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High-concentration nitrogen removal coupling with bioelectric power generation by a self-sustaining algal-bacterial biocathode photo-bioelectrochemical system under daily light/dark cycle

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HIGHLIGHTS

- Self-sustained power output and N removal in PBES were achieved by day/night cycle.
- Day/night cycle sustained high microbial diversity for power output and N removal.
- Daily light/dark cycle enable multi-approach N removal in algal-bacterial cathode.
- Content of N, P and TOC affect PBES performance mainly through alga activity.

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ABSTRACT

High-concentration nitrogen removal coupled with bioelectric power generation in an algal-bacterial biocathode photo-bioelectrochemical system (PBES) was investigated. The PBES can self-sustaining operation with continuous power output under day/night cycle by alternately using photosynthetic dissolved oxygen and nitrate/nitrite as cathodic electron acceptors. The PBES generated a high maximum power of 110mw/m^2 under illumination and relatively lower power of 40mw/m^2 under dark. The bioelectricity generation was accompanied by high-concentration nitrogen removal in the algal-bacterial biocathode. The NH_4–N was removed completely within 120 h while maximum NO_3–N removal efficiency of 86% and maximum total nitrogen removal efficiency of 83% can be reached after 192 h at initial NH_4–N concentration of 314 mg/L and NO_3–N concentration of 330 mg/L. Combined processes of bioelectrochemical reduction and algal-bacterial interactions provided multiple approaches for nitrogen removal in the biocathode, including nitrifying using photosynthetic oxygen, bioelectrochemical denitrification using the cathode as electron donor, heterotrophic denitrification using photosynthetically produced dissolved organic matters as carbon source and algal-bacterial uptake. Accelerated nitrogen removal with simultaneously improved cathode performance was observed at high concentration of nitrogen and phosphate buffer due to enhanced algal activities for photosynthetic oxygen release and enhanced algal-bacterial interactions for nitrogen transformation. Addition of external organic carbon negatively affected nitrification and decreased cathode potential due to oxygen consumption by aerobic carbon oxidation but enhanced denitrification due to continuous release of high concentration of

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photosynthetically produced dissolved organic matters by alga. The PBEC was demonstrated as an energy-saving approach for high-strengthen nitrogenous wastewater treatment.

1. Introduction

Discharge and inadequate treatment of high-strength nitrogenous wastewater, such as wastewater from animal farms, food processing facilities, and landfill leachate (Kim et al., 2004; Cristian, 2010; Paskuliakova et al., 2016) pose a serious ecological threat to aquatic environment due to the toxicity of nitrogenous compounds and eutrophication (Ono et al., 2000; Yu et al., 2015).

In a conventional two-stage biological nitrogen removal process, high dissolved oxygen (DO) and additional carbon source are required for effective nitrification and denitrification, which is not sustainable and cost-effective due to extensive energy requirements for aeration and extra cost for external organic carbon source supply. Therefore, development of a high-efficient, cost-effective and self-sustaining process for treatment of wastewater containing high concentration of nitrogen has been the need of the day.

Photo-bioelectrochemical system (PBES) which based on the synergistic cooperation of exoelectrogens and photosynthetic microorganisms has recently drawn increased attention because it provides a new approach to conversion of solar into bioelectricity while simultaneously biodegrade various organic pollutants and convert inorganic pollutants in wastewater (Rosenbaum et al., 2010; Xiao et al., 2012; Luo et al., 2017). Recently, several works demonstrated the use of algal-bacterial photo-biocathode PBES for efficient removal of nutrient from wastewater with extra net energy output (Xiao et al., 2012). However, directly feeding wastewater for the aerobic biocathode for nitrification and returning the catholyte to the anode compartment for denitrification will deteriorate the performance of the cathode and the anode due to negative effect of excessive dissolved organic carbon entering (Huang et al., 2011) and oxygen invasion and the presence of competitive terminal electron acceptor (Chen et al., 2014; Feng et al., 2015), respectively. Although simultaneous nitrification and denitrification by maintaining DO at specific level at aerated biocathode has been previously reported (Virdis et al., 2010), there is no study referring to high-strength nitrogen removal via simultaneous or continuous nitrification and denitrification using the algal-bacterial biocathode of the PBES by operating the cathode with daily light/dark cycle, which comply with the natural 24 h day/night cycle without extra energy input for artificial illumination. Moreover, the algae activity under daily light/dark cycle can construct alternate aerobic/microaerobic/anaerobic environment, resulting in periodic variation in availability of different terminal electron acceptors and consequent variation in metabolic activity of specific bacterial populations, which may provide heterogeneous niches to sustain diverse microbial communities and thereby providing multiple approaches for nitrogen removal. However, how the algal–bacterial communities may function together for synergistic nitrogen removal at the algal–bacterial biocathode with daily light/dark cycle has not been systematically explored.

In this study, a novel algal–bacterial biocathode PBES was developed to remove high-concentration nitrogen removal from synthetic high-strength nitrogenous wastewater. The algal–bacterial PBES was operated with daily light/dark cycle to comply with the metabolic pattern of alga under natural day/night cycle while simultaneously enabling a spontaneous bioelectrochemical reaction by alternately using photosynthetic oxygen and nitrate/nitrite as cathodic electron acceptors and thus can achieve self-sustaining nitrogen removal via alternating (or simultaneous) nitrification and denitrification in the biocathode half-cell without need of additional energy input and manual operation and minimize the negative effect of the wastewater treatment on the bioelectrochemical performance of the PBES. The performance and mechanisms of nitrogen removal by the algal–bacterial biocathode were investigated in terms of NH_4–N, NO_3–N and NO_2–N removal, cathode potential, algal activity, DO, pH and microbial diversity. In addition, some important operational parameters for the nitrogen removal including external resistance, initial nitrogen content, organic carbon addition and phosphate buffer capacity were also investigated. These data can help to discover the essence of nitrogen removal in the algal–bacterial biocathode, which is of significance for potential application of the PBES with daily light/dark cycle for high-strength nitrogenous wastewater treatment.

2. Materials and methods

2.1. PBES configuration

The PBESs were constructed by two equal volume plexi-glass cubic chambers (8 cm × 8 cm × 4 cm) separated by an cation exchange membrane. Nickel foam (5 × 6 × 0.2 cm) was chosen as a base material for anode and cathode due to the porous structure and high conductivity. The nickel foam was pretreated by sonication in acetone for 10 min followed by immersion in 5% HCl for 1 min to remove any surface impurities and finally rinsed thoroughly with distilled water (Karthikeyan et al., 2016). The anode and cathode was placed parallel to each other at approximately 1 cm from the CEM, and connected with titanium wire via a resistor of 50 Ω unless otherwise specified. The PBES configuration was illustrated in Figure S1 (Supporting Information).

