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Enhanced NH_4^+–N removal in a bioelectrochemical system with fabricated activated carbon-polytetrafluoroethylene electrodes

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ABSTRACT

Activated carbon (AC) is a promising electrode material for a bioelectrochemical system (BES) because of its high performance and low cost. Here, AC was flat-pressed with carbon black and polytetrafluoroethylene to fabricate the electrodes. These AC-based electrodes were applied in a single-chamber BES to achieve autotrophic NH_4^+–N removal under organic carbon-free conditions. In batch experiments, NH_4^+–N removal could be enhanced by applying voltage to the BES, in which case the NH_4^+–N removal efficiency gradually increased as the applied voltage increased from 0.1 to 0.25 V. The average NH_4^+–N removal rate in a BES was 47.8 mg L^{-1} d^{-1} in this study, which was comparable with the results of previous studies under similar conditions. Cyclic voltammetry results revealed the presence of several redox-active components on the anode and cathode surfaces, indicating that the AC-based bioanode and biocathode had good electroactivities. Microbial community analysis of 16S rRNA genes based on high-throughput sequencing indicated that the diversity of the microbial community increased after electric power application and that *Pseudomonas* and *Paracoccus* could be important for nitrogen-compound removal in the BES.

Keywords: Activated carbon; Biofilm; Ammonia removal; Bioelectrochemical system (BES)

1. Introduction

With a rapidly developed industrial and agricultural economy, the environmental problems caused by improper industrial waste disposal and fertilizer usage have become a concerning issue that affects human lives [1,2]. Nitrogen-containing compounds are considered the main pollutants [3], with NH_4^+–N being the main nitrogen-containing compound in agricultural wastewater (wastewater from livestock and poultry manure), especially wastewater from anaerobic digester liquor or fertilizer production factories containing high concentrations of NH_4^+–N [4,5]. Excessive amounts of NH_4^+–N in the water may cause eutrophication or pose a health risk to people when polluted water is consumed. Thus, it is important to remove excess nitrogen from polluted water.

In traditional biological removal processes, NH_4^+–N was first oxidized to NO_3^-–N via aerobic nitrification and then reduced to N_2 by using an anaerobic denitrification process [6]. However, the denitrification process needs an additional carbon source, which would increase the cost of treatment, and may also induce secondary pollution. Therefore, this traditional nitrification–denitrification process is difficult to apply when treating wastewater with a low C/N ratio or no organic carbon. The anaerobic ammonia oxidation process (annamox), which is an autotrophic process, could

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simultaneously remove NH_4^+–N and NO_2^-–N; however, annamox bacteria are difficult to cultivate [7], and would therefore be impractical for widespread application.

In recent years, bioelectrochemical systems (BESs) have been proposed as an alternative technology to achieve both nitrification and denitrification [8–10]. The key feature of these BESs is the use of solid-state electrodes as electron donors/acceptors to drive microbial reactions by way of direct electron transfer or mediated by electron shuttles or hydrogen gas. For instance, Virdis et al. [8] obtained simultaneous nitrification and denitrification in a microbial fuel cell with a solid-state electrode as electrode donor. However, it is difficult to use such a system for low C/N ratio wastewater treatment. Zhan et al. [10] achieved nitrification and denitrification in a microbial electrolysis cell, in which the cathode and anode were employed for denitrifying and nitrifying bacteria to achieve denitrification and nitrification, respectively. To achieve acceptable performance, the setting most researchers used for electric power was higher than 1.8 V [11,12], although Zhan et al. [10] verified that 0.2 V was sufficient to catalyze the reactions.

