Chapter 11
The Role of Denitrifying Bacteria Within the Bioelectrochemical System for Nitrate-Containing Wastewater Treatment

Xiaojun Jin and Hong Liu

Abstract Bioelectrochemical systems (BESs) with the coexistence of denitrifiers and electricigens were generally observed for simultaneous nitrogen removal and electricity production. As the increasing of nitrate, the percentage of denitrifiers increased and the percentage of electricigens relatively decreased until it lost its dominant position. In denitrifying BES, anodic heterotrophic denitrification could improve organics removal and energy recovery efficiency during the treatment of nitrate-containing wastewater. In this chapter, the developments of denitrifying BES as well as the evolution of the microbial community were comprehensively introduced. Furthermore, a special type of bacteria, denitrifying electricigens, was also introduced and utilized in BES for the treatment of nitrate-contaminated waters.

11.1 Introduction

Nitrogen pollution has become an increasing problem in the environment, especially the excessive emission of nitrate. Nitrate is generally found in the effluent of the aerobic ammonium oxidation process during the wastewater treatment. The discharge of excessive nitrate poses a growing threat to public health around the world, especially causes water eutrophication in the environment (Manassaram et al. 2006). Biological treatment seems a less costly technology for nitrogen removal in terms of operation and maintenance costs, comparing to the physical and chemical processes in traditional methods (Butz and Jackson 1977).

During a complete denitrification process, liquid nitrate is firstly reduced to liquid nitrite by the nitrate reductase. Next, nitrite was gradually reduced to gaseous nitric oxide, gaseous nitrous oxide, and nitrogen gas by the enzyme catalyst of nitrite

---

X. Jin · H. Liu (✉)
Chongqing Institutes of Green and Intelligent Technology (CIGIT) of Chinese Academy of Sciences (CAS), Chongqing, China
e-mail: jinxiaojun@cigit.ac.cn; liuhong@cigit.ac.cn

P. Kumar, C. Kuppam (eds.), *Bioelectrochemical Systems*,
https://doi.org/10.1007/978-981-15-6868-8_11

Table 11.1 The distribution of the denitrifying genera in taxonomy

Archaea	*Haloarcula*, *Halobacterium*, *Haloferax*, *Ferrogiobus*, *Pyrobaculum*	
Bacteria	Not Proteobacter	*Gram-positive*: *Bacillus*, *Corynebacterium*, *Prankia*, *Dactylosporangium*, *Dermatophilus*, *Gemella*, *Listeria*, *Kineasporia*, *Micromonospora*, *Microtetraspora*, *Nocardia*, *Pilimelia*, *Propionibacterium*, *Saccharomonospora*, *Saccharothrix*, *Spirrilospora*, *Streptomyces*, *Streptoverticillium*
		Gram-negative: *Aquifex*, *Flexibacter*, *Empedobacter*, *Flavobacterium*, *Sphingobacterium*, *Synechocystis* sp. PCCC 6803
	Proteobacter	*α-proteobacteria*: *Agrobacterium*, *Aquaspirillum*, *Azospirillum*, *Blastobacter*, *Bradyrhizobium*, *Gluconobacter*, *Hyphomicrobium*, *Magnetospirillum*, *Nitrobacter*, *Paracoccus*, *Pseudomonas*, *Rhizobium*, *Rhodobacter*, *Rhodoplanes*, *Rhodopseudomonas*, *Roseobacter*, *Sinorhizobium*, *Thiobacillus*
		β-proteobacteria: *Achromobacter*, *Acidovorax*, *Alcaligenes*, *Azoarcus*, *Brachymonas*, *Burkholderia*, *Chromobacterium*, *Comabacter*, *Eikenella*, *Hydrogenophaga*, *Janthinobacterium*, *Kingella*, *Microcirgula*, *Neisseria*, *Nitrosomonas*, *Ochrobactrum*, *Oligella*, *Ralstonia*, *Rubrivivax*, *Thauera*, *Thermothrix*, *Thiobacillus*, *Vogesella*, *Zoogloea*
		γ-proteobacteria: *Acinetobacter*, *Alteromonas*, *Azomonas*, *Beggiatoa*, *Deleya*, *Halomonas*, *Marinobacter*, *Moraxella*, *Pseudoalteromonas*, *Pseudomonas*, *Rugamonas*, *Shewanella*, *Thioploca*, *Thiomargarita*, *Xanthomonas*
		ε-proteobacteria: *Wolinella*, *Campylobacter*, *Thiomicrospiro*