2.2. PBES start-up and operation

The anode was inoculated with a mixture of aerobic sludge and anaerobic sludge while the same sludge plus *Chlorella vulgaris* were used to inoculate cathode. The anode growth medium contain glucose (500 mg COD/L), 50 mM phosphate buffer solution (PBS, pH = 7) and nutrients as described previously (Sun et al., 2015). The medium used in the cathode was identical to that of the anode, except for addition of 1.2 g/L NH_4Cl (corresponding to 314 mg/L NH_4–N) and 2 g/L $NaNO_3$ (corresponding to 330 mg/L NO_3–N), and replacement of glucose by $NaHCO_3$ (0.48 g/L). The details concerning acclimation of exoelectrogenic anode biofilm and algal-bacterial biocathode biofilm are provided in the electronic supplementary material. The PBES was operated under alternating 12 h light/12 h dark cycles to simulate natural day/night cycle. A light emitting diode (20 W, cover the entire visible light wavelength range from 380 to 780 nm) was used as light source for cathode illumination and placed at a distance of 5 cm from the cathode.

2.3. Nitrogen removal test at the algal-bacterial biocathode

Four controls were used to investigate the performance and the mechanisms of nitrogen removal in the algal-bacterial biocathode. The first was operation of the PBES under closed circuit and external resistor of 500 Ω, the second was operation of the PBES under closed circuit but covering the cathode with tinfoil to prevent photosynthesis of alga and providing intermittent aeration at daytime, the third was operation of the PBES under open circuit condition, and the fourth was operation of an equal reactor to the PBES with a closed circuit but without inoculation of the anode and cathode. In addition, a series of experiments were conducted to investigate the effect of some important operational parameters including external resistance (50 and 500 Ω), initial nitrogen content (0.6 g/L of NH_4Cl, 1 g/L $NaNO_3$ and 1.2 g/L of NH_4Cl, 2 g/L $NaNO_3$), organic carbon concentration (50 and 500 mg COD/L glucose), phosphate buffer capacity (10 and 50 mM) and external applied voltage (0.1, 0.2 and 0.4 V) on algal-bacterial biocathode performance with respect to nitrogen removal. All experiments were conducted at least in triplicate, in a constant temperature room (30 ± 1 °C), and the average values and standard deviations were reported.

2.4. Measurements and analyses

2.4.1. Chemical analysis

For nitrogen removal test, samples were collected from the algal-bacterial cathode every 3 h during the light period and at 3 h and 12 h from beginning of dark period during the 24 h light/dark cycle. The concentrations of ammonia (NH_4–N), nitrate (NO_3–N) and nitrite (NO_2–N) were analyzed according to Standard Method (APHA, 2005). Samples for total organic carbon (TOC) analysis were collected from the cathode with the same time interval and were measured by using a TOC analyzer (Shimadzu, Kyoto, Japan). Each example was filtrated through a 0.22 nm membrane before measurement.

The pH and DO were continuously monitored with a pH meter (Mettler–Toledo, Switzerland) and optic oxygen probe (Mettler–Toledo, Switzerland) connected to a personal computer.

2.4.2. Electrochemical analysis

The voltage across the external resistance was recorded every 10 min with a data acquisition device (Model 2700, Keithly Instruments, USA) connected to a personal computer. Both the cathodic and anodic potential were measured against saturated calomel electrode.

2.4.3. Microbial analysis

Algal biomasses were measured by optical density measurements on a UV/vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 680 nm. Optical density was then converted to dry weight of algae cells using a previously prepared calibration curve (Prajapati et al., 2014).

Bacterial diversity within the algal-bacterial biofilm was analyzed by high throughput sequencing. Samples of the algal-bacterial biofilm were collected at the end of experiments (at day 216). Total genomic DNA of the samples was extracted using Power Soil DNA Isolation Kit (MO BIO Laboratories, USA) according to the manufacturer's instructions. PCR was performed using the Gene AmpPCR-System®9700 (Applied Biosystems, USA), as previously described (Sun et al., 2015). The PCR products were then purified with the Gel Extraction Kit (Sangon Biotech Co., Ltd., Shanghai, China) and cloned into PCR2.1 vector using the TOPO ® TA cloning kit (Invitrogen, Carlsbad, CA), and then sent for sequencing (Invitrogen Life technologies Co., USA). The DNA sequences were examined using NCBI BLAST algorithm and the Ribosomal Database Project II Classifier at a confidence level of 80%, to identify similar sequences.

3. Results and discussion

3.1. Electrochemical performance of the PBES with daily light/dark cycle

Electrical energy generation and electrode potential are useful indicators of the bioelectrochemical performance of the PBES. A reproducible and stable light-dependent voltage output was observed and kept for more than 120 h after one month operation (Fig. 1A). The voltage output of the PBES sharply increased to a peak value of 0.18 V and then decreased gradually to a steady state value of 0.13 V during the 12 illumination period. The current output drop was due to the suspended algal biomass precipitation after mixing at the beginning operation of the illumination period.

Illumination induced a significant increase in cathode potential and moderate increase in anode potential (Fig. 1B), which indicates an accelerated electron transfer from anode to cathode. Similar to current and cathodic potential, periodic oscillation of DO level was observed under daily light/dark cycles due to the light-dependent photosynthetic oxygen evolution (Fig. 2B). Under illumination, DO reached a maximum concentration of 14 mg/L which can be maintained until the end of the 12 h illumination period. When the light was turned off, the DO gradually dropped to zero. Research found that photosynthetic oxygen production by algae could be inhibited by high light intensity and continuous illumination (Wu et al., 2014). In this study, intermittent illumination of 12-h light and dark periods at a light intensity of 2500 lux did not obviously reduce photosynthetic oxygen production by *chlorella*, indicating that the illumination scheme was a rational strategy for sustainable operation of the PBES. Although a significant decrease in current and cathode potential occurred during dark period due to lack of photosynthetic oxygen production, however, the current was much higher than the background current since the nitrate (or nitrite) can serve as another electron acceptor in the absence of oxygen (Virdis et al., 2010).

Similar results were observed for the polarization test (Fig. 1C and D). Under illumination, the PBES generated a high maximum power of 110 mw/m^2. The PBES also generated power in the dark, albeit at lower levels compared to under illumination (40 mw/m^2). For the electrode polarization, similar profiles were obtained for the anodic potentials, however, very different profiles were observed for the cathodic potentials depending on the illumination and dark. The open circuit half-cell potential for cathode was much higher under illumination (−0.12 V vs SCE) than that in the dark (−0.37 V vs SCE). These results suggest that a potential exoelectrogenic biofilm and an algal–bacterial biofilm have developed on anode and cathode respectively. Additionally, the PBES enabled spontaneous bioelectrochemical reactions by alternately using photosynthetic oxygen and nitrate/nitrite as cathodic electron acceptors and thus achieved self-sustaining operation without need of additional energy input which is important for practical application of the bioelectrochemical system for wastewater treatment (Do et al., 2018).