The electrode materials that were used in the above-mentioned studies (e.g., carbon felt, carbon cloth) present great challenges for large-scale application, either because of their high cost (100,000–500,000 US$ m^{-2}) [13] or operational difficulties, such as their inability to transfer electrons at long distances (carbon lacks the electrical conductivity of metals) [14]. It is reported that the cost of the electrode materials accounts for 20%–50% of the total BES cost [13,15,16]. Thus, lowering the manufacturing cost of an electrode is crucial to promote the commercialization of BES technology. Although biochar has recently been recommended to be a low cost and sustainable electrode material used for both anode or cathode of BESs [15,17]. However, the relative low conductivity of biochar is still an issue that needs to be considered for real application [18]. Activated carbon (AC) is known as an inexpensive yet effective electrode material (30-1,600 US$ m^{-2}) [19] with a specific surface area of 1,000–2,000 $m^2 g^{-1}$ [20] and a porosity that is higher than that of other carbon-based electrode materials [21]. AC can be fabricated with a stainless mesh (current collector) by the rolling-press method. This pressed activated carbon plate (ACP) electrode has significant advantages in terms of its low cost, high reproducibility and scalability, as well as its comparatively high performance [22,23]. The main mechanism is the enhancement of the transfer of electrons and ions, and the growth of biofilm on the surface of the electrode [19].

However, related studies have mostly been concentrated on the cathode, and little is known about using ACP as the anode of BES. Furthermore, studies published to date have mainly focused on electric power generation, and information on coupled wastewater treatment in an ACP-based BES is scarce. To the best of our knowledge, the ACP electrode has not yet been used in the BES for simultaneous nitrification and denitrification.

Thus, as the initial step, the aim of this study is to investigate the feasibility of employing a fabricated ACP anode and cathode in a BES for treating wastewater containing NH_4^+–N. The microbial community in the ACP electrode biofilm was also analyzed to understand the diversity of nitrifying and denitrifying bacteria.

2. Materials and methods

2.1. Fabrication of ACP electrode

The ACP electrode consisted of ultracapacitor AC powder (1,500 $m^2 g^{-1}$, Xinsen Carbon Co. Ltd, Fujian, China), carbon black powder and polytetrafluoroethylene (PTFE), with stainless steel mesh as the matrix. The ACP electrodes were prepared by a modified flat-platen pressing method [24]. Specifically, 4 g of AC powder and 0.4 g of carbon black powder were first mixed in an appropriate amount of 2-propanol in a beaker and ultrasonically agitated for 30 min at 30°C, after which 7.3 g of a PTFE suspension (10% w/v) was slowly dripped into it. After another 30 min of ultrasonic treatment, the blend was stirred and dried at 60°C to produce a dough-like paste. This paste was divided into two amounts, which were then flat-pressed into two thin films (each of 0.8 mm thickness) and were then pressed onto both sides of the stainless steel mesh to form a flat sheet of 0.4 mm thickness. This sheet was subsequently sintered for 25 min at 340°C to obtain an ACP electrode of 0.8 mm thickness. Before it was used, the ACP sheet was cut into a rectangular shape with L = 10 cm; W = 5 cm.

2.2. Reactor set up

Fig. 1 shows a detailed schematic of the BES that was used in this study and which was manufactured from plexiglass. Two ACP electrodes (10 cm × 5 cm) were placed into the BES and connected to a DC power supply. The effective volume of the BES was 1 L and a sampling port was opened at the bottom of the reactor. For system start-up, activated sludge obtained from the Jiangxinzhou sewage treatment plant (Nanjing, China) was inoculated, and 0.1 V electric power was applied with an initial NH_4^+–N concentration of 200 mg L^{-1}. The components of the enrichment medium were 0.4 g of NaH_2PO_4, 0.6 g of $Na_2HPO_4 \cdot 12H_2O$, 0.2 g of NaCl, 1.6 g of $NaHCO_3$, 0.02 g of $CaCl_2 \cdot H_2O$ and 0.05 g of $MgSO_4 \cdot 7H_2O$. Once initiated, the BES was stabilized in fed-batch mode at room temperature for a period of at least 30 d until 90% of NH_4^+–N was removed.