reductase, nitric oxide reductase, and nitrous oxide reductase, respectively (Knowles 1982). And these genes of oxidoreductases are correspondingly encoded by the narGHI, nirK or nirS, norBC, and nosZ, respectively. The genes of these enzymes were also used as molecular markers for the cultivation-independent analysis of denitrifying bacteria in the environment. Denitrifying bacteria, as the carriers of denitrifying genes, are widely appeared at the natural or contaminated environment. These microorganisms are taxonomically and biochemically very diverse (Table 11.1). Most are heterotrophic bacteria, and even some utilize one-carbon compounds, whereas others can spontaneously grow on hydrogen and carbon dioxide or reduced sulfur compounds (Hwang et al. 2009). One group is photosynthetic (Kim et al. 1999). Most of them possess the complete reductases for reducing nitrate to nitrogen gas. But some are termed nitrite dependent because there is no nitrate reductase or nitrous oxide reductase in cells. Sometimes microorganisms cannot produce nitrous oxide from nitrate or nitrite, though they possess nitrous oxide reductase (Knowles 1982). Therefore, nitrate removal is closely related to the microbial characterization of both biofilm and activated sludge in bioreactors.

Biological denitrification includes autotrophic denitrification and heterotrophic denitrification. The former is generally suitable for polluted groundwater treatment, due to the carbon source does not need to be externally added. However, the removal efficiency of nitrogen is commonly limited. The latter seems to have higher efficiency with the wastewater treatment than the former. But, to increase the removal efficiency of heterotrophic denitrification, sufficient organic carbon matter is required, which produces a large amount of excess sludge. Electrochemical technique (Bioelectrochemical system, BES) has been considered an alternative strategy because of the lack of additional chemical reagent, active sludge decrement, and high efficiency (Park et al. 2005; Chandrasekhar and Ahn 2017). Besides the nitrate reduction at anode, biocathode inoculated with either autotrophic or heterotrophic denitrifiers could also be adopted for nitrate removal in BES. Many researchers have reported the microbial communities of BES for denitrification. However, further analysis and comparison between the denitrifying bacteria in BES and bioreactor have been not investigated. This chapter aimed to review the microbial communities of BES for nitrate removal, and the electricity performance was also conjointly analyzed.

11.2 Main Text

Recent progress in wastewater treatment has led to the development of BES which uses microorganisms capable of electrochemically active and extracellular electron transfer, that facilitates the electron transfer to the anode where oxidation of pollutants (Debabov 2008; Chandrasekhar et al. 2014a). BES contains microbial electrolysis cell (MEC) and microbial fuel cell (MFC). Both of them use microorganisms as a biological catalyst on the electrode. MEC also needs to connect a counter electrode and an external power source (Abudukeremu et al. 2015a, b, 2016b). MEC seems a sustainable and energy-saving technology for H_2 generation and contaminant degradation. MFC is generally used to treat wastewater as well as harvest energy. Recent progress has led to the rapid development of BES for the treatment of various waste waters (Animesh et al. 2016; Rijuta et al. 2017). Furthermore, substantial advancement has been made in enhancing BES as a potential technology towards industrial applications for wastewater treatment (Ghafari et al. 2008). Combination of electrochemical method along with biological denitrification accelerates the denitrification process, and simultaneous declines the cost (Chandrasekhar et al. 2015). To date, it has received an increasing attention in denitrifying BESs.

Since nitrate can be reduced to nitrogen, it can be used as a potential electron acceptor at the cathode in BESs. Thus, using BES for nitrate-contained wastewater treatment can achieve simultaneous electricity generation and nitrate removal. Comparing to the activated sludge from the bioreactor and BES with different cathodes, the overall 8–97% higher nitrate removal rate could be obtained in BES with the bioelectrode (Animesh et al. 2016). The denitrifying MFC using biocathode showed high efficient nitrate removal and current density (Jin et al. 2018).

To date, the enhancing mechanism is still unclear, and which seriously block the development of the denitrifying BES. According to the previous researches, three assumptions about the enhancement of electrochemical denitrification were proposed, containing micro-surrounding pathway, H_2 pathway, and directly electron pathway. The first two pathways were proposed basing on the traditional mechanism of biological denitrification. As known to all, most denitrifying microorganisms are anaerobic or facultative bacteria. In the cathode of BES system, ORR consumes oxygen and thus contributes to the anaerobic microenvironment for biologically denitrification. As known, denitrifiers are common facultative anaerobic or strictly anaerobic bacteria. However, even oxygen exposes on the headspace, most denitrifiers can still reduce nitrate once in a while. The H_2 pathway is based on the speculation of H_2 could be generated through cathode, and next utilized by autotrophic denitrifiers as an electron donor in BESs (Abudukeremu et al. 2017, 2016b; Gopalakrishnan et al. 2017). The third directly electron pathway means the electrons from cathode are directly transferred to the denitrifying bacteria in the cathode chamber. The development of extracellular electron transfer (EET), especially the direct electron transfer (DET), greatly promoted the attention of the directly electron pathway. Currently, more and more studies about the DET had elaborated the pathway of electron transfer.