3.2. Nitrogen removal performance and mechanisms in the algal–bacterial biocathode with daily light/dark cycle

3.2.1. Nitrogen removal performance and contribution of bioelectrochemical process to nitrogen removal

To gain an understanding of nitrogen removal processes in the algal–bacterial biocathode with daily light/dark cycle, samples

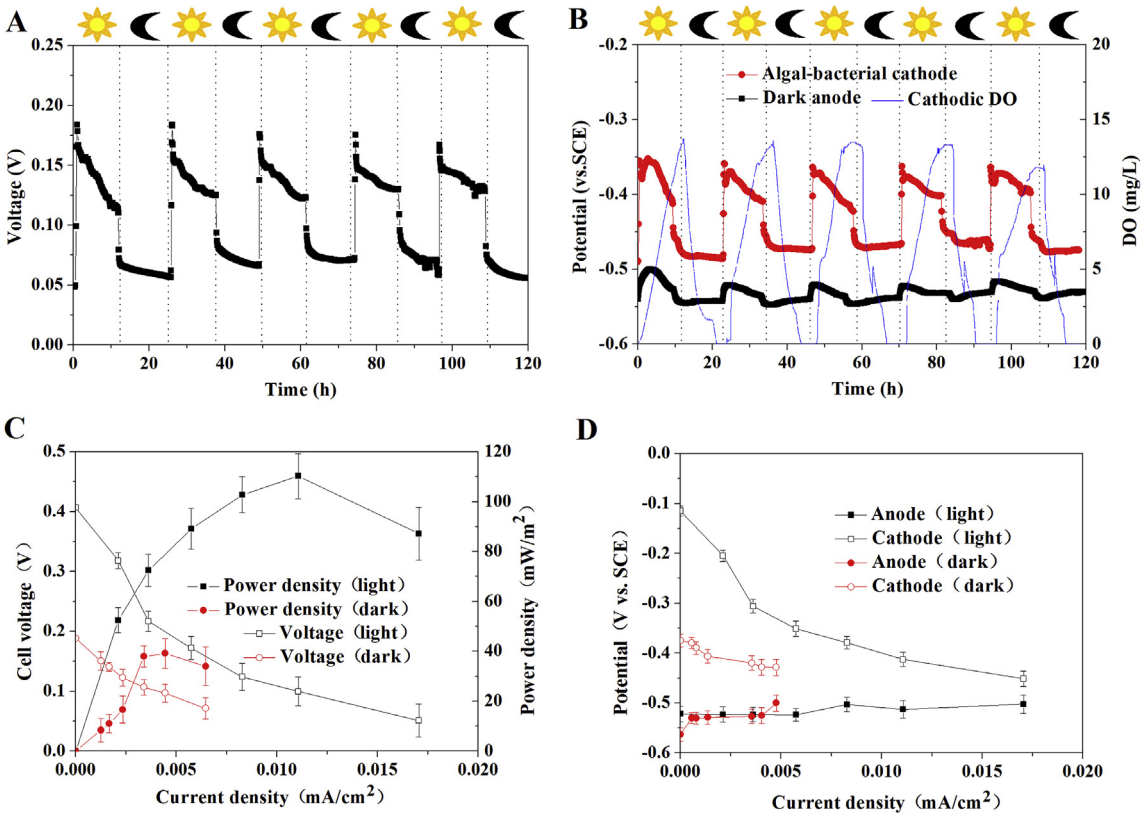

Fig. 1. Voltage output (A), electrode potential and DO (B), power output (C), electrode polarization (D) and variation of concentration of nitrogen species (E) during nitrogen removal test in the algal–bacterial biocathode PBES with daily light-dark cycle (12:12 h to simulate the natural day/night cycle). Error bars represent standard deviations of replicate samples (n = 3).

were collected at regular time interval during the entire operation period (120 h). Fig. 2A shows the time courses of different nitrogen species concentration in the algal–bacterial biocathode at a fixed external resistor of 500 Ω. The NH_4–N concentration experienced a continuous decrease from the beginning of the operation and the removal rate of the NH_4–N was obviously faster under illumination than that in dark, most likely due to the activity of nitrifying bacteria which can transform ammonia to nitrate in the presence of DO. Complete removal of 314 mg/L NH_4–N was achieved within 120 h. Continuous decrease in NH_4–N concentration and complete removal of NH_4–N also suggested that the dissimilatory nitrate reduction to ammonia was significantly slower than that of ammonia oxidation or did not take place in the algal–bacterial biocathode.

The trend for NO_3–N removal was different from that of NH_4–N. The concentration of NO_3–N was fluctuated throughout the entire operation period depending on the daily light/dark cycle, but showed a continuously decreasing trend. The fluctuation in concentration of NO_3–N could be resulted from the coinstantaneous process of ammonia oxidation to nitrate and nitrate removal. If all NH_4–N was oxidized to NO_3–N and the NO_3–N was not further removed, the NO_3–N accumulated at the cathode could up to 643 mg/L (sum of concentration of NO_3–N from NH_4–N oxidation and initial NO_3–N concentration). However, there was no accumulation of large amount of NO_3–N at the cathode and 86% NO_3–N was removed with 192 h (based on complete transformation of NH_4–N to NO_3–N). It is worth noting that decrease in the concentration of NO_3–N was also observed during light period cycle, which is most likely attributed to the NO_3–N removal through aerobic and anaerobic denitrification processes due to coexistence of aerobic, anoxic and anaerobic zones (inside and back of the electrode without illumination) within the cathode. Meanwhile, the NO_2–N increased sharply and accumulated in the initial stage, but its concentration gradually decreased in the later phase with decrease of NH_4–N and NO_3–N concentration. The possible reason for this trend is that nitrite oxidizers lagged behind ammonia oxidizer activity, particularly when they had to compete for oxygen when it was limiting since the NO_2–N is not only the product of ammonia oxidizers but also the substrate of nitrite oxidizers. Based on nitrogen mass balance calculation, maximum nitrogen removal efficiency of 83% can be achieved.

In order to investigate how the bioelectrochemical process contributes to the nitrogen removal in the algal-bacterial biocathode of the PBES with daily light/dark cycle, different modes were tested for comparison. Fig. 2B shows the time courses of NH_4–N under different modes. It was observed that the NH_4–N removal rate under close circuit condition (500 Ω) was similar to that under open circuit condition, indicating electric current under this resistor has insignificant effect on NH_4–N removal. Comparatively, the NH_4–N removal rate was slowed down slightly under close circuit condition of 50 Ω as compared to that under open circuit condition. In the biocathode, nitrification of NH_4–N would consume a portion of DO which was simultaneously served as the terminal electron acceptor for the cathode. Thus, there seems to exist a competition between NH_4–N and the cathode for DO. Increase of current by incorporating a lower resistor into the electric circuit will result in increased consumption of the DO in the biocathode, making less DO available for the nitrification of NH_4–N. It should be noted that the abiotic control experiment also showed 30% of NH_4–N removal after 120 h-operation, mainly attributed to