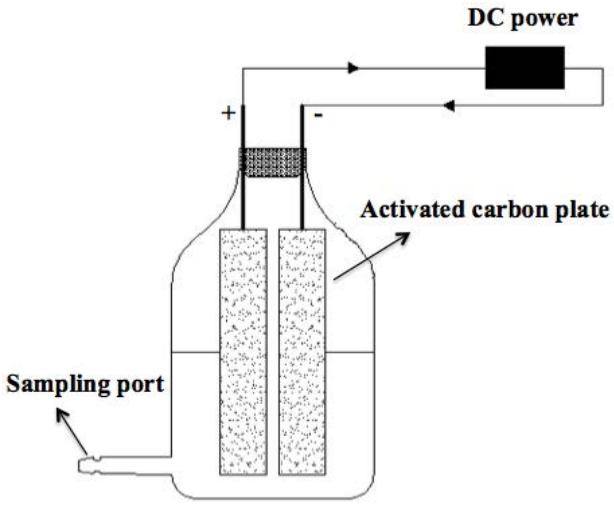

Fig. 1. Schematic diagram of BES for NH_4^+–N removal.

2.3. Effect of electric power on the removal of nitrogen in BES

The effect of electric power was investigated by comparing the NH_4^+–N removal performance of BES operated at 0.25, 0.15, 0.1 and 0 V. The reactor was operated in fed-batch mode at room temperature, the initial NH_4^+–N content was 200 mg/L, and the initial pH was pre-adjusted to 7.0. The other experimental conditions were the same as described above. Abiotic control was also carried out to evaluate the contribution of the direct electrochemical effect of BES for NH_4^+–N removal. Liquid samples were taken every day and the concentrations of NO_3^-–N and NO_2^-–N were measured by a Metrohm 761 compact ion chromatograph (Herisau, Switzerland) equipped with a SI-90 4E column and a conductivity detector. The NH_4^+–N concentration was determined using Nessler's method with a UV-visible spectrophotometer (DR-2800, Hach, Loveland, CO., USA). All experiments were run in triplicate and one-way analysis of variance was applied to assess the statistical difference of the results at a significance level of $p = 0.05$.

2.4. Effect of initial concentrations of NH_4^+–N on the removal of nitrogen in BES

The effect of different initial concentrations of NH_4^+–N (150, 250 and 300 mg/L) on the removal of nitrogen was examined at an applied electric power of 0.15 V, with the other parameters remaining the same. The concentration of NH_4^+–N in this study was selected by reviewing and comparing the sludge digester liquor, some industrial wastewaters (fertilizer production, etc.) or some synthetic wastewaters from previous related studies [5,25–29]. The concentrations of ammonium NH_4^+–N, NO_3^-–N and NO_2^-–N in the BES were measured daily. All experiments were run in triplicate.

2.5. Cyclic voltammetry (CV) measurement

After the batch experiments, the ACP anode and cathode with biofilm were removed from the reactor and separately placed into two new electronic cells for CV measurement under anaerobic conditions. The CV measurements were carried out with a potentiostat (WMPG1000K8; WonATech Co. Ltd., Seoul, Korea), an Ag/AgCl reference electrode (HX-R8; Hokuto Denko Inc., Osaka, Japan) and a twisted platinum counter electrode (0.8 mm × 1 m; Nilaco, Tokyo, Japan). The scan rate was 10 mV/s with a potential range from –0.8 to 0.6 V (vs. Ag/AgCl). The composition of the electrolyte for the CV measurement was 25 mM phosphate buffer (4.58 g L^{-1} Na_2HPO_4 and 2.45 g L^{-1} $NaH_2PO_4{\cdot}H_2O$; 0.13 g L^{-1} KCl, pH 7.0).