The recent development of biocathode MFC can provide final alternative electron acceptor (nitrate instand of oxygen) and it can also produce bioelectricity generation. Clauwaert et al. (2007) first realized simultaneous organics oxidation by biological anodic and nitrate reduction by denitrifiers on the cathode of a two-chambered MFC without external power supply (Clauwaert et al. 2007). This biocathode MFC could simultaneously achieve the maximum power density (MPD) of 8 W/m^3 and a nitrate removal rate of 0.146 kg NO_3^--$N/d/m^3$, demonstrating that the feasibility of combining biological denitrification with organics removal in biocathode MFC for nitrate-contaminated wastewater treatment. Bioelectrochemical technology can be utilized for nitrogen removal mainly originated from groundwater, surface water, and waste water. Up to now, nitrate removal using biocathode BES has been realized through either heterotrophic denitrification or autotrophic denitrification. Denitrifiers on the cathode directly use the electrons from the anode for nitrate reduction. Gregory et al. (2004) reported that autotrophic denitrifying bacteria preferentially utilized an electrode as the electron donor for nitrate reduction. Then several researchers have confirmed that nitrate can be treated using biocathodes in either an autonomous MFC or a MEC with an external power source (Virdis et al. 2009). These methods using BESs are usually useful for in situ remediation of nitrate-contaminating groundwater (Knoche et al. 2016). For example, Zhang and Angelidaki (2013) assembled a novel bioreactor, named submerged microbial desalination denitrification cell (SMDDC) for nitrate-contaminated groundwater treatment. This special reactor could simultaneously realize electricity generation and biological desalination in continuous mode. Nitrate from the groundwater was transferred into the anode chamber through the separator (cation exchange membrane, CEM) and the anolyte then was directly flowed to the cathode chamber for nitrate removal. Pous et al. (2015) reported a cost-effective strategy using BES with nitrate as electron acceptor and organic matter or water as electron donor for groundwater treatment. This technology imposed an extra power source for enhancing the nitrate reduction. Furthermore, some factors were also reported to evaluate the performance of denitrifying MFCs (Zhao et al. 2016). Also, ammonia could be oxidized in the cathode and then nitrate was in situ reduced via biological or electrochemical process. The usefulness of coupling short-cut nitrification and bioelectrochemical denitrification in the cathode chamber of MFC was reported for nitrogen removal and obtained a removal rate of 0.0125 kg N/m^3 (Li et al. 2016).

Generally, a complete BES contains physicochemical and biological processes. And the latter requires the electrogenic microorganism (e.g., *Geobacter* species) to support its work (Kashima and Regan 2015). For denitrifying BES, the electrogenic denitrifiers could rapidly adapt and then enrich on the surface of the anode. In single-chambered MFCs (SCMFCs), nitrate reduction not only occurred in the cathode with bioelectrochemical denitrification but also in the anode with heterotrophic denitrification (Huang et al. 2018; Drewnowski and Fernandez-Morales 2016). Researchers fabricated an air cathode SCMFC coupling heterotrophic denitrification with anodic respiring and obtained a nitrate reduction rate of 60 mg/L/h (Drewnowski and Fernandez-Morales 2016). Unfortunately, some conditions, like the original construction, the type of substrates, and nitrate concentration, inevitably affect the dominated genera of anodic microbial community. For example, with the initial nitrate concentration increasing (from 0 to 800 mg/L), the percentage of denitrifying bacteria increased from 11.2% to 79.5%, while the percentage of electricigens decreased from 71% to 8.1% in SCMFC (Huang et al. 2018). The genera of *Thauera* and *Geobacter* were, respectively, considered as the dominant genus of denitrifiers and electricigens. The proportion of electricigens in anodic biofilm was obviously decreased when the addition of initial nitrate concentrations to the anode of SCMFC was increased. Although the construction of the biological community is obviously different, MPDs had little affected by nitrate in SCMFC. It is speculated that the amount of electricigens were not a limiting factor in MFCs. A cooperation mode (e.g., direct interspecies electron transfer, DIET) between denitrifiers and electricigens was used to improve the electron transfer from bacteria to the solid electrode (Kumar et al. 2018). Another reason suggested that the denitrifying bacteria might have the capability of extracellular electron transfer. Yang et al. reported a denitrifying SCMFC with 74.5% *Thauera*, demonstrating that *Thauera* has both the capability of extracellular electron transfer and nitrate reduction (Yang et al. 2019). Pous et al. fabricated a dominated—*Thiobacillus* (involved in NO_2 and N_2O reduction) biocathode BES for denitrification, and the bioelectrochemical reduction of nitrate was realized (Pous et al. 2015). Although a wider number of sub communities were involved in denitrification, *Thiobacillus* was enriched from 0% to 33% in the biocathode.