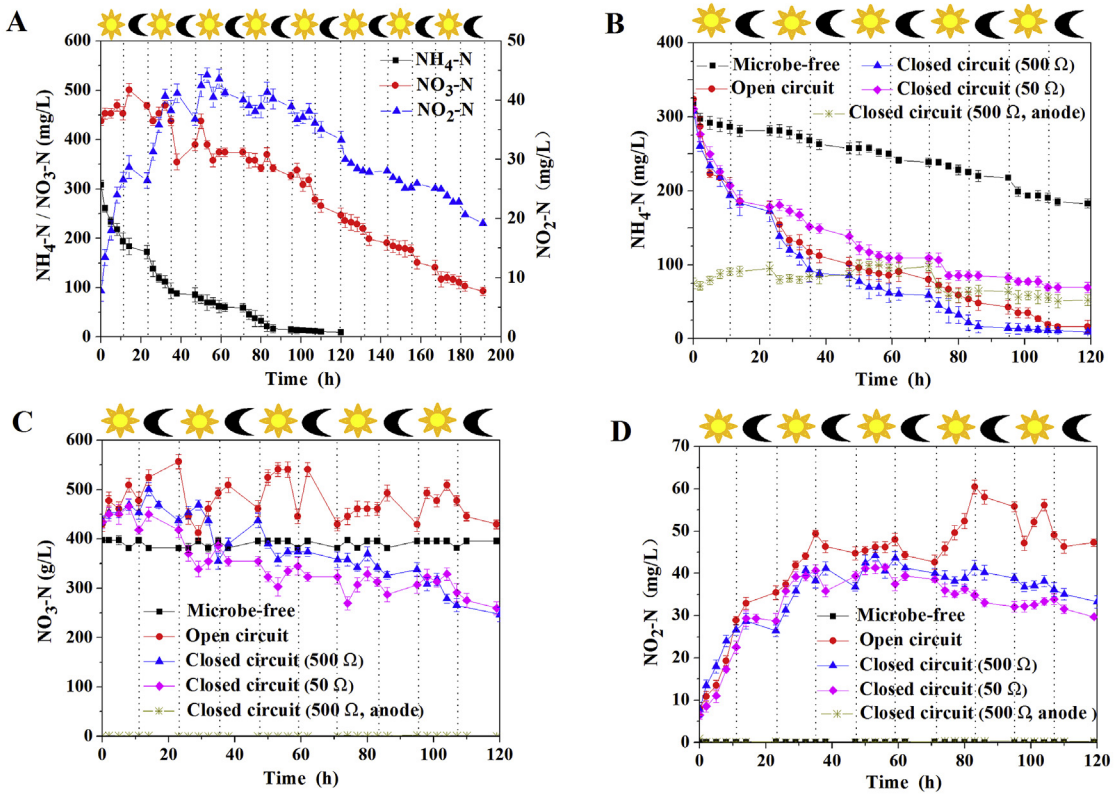

Fig. 2. Variation of concentration of NH_4–N (A), NO_3–N (B) and NO_2–N (C) during nitrogen removal test in the algal–bacterial biocathode of the PBES with daily light/dark cycle under different conditions.

spontaneous NH_4^+ diffusion through the cation exchange membrane from cathode to anode because of its positive charge and concentration gradient which was significantly diminished in the PBES due to bio-generated electric field driven actions diffusion from anode to cathode (Fig. 2B). Thus, the NH_4–N loss in the cathode of the PBES was significantly less than that in the abiotic control reactor.

Different from NH_4–N removal, the NO_3–N removal rate under close circuit condition was significantly higher than that of the open circuit control (Fig. 2C). With an initial NO_3–N concentration of 643 mg/L (sum of concentration of NO_3–N from NH_4–N oxidation and initial NO_3–N concentration), the removal efficiency could reach 68% after 120 h under close circuit condition (500 Ω), whereas only 33% of NO_3–N was removed under open circuit condition, indicating that the electricity generation was responsible for approximately 35% NO_3–N removal. The total Coulombs calculated by integrating current over time (120 h) are 887 C which can be used to reduce 105 mg/L NO_3–N to N_2. Moreover, the cathodic bioelectrochemical process could enhance the metabolism of the bacteria by using the cathode as electron donor and the nitrate as electron acceptor, and thus enhance the NO_3–N removal through bacterial nitrogen assimilation because the cathode potential has a positive effect on microbial physiology, which include changing the cell surface properties, increasing the enzyme activity, as well as shortening the doubling time of the bacteria (Huang et al., 2011). The slight NO_3–N removal under open circuit condition could be due to the heterotrophic denitrification with organic matters produced by photosynthesis of alga as carbon source and direct electrochemical reduction (Zhang et al., 2012). No obvious change in NO_3–N concentration was observed in abiotic cathode, indicating that neither adsorption nor degradation occurred in the abiotic processes. A substantial accumulation of NO_2–N was observed under open circuit control, whereas the accumulation of NO_2–N was alleviated significantly under close circuit condition (500 Ω) (Fig. 2D). The removal of NO_3–N and NO_2–N was further accelerated with a lower resistor of 50 Ω than that with 500 Ω. Although lower nitrite oxidation rate compared to ammonia oxidation rate could contribute to the accumulation of NO_2–N, however, the nitrite oxidation was not a current independent process at the biocathode. Thus, the faster removals of NO_3–N and NO_2–N under closed circuit condition compared to that under open circuit condition and further accelerations in NO_3–N and NO_2–N removal by using a low external resistor were mainly attributed to the bioelectrochemical reduction with the cathode directly serving as the electron donor. It should be noted that the acceleration in NO_3–N removal was gradually reduced at 50 Ω compared to that at 500 Ω after 96 h of operation due to decreased electrons supply for cathodic reduction of nitrate which resulted from continuous consumption of glucose in the anode. There was no detectable NO_3–N and NO_2–N at the anode compartment throughout the 120 h experiment period because the anions can not pass through the cation exchange membrane and thus excluding the possibility of NO_3^- and NO_2^- diffusion from the cathode to the anode. These results indicate that the algal–bacterial biocathode with daily light/dark cycle can provide multiple approaches for NO_x-N removal, in which the bioelectrochemical denitrification by using the cathode as the sole electron donor played a major role (Cecconet et al., 2018), whereas a minor role might be attributed to heterotrophic denitrification using photosynthetically produced dissolved organic matters as carbon source.

3.2.2. Contribution of photosynthesis to nitrogen removal

To understand the contribution of photosynthesis of alga to the nitrogen removal in the algal–bacterial biocathode, a comparison was made between the algal–bacterial biocathode and the aerated biocathode. As shown in Fig. 3A, accelerated removal of NH_4–N was observed in the algal–bacterial biocathode as compared to the aerated biocathode at any giving time during the 120 h nitrogen removal experiment. The NH_4–N was removed almost completely after 120 h in the algal–bacterial biocathode while 88% was removed for the aerated biocathode. Accelerated NH_4–N removal in the algal–bacterial biocathode was attributed to the high concentration of DO released by alga, which increases the metabolic rate of nitrifying bacteria (Ginestet et al., 1998). The DO concentration was maintained at around 4 mg/L by controlling the aeration rate to avoid biofilm destruction in the aerated biocathode (Fig. 3E). Although further improvement in NH_4–N removal by increasing aeration rate could be expected, however, the energy consumption could also be increased substantially. It is vital that the alga produced a much high DO concentration of 14 mg/L through photosynthesis under illumination which is 1.85 folds higher than that in oxygen-saturated deionized water at the same temperature (7.56 mg/L), and thus enhanced NH_4–N removal was achieved due to the super saturation of electrolyte with DO in the algal–bacterial biocathode. The NH_4–N concentration also decreased slightly at a very low rate during dark period, which might be attributed to algal-bacterial nitrogen uptake (Xiao et al., 2012).