2.6. Pyrosequencing of biofilm samples

At the end of the experiment, the biofilm samples were collected from the surface of the ACP anode and cathode in a glove chamber (AW300SG, ELECTROTEK, UK). A Power Soil DNA isolation kit (MOBIO, USA) was used to extract 1 mL of genomic DNA from the biofilm samples. The quality and concentrations of the DNA were examined using a Qubit 2.0 DNA detection kit. The V3-V4 region of the bacterial 16S rRNA fragments was amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Then, high-throughput sequencing and analyses were carried out on an Illumina MiSeq PE300 sequencing platform by Sangon Biotech (Sangon, Shanghai, China) as described previously [30]. The alpha diversity parameters (Shannon index, ACE index, Chao1 index, Good's coverage and Simpson index) were calculated using the mothur program.

3. Results and discussion

3.1. Electrically enhanced NH_4^+–N removal in BES

The performance of BES with and without applied electric power was compared to elucidate the effect of additional power (0.1, 0.15 and 0.25 V) on the NH_4^+–N removal efficiency, with an initial NH_4^+–N concentration of 200 mg L^{-1}. As shown in Fig. 2a, the NH_4^+–N removal efficiency of closed circuit systems was nearly 98% in 4 d with a higher removal rate compared with the open circuit control. Further, approximately 36.5 mg L^{-1} NO_3^-–N and 20.5 mg L^{-1} NO_2^-–N accumulated in the open circuit control (Figs. 2b and 2c). In contrast, almost no residual NO_3^-–N and NO_2^-–N remained in the system to which electric power was applied, suggesting that the total nitrogen removal capacity of BES was enhanced by applying electric power. No NH_4^+–N removal was observed in the abiotic experiment (data not shown) suggesting that the enhanced NH_4^+–N removal may be attributed to enhanced microbial activity. The average NH_4^+–N removal rate was 47.8 mg L^{-1} d^{-1} in this study, and was comparable with the results of previous studies under organic carbon-free conditions (31–60 mg L^{-1} d^{-1}) [10,31]. However, it should be noted that the ACP used in this study was less expensive and had better reproducibility than that used in previous studies by others, in which carbon felt was normally used [10,31].

3.2. Effect of NH_4^+–N concentration on the removal of nitrogen

The effect of the initial NH_4^+–N concentration on the removal of nitrogen was investigated by setting the NH_4^+–N concentration to 150, 250 or 300 mg L^{-1}, with an external voltage of 0.15 V in all cases. As shown in Fig. 3, complete NH_4^+–N removal was achieved when 150 mg L^{-1} NH_4^+–N was initially added to the system. This is consistent with the results demonstrated in Fig. 2, in which the 200 mg L^{-1} NH_4^+–N that was initially present was removed at 0.15 V with almost no residue. However, when the initial NH_4^+–N concentration was increased (250 and 300 mg L^{-1}), NO_3^-–N and NO_2^-–N started accumulating in the system. Especially, in the system to which 300 mg L^{-1} was added, approximately 52.2 and 24.5 mg L^{-1} NO_3^-–N and NO_2^-–N accumulated, respectively. Similar trends were also observed by Zhan et al. [10], in which NO_3^-–N started accumulating when 150 mg L^{-1} NH_4^+–N was added to the system.

3.3. CV measurement

The CV analysis of the ACP bioanode (red line) after incubation showed much higher anodic catalytic current (1,300 μA) at 600 mV (vs. Ag/AgCl) than the bare electrode