In our research, acetate as an electron donor was cultured in MFCs, and nitrate as an alternative substrate was added to the anode chamber. The electricity performance and anodic microbial communities of MFCs with nitrate or not were analyzed and compared (Jin et al. 2019). Results showed that nitrate significantly affected the genus of anodic microbial communities. As shown in Fig. 11.1, the proportion of denitrifying bacteria increased significantly from 16.2% in MFC without nitrate (Fig. 11.1A) to 37.0% in MFC with nitrate (Fig. 11.1B), whereas the exoelectronic bacteria decreased from 73.3% to 39.6%. Furthermore, the type of electricigens also increased from *Geobacter* in MFC without nitrate (MFC-C) to the combination of *Geobacter* and *Pseudomonas* in MFC with nitrate (MFC-D). *Geobacter* has been

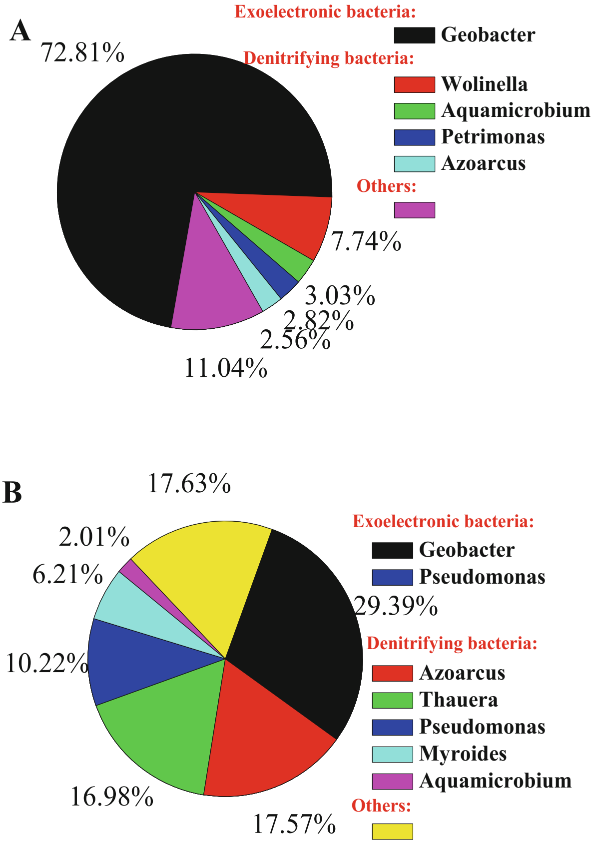

Fig. 11.1 Composition of bacterial community in the anodic biofilms of MFC without or with nitrate. (Referenced by Jin et al. 2019)

confirmed its ability of extracellular electron transfer and plays a crucial role in anodic biofilms of BESs. Another genus *Pseudomonas* was accounted for 1.5% in MFC without nitrate. However, the proportion was significantly increased to 10.2% in MFC with nitrate. *Pseudomonas* species have been verified the capacity of extracellular electrons transfer from bacteria to the solid electrode in BES or reduce nitrate to nitrogen in bioreaction. Further analysis about the characteristic sequences (*NirS*) of denitrifiers showed that the denitrifying bacteria was composed of *Azoarcus*, *Pseudomonas*, and *Thauera*, which were accounted for 40.3%, 36.3%, and 21.2%, respectively (Fig. 11.2). Importantly, with a long-term operation, the proportion of *Pseudomonas* continuously increased to 26.3%, until considering as a dominant genus. In this system, Geobacter could not use nitrate as an electron acceptor, and no negative effect of nitrate on power production was detected. In a word, *Pseudomonas* was considered the denitrifying electricigens in this system (Jin et al. 2019).

Based on the difference in microbial communities, the performance of nitrate removal and electricity generation was also changed. Though no significant changes in voltage output, the cycles of power generation sharply shorted in the presence of nitrate (Fig. 11.3a). The MPD increased by 14.1% (Fig. 11.3b) and the internal

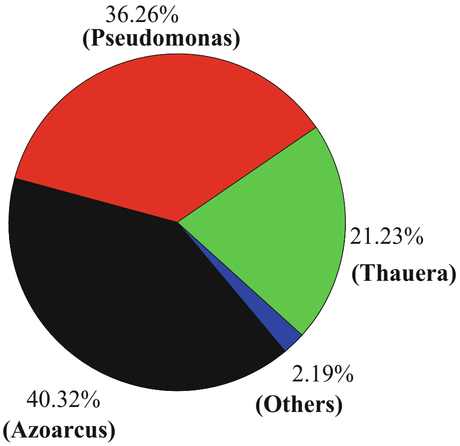

Fig. 11.2 The relative abundance of the denitrification genes (*NirS*) in the anodic biofilm of MFC. (Referenced by Jin et al. 2019)