The time-course of NO_3–N and NO_2–N concentration in the algal–bacterial biocathode and aerated biocathode were presented in Fig. 3B and C. The NO_3–N removal rate was relatively lower in the algal–bacterial biocathode than that in the aerated biocathode in the early phase, however, it exceed gradually that of aerated biocathode in the later phase during the 120 h nitrogen removal experiment. Similarly, NO_2–N removal rate in the algal–bacterial biocathode was similar to that in the aerated biocathode during the first 53 h and much higher that of the aerated biocathode during later hours. Slow NO_3–N removal in the algal–bacterial biocathode compared to that in the aerated biocathode in the early phase might be due to high concentration of potosynthetic DO production by alga suppress the expression of necessary enzyme

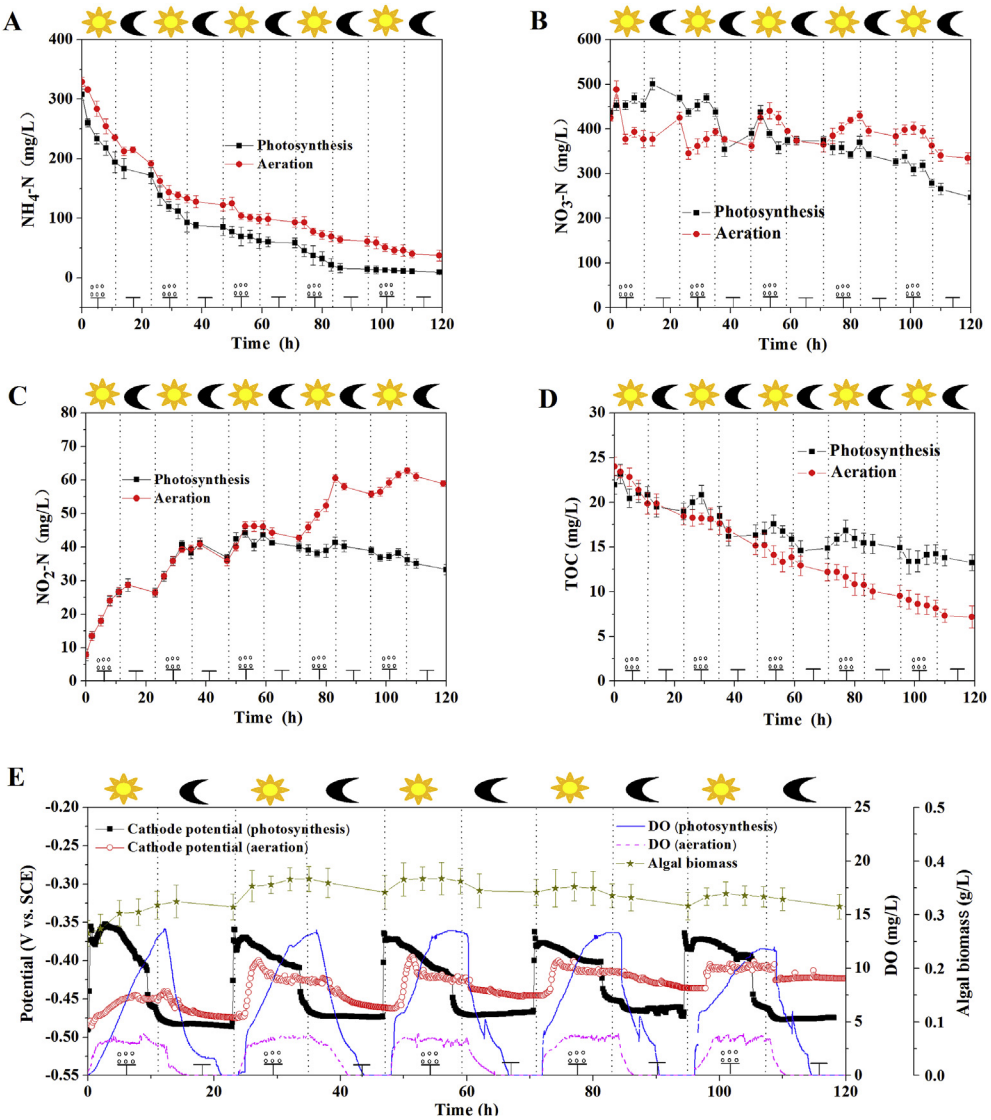

Fig. 3. Comparison of removal of NH_4–N (A), NO_3–N (B), NO_2–N (C), TOC (D) and change of cathode potential, DO and algal biomass (E) in the algal–bacterial biocathode using photosynthesis and aeration for oxygen supply during light period.

system in denitrification process, resulting in a large amount of nitrate as electron accepters for denitrification could not be reduced during the light period. Comparatively, the DO concentration was maintained at 4 mg/L in the aerated biocathode which could not sufficient to significantly inhibit denitrification process since limited denitrification was observed at DO level around 7.24 mg/L according to previous study (Virdis et al., 2010). Meanwhile, the algal–bacterial biocathode resulted in relatively low cathode potential than that of the aerated biocathode during dark, might be due to coinciding release of carbon dioxide and consumption of oxygen by the process of algal respiration in the absence of light which could help to maintain an anaerobic condition for denitrification.

It was important to note that the rate of denitrification in the aerated biocathode was greatly slowed down in the later phase while the algal–bacterial biocathode achieved a high denitrification performance until the end of the experiment. This observation was attributed to the difference in availability of organic carbon source for denitrification between the two types of biocathodes. As illustrated in Fig. 3D, TOC concentration in the aerated biocathode decreased as a function of time due to continuous consumption of organic carbon by aerobic carbon oxidation and denitrification, whereas its concentration in the algal–bacterial biocathode fluctuated depending on the light/dark cycle and maintained at a relatively high concentration throughout the 120 h experimental period. This result underlines that the photosynthesis of alga can provide additional organic carbon source for denitrification which contribute to the sustainable NO_x-N removal through heterotrophic denitrification in the algal–bacterial biocathode. In addition, a certain amount of biodegradable organics may keep a proper growth of heterotrophic biofilm which may helpful for improvement of the NO_x-N reduction in the biocathode during the dark period (Huang et al., 2011).

3.2.3. Bacterial community analysis

To discover functional and taxonomic diversity of the algal–bacterial biofilm bacterial communities, the bacterial community structure in the biofilm of algal–bacterial biocathode was analyzed and compared with that of aerated biocathode and dark anode by high-throughput sequencing. The taxonomic classification and bacterial diversity at the phylum and genus levels were presented in Fig. 4. On the phylum level (Fig. 4A), the *Proteobacteria* was the largest phylum in the biofilm of algal–bacterial biocathode, accounting for 51.1% of the total effective bacterial sequences, which was obviously higher than that of the dark anode (40.56%) and the aerated biocathode (46.84%) indicating selective enrichment of *Proteobacteria* in the algal–bacterial biocathode. It was reported that most of the known nitrifying (*Nitrosomonas*, *Nitrosococcus*, *Nitrobacter*, *Nitrotoga*, *Nitrococcus*, and *Nitrospina*) and denitrifying genera (*Bradyrhizobium, Azospirillum* and *Pseudomonas*) belonged to *Proteobacteria*, which played an important role in nitrogen removal (Isobe and Ohte, 2014; Gómez-Villalba et al., 2006). Other dominant phylums within the biofilm of algal–bacterial biocathode were *Firmicutes* (19.99%), *Bacteroidetes* (18.98%) and *Actinobacteria* (5.16%). *Firmicutes* and *Bacteroidetes* were frequently detected in heterotrophic denitrification systems (Demaneche et al., 2009; Fernandez et al., 2008) while *Actinobacteria* comprise diverse groups of bacteria capable of degradation of complex organic matters (Alvarez et al., 2017). The abundance of *Firmicutes* and *Actinobacteria* were found to be higher in the biofilm of algal–bacterial biocathode than that of aerated biocathode but lower than that of the dark anode. In contrast, the abundance of *Bacteroidetes* was much higher in the biofilm of algal–bacterial biocathode than that of dark anode but slightly lower than that of aerated biocathode. *Planctomycetes* capable of anaerobic ammonium oxidation (anammox) were also detected in the three biofilm samples. However, its abundance is extremely low and thus has a negligible effect on nitrogen removal in the biocathode. The observed differences in abundance of dominant phylums within the three biofilm samples could be largely attributed to the differences in oxygen exposure duration and organic and inorganic carbon source concentration in their growth medium. Daily light/dark cycle-induced photosynthesis and respiration of algae created an alternate aerobic/anaerobic condition and intermittent organic and inorganic carbon source supply, when an anaerobic organic-rich condition and an autotrophic condition were maintained in the dark anode and the aerated biocathode, respectively.