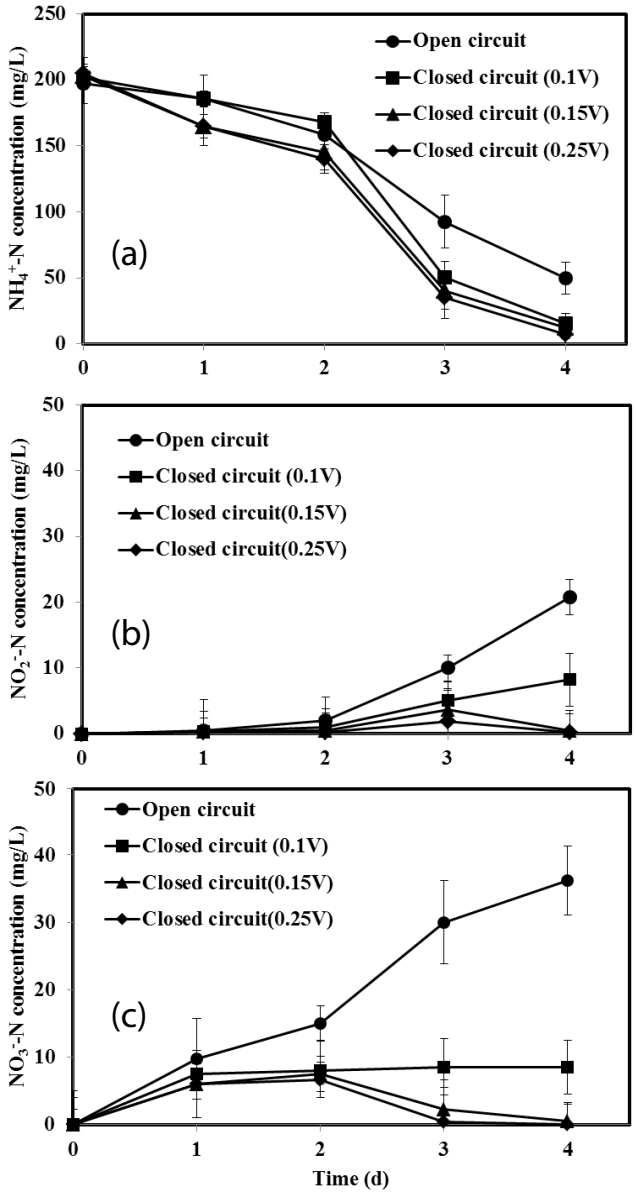

Fig. 2. Concentration variation of NH_4^+–N (a), NO_2^-–N (b), NO_3^-–N (c) in BES with and without applied electric power.