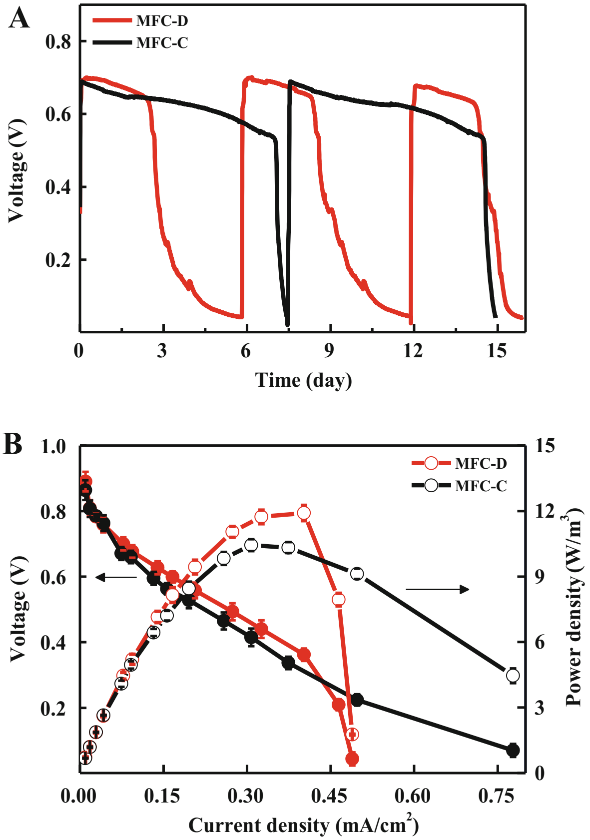

Fig. 11.3 Voltages (**a**) and power densities (**b**) of MFC without nitrate (MFC-C) and with nitrate (MFC-D). (Referenced by Jin et al. 2019)

Table 11.2 The denitrifying electricigens in BESs in the literature

Genus	Electron donor	Power density (mW/m^2)	Ref.
Shewanella oneidensis	–	–	Cruz-Garcia et al. (2007)
Ochrobactrum anthropi	Acetate	89	Zuo et al. (2008)
Comamonas denitrificans	Acetate	35	Xing et al. (2010)
Calditerrivibrio nitroreducens	Acetate	823	Fu et al. (2013)
Pseudomonas aeruginosa	Wastewater	173.3	Manogari and Daniel (2015)
Geobacter metallireducens	Acetate	–	Kashima and Regan (2015)

resistance (R_{in}) relatively decreased from 150 to 100 Ω. The changes of MPD and R_{in} could be explained by alleviating anolyte acidification and DIET between denitrifiers and electricigens. Therefore, the extracellular electron transfer by electricigens was stimulated. With a low nitrate concentration, MFC performance has not obvious negative influence as previous reports (Fu et al. 2013). Compare to single chamber MFCs, the effect of nitrate on electricity performance was more sensitive in dual chamber MFCs when nitrate was added to the anode chamber. For example, nitrate nitrogen of 20 mg/L could make current output decreased in a dual-chamber MFC. The inhibition concentration is also related to the configuration of the reactor. In a micro-scale dual-chamber MFC, only nitrate nitrogen of 4 mg/L could also make current output decreased too. It seems that dual-chamber MFC was more sensitive to nitrate than single-chamber MFCs. This discrepancy was mainly attributed to the characterization of functional microbes. Generally, nitrate can be removed by anodic denitrifiers and electrons can be transferred to anode by electricigens for generating electricity in MFCs. Once these two processes happen independently, denitrification will no negative effect on power generation. Interestingly, specific functional bacteria with simultaneous denitrification and electricity performance must be considered in denitrifying MFCs. To date, the mechanism of denitrifying electricigens is still unclear and resulted in the optimal conditions in denitrifying MFCs are still uncontrolled.