Phylogenetic analysis on genus level further reveals potential functions of the dominant bacterial species (Fig. 4B). *Azospira* showed the greatest abundance (12.58%) within the biofilm of the algal–bacterial biocathode but rare within the biofilm of dark anode (4.08%) and aerated biocathode (less than 1%). It is widely reported that the *Azospira* is functionally heterotrophic denitrifying-related genus in groundwater and biological nitrogen removal systems (Bae et al., 2007; Zhou et al., 2016). Consequently, *Azospira* potentially played the largest role in anaerobic denitrification in the algal–bacterial biocathode. *Proteiniclasticum* (6.75%), *Azospirillum* (4.12%), *Bacillus* (3.99%), *Chryseobacterium* (3.82%), *Comamonas* (2.67%), *Brucella* (2.4%) *Shinella* (2.38%), *Rhodopseudomonas* (2.17%) and *Nitrosomonas* (1.66%) were sub-dominant genus in the algal–bacterial biocathode. *Azospirillum* was only enriched in algal–bacterial biocathode and absent in other two samples. Many species of the genus *Azospirillum* are reported as important autotrophic denitrifiers (Kloos et al., 2001), with some being able to perform aerobic denitrification (Molina-Favero et al., 2008). *Bacillus*, *Comamonas* and *Chryseobacterium* have heterotrophic nitrification and aerobic denitrification abilities, converting ammonia to nitrogen aerobically (Zhang et al., 2012; Chen and Ni, 2011). *Brucella* has the capacity to utilize nitrate as an alternative electron acceptor for respiration (Haine et al., 2006). *Rhodopseudomonas* as potential photoautotrophic denitrifying bacteria can use electrons and reducing power from cathodes of BES (Li et al., 2016). *Nitrosomonas* belongs to the typical ammonia-oxidizing bacteria was distinctly enriched in algal–bacterial biocathode and aerated biocathode but not in the dark anode, indicating oxygen exposure is essential for its growth. The presence of *Nitrosomonas* could largely contribute to excellent nitrification in the algal–bacterial biocathode. Both *Shinella* and *Proteiniclasticum* are heterotrophic decomposers. The former is facultative anaerobic bacteria and proficient in degrading biopolymer (Bai et al., 2009), while the latter is capable of aerobic degradation of heterocyclic compounds (Zhang et al., 2010).

Based on the results discussed above, algal activity under daily light/dark cycle created an alternated aerobic/anaerobic environment and intermittent organic and inorganic carbon source supply, resulting in co-existence of nitrifying and denitrifying bacteria, as well as organic degradation bacteria and contributing to nitrogen removal in the algal–bacterial biocathode.

3.3. Effect of important operating parameters on nitrogen removal and cathode performance

3.3.1. Nitrogen content

The effect of nitrogen content on the nitrogen removal in the algal–bacterial biocathode was investigated at two different initial nitrogen contents: 314 mg/L NH_3–N, 330 mg/L NO_3–N and 157 mg/L NH_3–N, 165 mg/L NO_3–N. As shown in Fig. 5A, NH_4–N removal was significantly faster at high initial nitrogen concentration than that at low initial nitrogen concentration at the beginning of the nitrogen removal test which resulted in almost similar final NH_4–N

