, N. Rodriguez, R. Amils, V. M. Fernandez and A. L. De Lacey, *Biosens Bioelectron*, 2010, **26**, 877-880.
20. J. C. Thrash and J. D. Coates, *Environ Sci Technol*, 2008, **42**, 3921-3931.
21. Y. Feng, Q. Yang, X. Wang and B. E. Logan, *J Power Sources*, 2010, **195**, 1841-1844.
22. X. Wang, Y. Feng, J. Liu, H. Lee, C. Li, N. Li and N. Ren, *Biosens Bioelectron*, 2010, **25**, 2639-2643.
23. D. R. Lovley and E. J. P. Phillips, *Appl Environ Microbiol*, 1988, **54**, 1472-1480.
24. B. E. Logan, S. Cheng and H. Liu, *Environ Sci Technol*, 2006, **40**, 2426-2432.
25. APHA, *Washington DC, USA.*, 1999.
26. Y. Qu, Y. Feng, J. Liu, W. He, X. Shi, Q. Yang, J. Lv and B. E. Logan, *Desalination*, 2013, **317**, 17-22.
27. X. Xia, Y. Sun, P. Liang and X. Huang, *Bioresour Technol*, 2012, **120**, 26-33.
28. T. P. Sciarria, A. Tenca, A. D'Epifanio, B. Mecheri, G. Merlino, M. Barbato, S. Borin, S. Licoccia, V. Garavaglia and F. Adani, *Bioresour Technol*, 2013, **147**, 246-253.
29. K. Isobe, K. Koba, S. Otsuka and K. Senoo, *J Forest Res*, 2011, **16**, 351-362.
30. S. Puig, M. Serra, A. Vilar-Sanz, M. Cabre, L. Baneras, J. Colprim and M. Dolors Balaguer, *Bioresour Technol*, 2011, **102**, 4462-4467.
31. J. Keller, B. Virdis, K. Rabaey and Z. Yuan, *Water Res*, 2008, **42**, 3013-3024.
32. S. J. You, N. Q. Ren, Q. L. Zhao, P. D. Kiely, J. Y. Wang, F. L. Yang, L. Fu and L. Peng, *Biosens Bioelectron*, 2009, **24**, 3698-3701.
33. A. E. Ingalls, S. R. Shah, R. L. Hansman, L. I. Aluwihare, G. M. Santos, E. R. M. Druffel and A. Pearson, *P Natl Acad Sci USA*, 2006, **103**, 6442-6447.
34. S. M. Strycharz-Glaven, R. H. Glaven, Z. Wang, J. Zhou, G. J. Vora and L. M. Tender, *Appl Environ Microbiol*, 2013, **79**, 3933-3942.
35. H.-T. Tran, D.-H. Kim, S.-J. Oh, K. Rasool, D.-H. Park, R.-H. Zhang and D.-H. Ahn, *Water Sci Technol*, 2009, **59**, 1803-1808.
36. S. Freguia, S. Tsujimura and K. Kano, *Electrochim Acta*, 2010, **55**, 813-818.
37. L. T. Angenent and Z. He, *Electroanal*, 2006, **18**, 2009-2015.
38. B. Liang, H.-Y. Cheng, D.-Y. Kong, S.-H. Gao, F. Sun, D. Cui, F.-Y. Kong, A.-J. Zhou, W.-Z. Liu, N.-Q. Ren, W.-M. Wu, A.-J. Wang and D.-J. Lee, *Environ Sci Technol*, 2013, **47**, 5353-5361.
39. D. Y. Sorokin, T. N. Zhilina, A. M. Lysenko, T. P. Tourova and E. M. Spiridonova, *Extremophiles*, 2006, **10**, 213-220.