Up to date, researchers have reported a few denitrifying electricigens possessing the denitrification capacity and anodic respiration in BESs (Table 11.2). Fu et al. (2013) reported a current output of MFCs with *Comamonas denitrificans* or *Calditerrivibrio nitroreducens* was negative effected by nitrate, suggesting that the possible change in electron transfer mechanism and resulted in the electricity performance negatively (Fu et al. 2013). The similar conclusion about a shift between anodic denitrification and anode respiration was also reported by Kashima and Regan (2015) when *Geobacter metallireducens* as a denitrifying electricigen was inoculated in a BES. The nitrate concentration determined the electricity performance and there existed a critical level in this system. Once the addition of nitrate was higher than the critical concentration, the electron flowed to the anode was severely restrained. Besides the above mentioned, other electricigens containing *Shewanella oneidensis* (Cruz-Garcia et al. 2007), *Pseudomonas aeruginosa* (Manogari and Daniel 2015), and *Ochrobactrum anthropi* (Zuo et al. 2008) also have the capacity of heterotrophic denitrification, but the comprehensive performance of denitrification and electricity generation is still unclear.

In our experiment, a novel denitrifying electricigen (named *Mycobacterium* sp. EB-1) was isolated and inoculated into a dual-chambered air cathode MFC (Jin et al. 2018). The MPD of 0.84 W/m^2 could be achieved for simultaneous electricity generation and nitrate removal. And further research suggested that no mediator referred to the extracellular electron transfer in MFC. The concurrent processes of anode respiration and anodic denitrification is a limitation rather than an inhibition of the electron donors in this system. The conclusion was greatly different from the previous reports. For instance, the isolated strain Yu37-1 as a denitrifying electricigen, belonged to *Calditerrivibrio nitroreducens*, was strongly inhibited when 20 mM nitrate was added to the anode chamber of MFC (Fu et al. 2013). Similarly, Xing et al. (2010) presented a MFC with *Comamonas denitrificans* DX-4, and the voltage output obviously decreased when 10 mM nitrate was added to the anolyte. Though it is confirmed that nitrate has a negative effect on the current generation in BES, the cooperation mode between anodic respiration and anodic denitrification was realized in our study (Jin et al. 2019). As known, organics oxidized by microbes and generated a large mass of electrons, which were, respectively, flowed into anodic respiration, anodic denitrification, and others contained electron losses for the overpotential and biomass synthesis in denitrifying MFC. The electron flux has a great association with the concurrent metabolism of anodic respiration and anodic denitrification (Virdis et al. 2009; Chandrasekhar et al. 2014b). With an increasing amount of nitrate, electrons for denitrification increased and electrons for anodic respiration relatively decreased, indicating the electron consumption rate for anodic denitrification was much faster than that for anodic respiration rate. When the sum of above rates is bigger than electron production rate, inhibition of the current generation will occur. Otherwise, the electron production rate can satisfy the sum of the rate for anodic denitrification and anodic respiration, electricity performance will not be influenced. It is indicated that a critical condition existed for the symbiotic metabolisms of denitrification and anode respiration in MFCs inoculated with exoelectrogenic denitrifying bacteria. Therefore, whether or not to inhibit the electricity performance mainly depends on the ability of organics metabolism and electron transportation by bacteria in a BES. Generally, the maximum metabolic rate of organic matters and anodic respiration rate are fixed in a stable system. However, nitrate concentration plays a key role in the anodic denitrification rate. In MFC with mixed culture, the amount and composition of the microbial community changed with the presence of nitrate, and the rates also changed relatively. Besides, DIET between bacteria interferes the analysis of experiment results. In a word, the complex condition makes the mechanism analysis of the biological community difficult.

11.3 Conclusion

This chapter reviews the development of denitrifying bacteria and electricigenic bacteria in BESs with nitrate-containing wastewater treatment in the previous study especially introduced the key role of denitrifying electricigens. Denitrifying bacteria was easily involved in denitrification with BESs for nitrate removal. BESs incorporating heterotrophic denitrification could improve electricity recovery and carbon removal efficiencies. In MFC with mixed culture, the proportion of electricigens decreased and the proportion of denitrifiers relatively increased with the increasing nitrate concentration. Within the anodic biofilm of denitrifying BES, denitrifying electricigens, capable of simultaneous denitrification and electricity generation, would finally occupy as dominant bacteria.

11.4 Opinion

Denitrifying electricigens are the amazing genera in denitrifying BESs for nitrogen removal. However, the electron fluxes for denitrification and electricity and the electron transfer mechanism are unclear. Further researches should pay attention to these special bacteria for enlarging the isolating scope. If we could extract certain genes like nitrate reductases, the performance of electrochemical activity bacteria might be analyzed by considering certain functional genes as molecular markers.

References

Abudukeremu, K., Mohd, S. K., Kuppam, C., Gunda, M., Ganesh, D. S., Rijuta, G. S., et al. (2017). Surpassing the current limitations of high purity H_2 production in microbial electrolysis cell (MECs): Strategies for inhibiting growth of methanogens. *Journal of Bioelectrochemistry, 119*, 211–219.