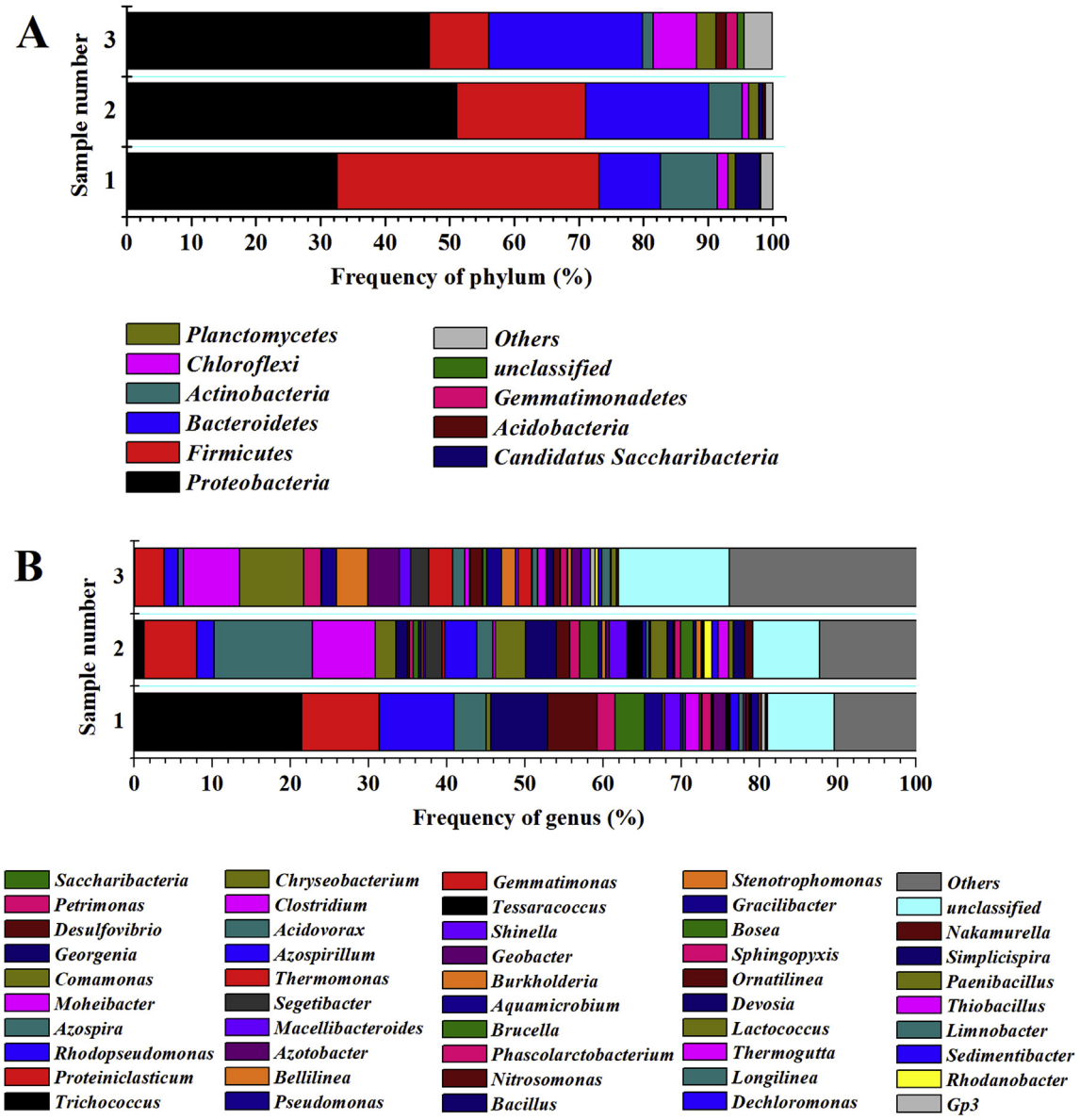

Fig. 4. Taxonomic classification of bacterial DNA sequences from communities of dark anode biofilm, algal–bacterial biocathode biofilm and aerated biocathode biofilm. at the phylum level (A) and (B) the genus level. The characters of "1", "2" and "3" represent the communities in dark anode biofilm, algal–bacterial biocathode biofilm and aerated biocathode biofilm, respectively.

removal efficiency for both the nitrogen content levels (97% vs. 92%). Meanwhile, NO_3–N was transiently accumulated as nitrogen concentration increased due to fast nitrification of NH_4–N to NO_3–N, and then rapidly declined. It is worth noting that high nitrogen content did not result in higher accumulation of NO_2–N compared to low nitrogen content due to accelerated removal of NO_2–N by the denitrification process. Based on nitrogen mass balance calculation, total nitrogen removal efficiency of 63% and 68% was obtained for the low and high nitrogen concentration, respectively. These results indicated that nitrogen content at the two levels did not appreciably affect total nitrogen removal efficiency but the removal of the three nitrogen species was accelerated at high nitrogen content as compared to that at low nitrogen content.

Cathodic potential, algal growth and DO revealed the potential mechanisms of the nitrogen content effect (Fig. 5B and C). The increases in the nitrogen content resulted in higher algal biomass and DO concentration, and can sustain a higher cathodic potential longer. This result was due to the fact that high nitrogen content promotes higher photosynthetic activity and the production of oxygen, which will be available for the nitration reaction of NH_4–N during light period. Thus, accelerated NH_4–N removal at high nitrogen content in the algal–bacterial biocathode is mainly attributed to enhanced nitration and algal uptake (Zhang et al., 2011). Furthermore, the enhanced photosynthesis of alga at high nitrogen content released more dissolved organic matters which could promote the aerobic and anaerobic heterotrophic denitrification during the light/dark cycle (Luo et al., 2017), resulting in accelerated removal of NO_x-N, since both aerobic and anaerobic heterotrophic denitrifying bacteria were detected simultaneously in the algal–bacterial biocathode (Fig. 4). Additionally, senescing algal biomass could also stimulate the denitrification rate by providing labile organic carbon fractions to denitrifiers (Mcmillan et al., 2008).

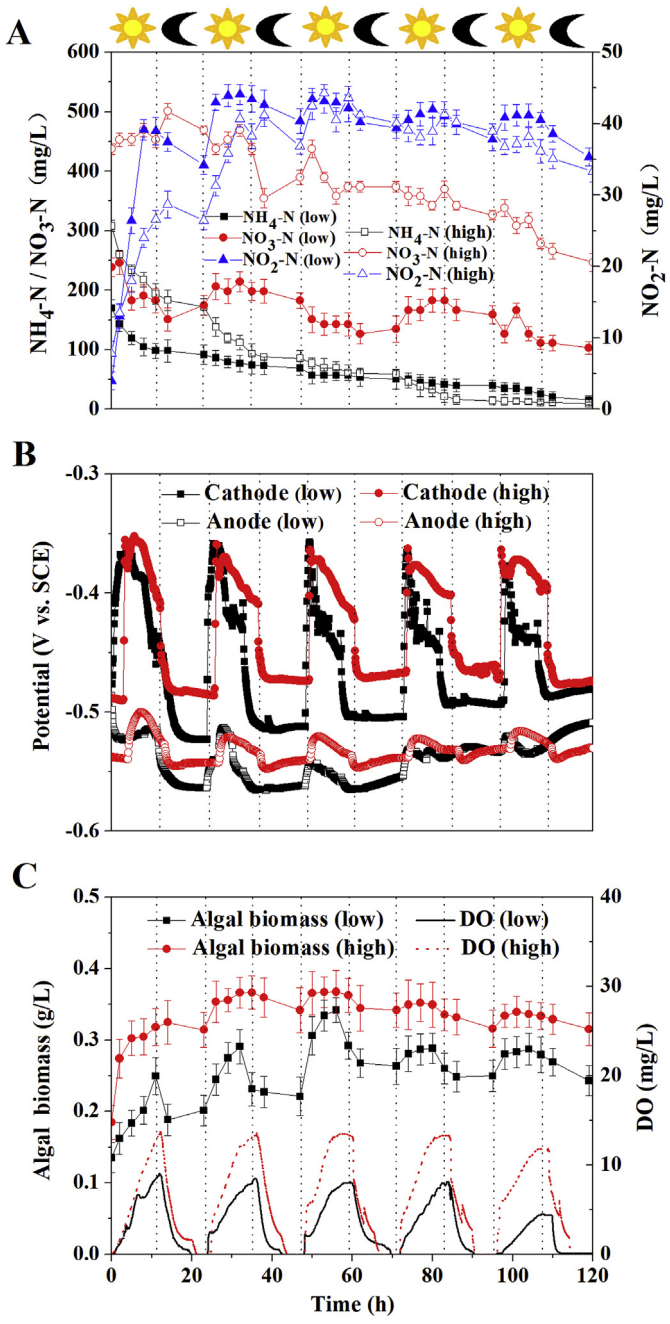

Fig. 5. Comparison of nitrogen removal performance (A) and change of electrode potential (B) and algal biomass and DO (C) in the algal–bacterial biocathode under different initial nitrogen content.

The biocathode at high initial nitrogen concentration also showed superior performance compared to that at low initial nitrogen concentration. The potential of biocathode was obviously higher and more durable at high initial nitrogen concentration than that at low initial nitrogen concentration during both the light and dark periods of the light-dark cycle (Fig. 5B). Enhanced photosynthetic DO release and accelerated production of nitrate from NH_4–N nitration were responsible for the improved biocathode performance due to increase in cathodic electron acceptors concentration (Fig. 5A and C).