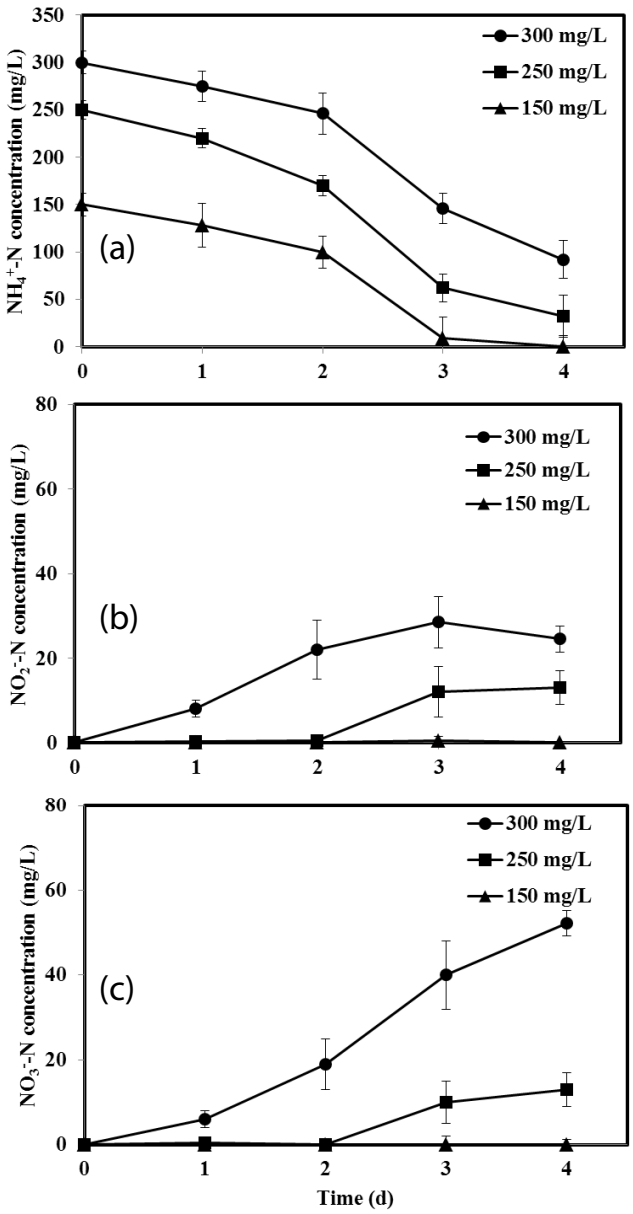

Fig. 3. Effects of different initial NH_4^+–N concentrations (150, 250 and 300 mg/L) on NH_4^+–N removal, NH_4^+–N (a), NO_2^-–N (b), NO_3^-–N (c) in BES at 0.15 V.

(24 μA) (Fig. 4), while the cathodic catalytic current of the ACP cathode (–1,440 μA) at –800 mV (vs. Ag/AgCl) was also higher than that of the bare electrode (–360 μA). The greater production of electricity after incubation of the microbial biomass was probably due to larger capacitance and smaller resistance after biofilm growth on the ACP electrode surface [32,33]. The oxidation and reduction waves on the cyclic voltammograms of the bioanode biofilms appeared at 0.25 and 0.23 V (vs. Ag/AgCl), respectively, and the redox potentials of these two waves were lower than that of the ammonium oxidation bioanode used in a previous study [31], probably due to the different microbial community compositions and functional microorganisms of the biofilm in this study. In addition, several oxidation and reduction waves appeared on the cyclic voltammograms of the biocathode, although the peaks are inconspicuous, which indicate the presence of several redox-active components with different redox potentials on the surfaces of these cathodes [34].

The current generated in anodic CV is similar to that in cathodic CV, suggesting that both bioanodic and biocathodic reactions were dominant in the BESs [35]. The insignificant difference in the shapes of the CVs for the bioanode and biocathode might be attributed to the fact that their microorganisms were derived from the same source (active sludge), and similar bacterial species in anodic biofilm and cathodic biofilm (a wide variety of bacteria possess both nitrification and denitrification capabilities [36]). Further,

the electrochemically active microorganisms or redox-active components in the biofilms of both the bioanode and biocathode may be similar. Several bacteria capable of nitrate reduction carried out bidirectional electron transfer (e.g., *Geobacter*, *Pseudomonas*) [37]. The detailed mechanism of bioanodic and biocathodic electron transfer warrants for further research.

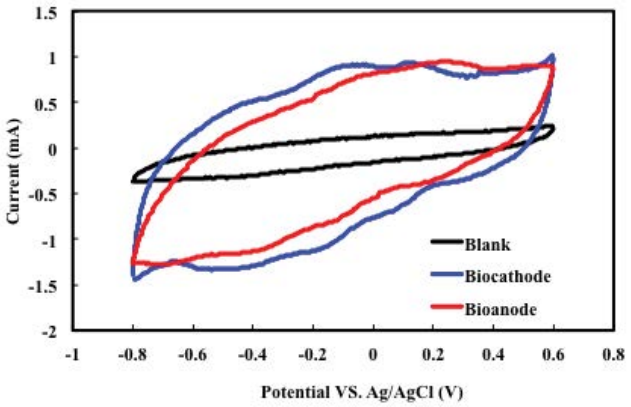

Fig. 4. Cyclic voltammograms of the bioanode and biocathode, respectively.

3.4. Microbial communities in the bioanode and biocathode biofilms

The microbial compositions of the original active sludge and the bioanode and biocathode biofilms were determined using high-throughput sequencing (Fig. 5). The Good's coverage, OTU number, Shannon index, Ace index, Chao1 index and Simpson index are listed in Table 1. The high Good's coverage of 0.96–0.98 means that the majority of microbial communities in the samples were sequenced. The Shannon index showed that the microbial diversity of the anode and cathode biofilms were more complex relative to the original activated sludge, probably because much more bacterial species involved in electron transfer for ammonium removal were enriched after acclimation in the BESs, which was in agreement with previously reported results [31]. The lower values of Ace and Chao1 indices of the anode and cathode biofilms indicated that their total microbial abundances were lower than those of the original activated sludge.