Abudukeremu, K., Mohd, S. K., Peyman, A., Chandrasekhar, K., Azah, M., Nadia, F. A., et al. (2016b). Recent advances and emerging challenges in microbial electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals. *Renewable and Sustainable Energy Reviews, 61*, 501–525.

Abudukeremu, K., Yibadatihan, S., Chandrasekhar, K., Manal, I., & Mohd, S. K. (2015a). Hydrogen gas production with an electroformed Ni mesh cathode catalysts in a single-chamber microbial electrolysis cell (MEC). *International Journal of Hydrogen Energy, 40*, 14095–14103.

Abudukeremu, K., Yibadatihan, S., Peyman, A., Nadia, F. A., Chandrasekhar, K., & Mohd, S. K. (2015b). A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. *Alexandria Engineering Journal, 55*, 427–443.

Animesh, S. D., Harita, A. P., Chandrasekhar, K., Bhagwat, A. M., & Dikshit, A. K. (2016). Sequential microbial activities mediated self-induced bioelectricity production from distillery wastewater using bio-electrochemical system with simultaneous waste remediation. *International Journal of Hydrogen Energy, 42*(12), 1130–1141.

Butz, R. G., & Jackson, W. A. (1977). Mechanism for nitrate transport and reduction. *Phytochemistry, 16*(4), 409–417.

Chandrasekhar, K., & Ahn, Y. (2017). Effectiveness of piggery waste treatment using microbial fuel cells coupled with elutriated-phased acid fermentation. *Bioresource Technology, 244*, 650–657.

Chandrasekhar, K., Lee, Y. J., & Lee, D. W. (2015). Biohydrogen production: Strategies to improve process efficiency through microbial fermentation. *International Journal of Molecular Sciences, 16*, 8266–8293.

Chandrasekhar, K., Venkata, M., & S. (2014a). Bio-electrohydrolysis as a pretreatment strategy to catabolize complex food waste in closed circuitry: Function of electron flux to enhance acidogenic biohydrogen production. *International Journal of Hydrogen Energy, 39*, 11411–11422.

Chandrasekhar, K., Venkata, M., & S. (2014b). Induced catabolic bio-electrohydrolysis of complex food waste by regulating external resistance for enhancing acidogenic biohydrogen production. *Bioresource Technology, 165*, 372–382.

Clauwaert, P., Rabaey, K., Aelterman, P., De Schamphelaire, L., Ham, T. H., Boeckx, P., et al. (2007). Biological denitrification in microbial fuel cells. *Environmental Science & Technology, 41*(9), 3354–3360.

Cruz-Garcia, C., Murray, A. E., Klappenbach, J. A., Stewart, V., & Tiedje, J. M. (2007). Respiratory nitrate ammonification by Shewanella oneidensis MR-1. *Journal of Bacteriology, 189*(2), 656–662.

Debabov, V. G. (2008). Electricity from microorganisms. *Microbiology, 77*(2), 123–131.

Drewnowski, J., & Fernandez-Morales, F. J. (2016). Heterotrophic anodic denitrification in microbial fuel cells. *Sustainability, 8*(6), 561.

Fu, Q., Kobayashi, H., Kawaguchi, H., Wakayama, T., Maeda, H., & Sato, K. (2013). A thermophilic gram-negative nitrate-reducing bacterium, Calditerrivibrio nitroreducens, exhibiting electricity generation capability. *Environmental Science & Technology, 47*(21), 12583–12590.

Ghafari, S., Hasan, M., & Aroua, M. K. (2008). Bio-electrochemical removal of nitrate from water and wastewater - A review. *Bioresource Technology, 99*(10), 3965–3974.

Gopalakrishnan, K., Periyasamy, S., Arivalagan, P., Dung, T. N. B., Zhen, G. Y., Chandrasekhar, K., et al. (2017). A comprehensive overview on light independent fermentative hydrogen production from wastewater feedstock and possible integrative options. *Energy Conversion and Management, 141*, 390–402.

Gregory, K. B., Bond, D. R., & Lovley, D. R. (2004). Graphite electrodes as electron donors for anaerobic respiration. *Environmental Microbiology, 6*(6), 596–604.

Huang, H. B., Cheng, S. A., Yang, J. W., Li, C. C., Sun, Y., & Cen, K. F. (2018). Effect of nitrate on electricity generation in single-chamber air cathode microbial fuel cells. *Chemical Engineering Journal, 337*, 661–670.

Hwang, J. H., Cicek, N., & Oleszkiewicz, J. A. (2009). Long-term operation of membrane biofilm reactors for nitrogen removal with autotrophic bacteria. *Water Science and Technology, 60*(9), 2405–2412.