The main genera identified in the original active sludge were *Lysinibacillus* (45.42%), *Brevibacillus* (16.30%), *Bacillus* (13.34%), *Alkaliphilus* (7.55%), *Tissierella* (2.90%) and *Paracoccus* (2.60%). Some of the nitrifiers and denitrifiers were already abundant in the original sludge [38–42]. *Lysinibacillus* sp. [38], *Bacillus* sp. [39] and *Paracoccus* sp. [40] were reportedly able to efficiently afford simultaneous

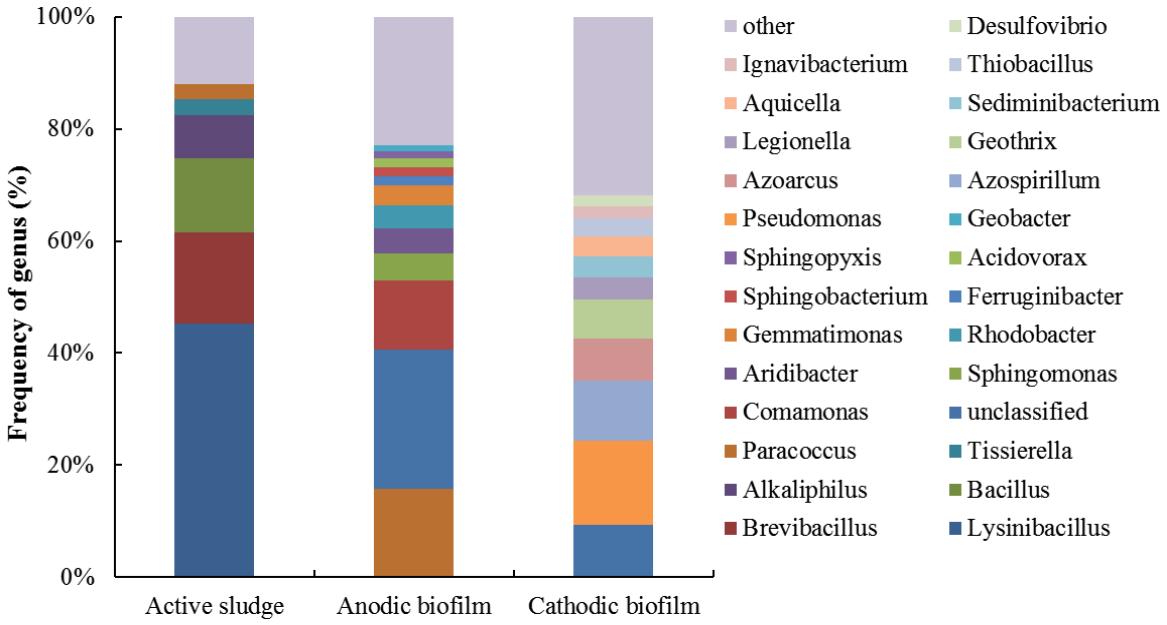

Fig. 5. Bacterial community composition of biofilms at the genus level.

Table 1
Sequence read and alpha diversity parameters

Sample	No. of Reads	No. of OTUs	Shannon index	Ace index	Chao1 index	Good's coverage	Simpson index
Active sludge	65,081	1,447	2.24	107,396.14	32,846.14	0.98	0.24
Anodic biofilm	64,231	2,323	4.37	30,141.05	14,797.24	0.97	0.05
Cathodic biofilm	70,959	3,311	4.53	38,760.37	18,161.26	0.96	0.04

heterotrophic nitrification–aerobic denitrification reactions, while *Brevibacillus,* as one of the gram-positive denitrifying bacteria, are widespread in the environment [41]. The growth of *Tissierella* was reported enhanced after the addition of nitrate to the medium and might be involved in the nitrate reduction [43].

After incubation in the BESs, *Paracoccus* sp. (15.86%) showed rapid growth in the nitrifying biofilm of the anode and *Pseudomonas* sp. (14.90%), as the newly appearing denitrifier, became the most dominant bacteria in the denitrifying biofilm of the cathode. *Pseudomonas* sp. has already been studied in detail and exhibits efficient denitrification under anaerobic conditions [41,42]. This indicated that *Paracoccus* sp. and *Pseudomonas* sp. might play the greatest role in the anodic nitrification and cathodic denitrification in this BES, which is in agreement with the results of Zhan et al. [31].

Other nitrifiers, such as *Comamonas* (12.24%), *Rhodobacter* (4.04%) (which are capable of heterotrophic nitrification–aerobic denitrification [44,45]), *Sphingomonas* (4.82%) (which has the potential to participate in ammonium oxidation via heterotrophic or autotrophic nitrification [46]) and *Acidovorax* (1.51%) (which are responsible for ammonium oxidation in the partial nitrification process [47]), and potential denitrifiers, such as *Azospirillum* (10.72%), *Azoarcus* (7.54%), *Geothrix* (6.95%), *Thiobacillus* (3.13%), *Ignavibacterium* (2.11%) and *Desulfovibrio* (2.07%), were also enriched in the nitrifying and denitrifying biofilms, respectively. The involvement of *Azospirillum* and *Ignavibacterium* [48], *Azoarcus* [49], *Geothrix* and *Thiobacillus* [50], and *Desulfovibrio* [51] in denitrification has been well studied. The growth of these nitrifiers and denitrifiers was attributed to the applied electrical power, and they might also participate in the nitrification and denitrification process in the BES.

Electrochemically active microorganisms are organisms capable of transferring electrons to and/or from solid-phase electrodes via direct extracellular electron transfer or redox-active components [52,53]. Among the denitrifiers in the cathodic biofilm, *Pseudomonas* [54] and *Thiobacillus* [55] were reported to exhibit electroactivity. Su et al. [54] have shown that *Pseudomonas alcaliphila* strain MBR could utilize the electrode as the electron donor to enhance the nitrate reduction process. Moreover, Kato et al. [55] revealed interspecies electron transfer via electric currents through conductive minerals from *Geobacter sulfurreducens* to *Thiobacillus denitrificans* for nitrate reduction. *Pseudomonas* and *Thiobacillus* may play important roles in denitrification in cathodic biofilm here. *Geobacter*, as the electrochemically active microorganisms, have been well documented [56-58] and were detected in the anode biofilm, although they only accounted for 1.15% of the total bacteria. These results were in good agreement with a previous study which showed that *Geobacter* was identified in the anode compartments of BESs for nitrogen removal through nitrification and denitrification processes [53]. *Geobacter* have not been reported as nitrifiers, and might not be directly involved in ammonium oxidation, but cooperate with nitrifiers. The electron transfer mechanisms of this relatively complicated microbial community of the anode and cathode biofilms could not be ascertained in the present study. Further studies on the synergistic interaction and mutual relation among electrochemically active microorganisms, nitrifiers and denitrifiers are warranted.

4. Conclusion

This study demonstrated enhanced NH_4^+–N removal in a BES with fabricated activated carbon plates as electrodes. These ACP electrodes exhibited strong electrochemical response. The removal of NH_4^+–N improved by increasing the applied voltage and this performance was comparable with that of BES with a carbon felt electrode. Analysis of the biofilm community suggested that the nitrifying and denitrifying bacteria that could survive on the surface of ACP were highly diverse and that there were also colonies of some electrochemically active microorganisms. The results of this work demonstrate the potential for developing a cost-effective electrode for efficient electro-catalysis in BES.

Acknowledgements

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