Jin, X. J., Guo, F., Liu, Z. M., Liu, Y., & Liu, H. (2018). Enhancing the electricity generation and nitrate removal of microbial fuel cells with a novel denitrifying exoelectrogenic strain EB-1. *Frontiers in Microbiology, 9*, 2633.

Jin, X. J., Guo, F., Ma, W. Q., Liu, Y., & Liu, H. (2019). Heterotrophic anodic denitrification improves carbon removal and electricity recovery efficiency in microbial fuel cells. *Chemical Engineering Journal, 370*, 527–535.

Kashima, H., & Regan, J. M. (2015). Facultative nitrate reduction by electrode-respiring *Geobacter metallireducens* biofilms as a competitive reaction to electrode reduction in a bioelectrochemical system. *Environmental Science & Technology, 49*(5), 3195–3202.

Kim, J. K., Lee, B. K., Kim, S. H., & Moon, J. H. (1999). Characterization of denitrifying photosynthetic bacteria isolated from photosynthetic sludge. *Aquacultural Engineering, 19*(3), 179–193.

Knoche, K. L., Renner, J. N., Gellett, W., Ayers, K. E., & Minteer, S. D. (2016). A self-sufficient nitrate groundwater remediation system: *Geobacter sulfurreducens* microbial fuel cell fed by hydrogen from a water electrolyzer. *Journal of the Electrochemical Society, 163*(7), F651–F656.

Knowles, R. (1982). Denitrification. *Microbiological Reviews, 46*(1), 43–70.

Kumar, P., Chandrasekhar, K., Kumari, A., Sathiyamoorthi, E., & Kim, B. S. (2018). Electro-fermentation in aid of bioenergy and biopolymers. *Energies, 11*(2), 343.

Li, Y., Williams, I., Xu, Z. H., Li, B. K., & Li, B. T. (2016). Energy-positive nitrogen removal using the integrated short-cut nitrification and autotrophic denitrification microbial fuel cells (MFCs). *Applied Energy, 163*, 352–360.

Manassaram, D. M., Backer, L. C., & Moll, D. M. (2006). A review of nitrates in drinking water: Maternal exposure and adverse reproductive and developmental outcomes. *Environmental Health Perspectives, 114*(3), 320–327.

Manogari, R., & Daniel, D. K. (2015). Isolation, characterization and assessment of Pseudomonas sp VITDM1 for electricity generation in a microbial fuel cell. *Indian Journal of Microbiology, 55*(1), 8–12.

Park, H. I., Kim, D. K., Choi, Y. J., & Pak, D. (2005). Nitrate reduction using an electrode as direct electron donor in a biofilm-electrode reactor. *Process Biochemistry, 40*(10), 3383–3388.

Pous, N., Koch, C., Vila-Rovira, A., Balaguer, M. D., Colprim, J., Muhlenberg, J., et al. (2015). Monitoring and engineering reactor microbiomes of denitrifying bioelectrochemical systems. *RSC Advances, 5*(84), 68326–68333.

Rijuta, G. S., Chandrasekhar, K., Ackmez, M., Ganesh, D. S., Periyasam, S., Zhen, G., et al. (2017). Bioelectrochemical systems using microalgae–A concise research update. *Chemosphere, 177*, 35–43.

Virdis, B., Rabaey, K., Yuan, Z. G., Rozendal, R. A., & Keller, J. (2009). Electron fluxes in a microbial fuel cell performing carbon and nitrogen removal. *Environmental Science & Technology, 43*(13), 5144–5149.

Xing, D. F., Cheng, S. A., Logan, B. E., & Regan, J. M. (2010). Isolation of the exoelectrogenic denitrifying bacterium Comamonas denitrificans based on dilution to extinction. *Applied Microbiology and Biotechnology, 85*, 1575–1587.

Yang, N., Zhan, G. Q., Li, D. P., Wang, X., He, X. H., & Liu, H. (2019). Complete nitrogen removal and electricity production in Thauera-dominated air-cathode single chambered microbial fuel cell. *Chemical Engineering Journal, 356*, 506–515.

Zhang, Y. F., & Angelidaki, I. (2013). A new method for in situ nitrate removal from groundwater using submerged microbial desalination-denitrification cell (SMDDC). *Water Research, 47*(5), 1827-1836.

Zhao, H. M., Zhao, J. Q., Li, F. H., & Li, X. L. (2016). Performance of denitrifying microbial fuel cell with biocathode over nitrite. *Frontiers in Microbiology, 7*, 344.

Zuo, Y., Xing, D. F., Regan, J. M., & Logan, B. E. (2008). Isolation of the exoelectrogenic bacterium Ochrobactrum anthropi YZ-1 by using a U-tube microbial fuel cell. *Applied and Environmental Microbiology, 74*(10), 3130–3137.