3.3.2. Phosphate buffer

The effect of phosphate buffer on nitrogen removal in the algal–bacterial biocathode was tested. Fig. 6A shows the time courses of three nitrogen species concentration in the algal–bacterial biocathode under different phosphate buffer concentrations (10 and 50 mM). The removal efficiencies of NH_4–N was decreased from 97% to 77% as the phosphate buffer concentrations decreased from 50 to 10 mM although the NH_4–N removal rates were comparable under the two different phosphate buffer concentrations during the initial 32 h. NO_3–N concentrations followed the same variation trend observed for NH_4–N. The NO_3–N had accumulated to a final concentration of 318 mg/L at the end of the operation cycle in the biocathode with 10 mM phosphate buffer which is 22% higher than that accumulated in the biocathode with 50 mM phosphate buffer. The deterioration in nitrogen removal efficiency at low phosphate buffer could be mainly caused by the limitation of algal growth under low phosphorus availability which could reduce the amount of the nitrogen removed by algal uptake (Fig. 6D) (Paskuliakova et al., 2016; Yu et al., 2015). The reduction in algal biomass also resulted in the decrease in DO concentration (Fig. 6D) along a rapid decrease in cathodic potential during light period (Fig. 6C), which might partly contribute to the low NH_4–N removal efficiency in the algal–bacterial biocathode with 10 mM phosphate buffer due to reduced DO supply for nitrification. Fig. 6B shows the pH variation of catholytes with different phosphate buffer concentration. The pH of the catholyte in the algal–bacterial biocathode was found to be fluctuated depending on the light/night cycle which tended to increase during light period and decrease during dark, but showed an overall increasing trend over time. In addition, compared to the low concentration phosphate buffer, high concentration phosphate buffer was more effective in maintaining the stabilization of catholyte pH. During the 120 h experimental period, the pH of the catholyte showed only a slight increase at 50 mM phosphate buffer (from 6.92 to 7.08) whereas the pH increased rapidly from 6.95 to 7.54 when the phosphate buffer concentration was reduced to 10 mM. The variation in pH in the algal–bacterial biocathode could be a result of the combined effect of the protons diffusion from the anode to the cathode, cathode oxygen reduction, nitrification and denitrification. During light period, the proton generated by nitrification and the proton migrated from the anode could compensate proton consumption by cathodic oxygen reduction (You et al., 2009) and thus maintain a stable catholyte pH while the alkalinity produced from denitrification (Cheng et al., 2012) during dark can only partially be neutralized by the acidity produced during light period of the next light/night cycle which caused gradually rise of catholyte pH. Overall, the pH of catholyte can be maintained at a level appropriate for algal and bacterial growth under 10 mM phosphate buffer (Tang et al., 2011; Xiao et al., 2014).

3.3.3. Organic carbon content

Besides nitrogen content, organic carbon content is another important operation parameter in the PBES system, especially for actual wastewater treatment. The effect of organic carbon content on the nitrogen removal in the algal–bacterial biocathode was investigated by adding glucose to the biocathode at the beginning of the operation of the PBES. Two different glucose contents (0.013 and 0.13 g) were used in the test. As shown in Fig. 7A–C, supply of additional glucose caused decreases of NH_4–N removal and increases of NO_x-N removal in the algal–bacterial biocathode during daily light/dark cycle, indicating that when additional organic carbon source was added, nitrification was weakened whereas denitrification was enhanced. The negative effect on nitrification and

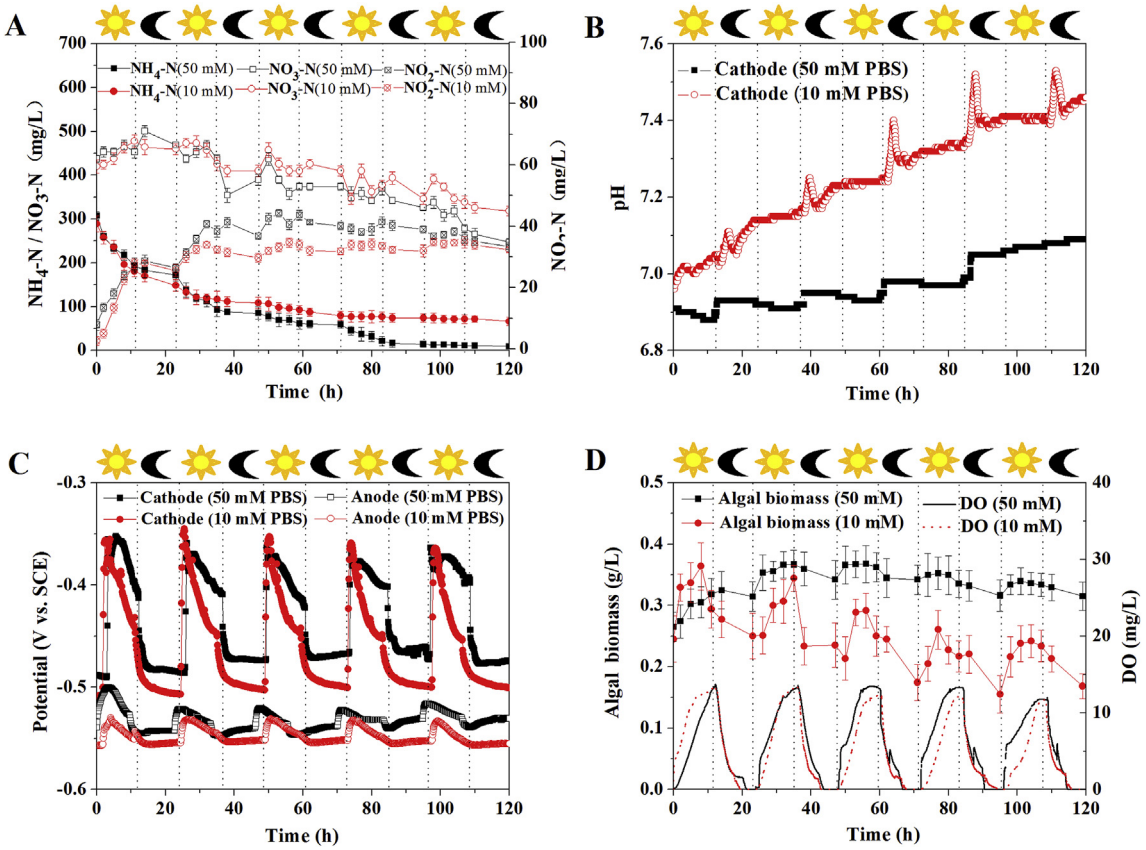

Fig. 6. Comparison of nitrogen removal performance (A) and change of pH (B), electrode potential (C) and algal biomass and DO (C) in the algal–bacterial biocathode under different phosphate buffer concentration.

positive effect on denitrification by glucose addition was further enhanced with the increase of glucose concentration (from 0.013 to 0.13 mg/L). The deterioration in performance of the biocathode for NH_4–N removal with the addition of glucose was likely due to the higher oxygen demand for glucose oxidation and the fact that glucose competes with the NH_4–N for oxygen (Hanaki et al., 1990). The enhanced denitrification with addition of glucose could have been expected since biodegradable organic carbon can serve as endogenous electron donor for heterotrophic denitrification, which was responsible for the enhanced removal of NO_x-N. Algal biomass and DO at the biocathode was monitored at regular intervals to correlate with the nitrogen removal (Fig. 7D). The addition of glucose led to fast growth of *chlorella* and the algal biomass concentration was significantly higher than that without the addition of glucose because algae grown under mixotrophic conditions metabolized both heterotrophically and autotrophically, this resulted in a stimulated growth rate. The fast growth of *chlorella* could lead to enhanced nitrogen removal, especially for NH_4–N because of more efficient utilization of NH_4–N than NO_x-N by *chlorella* (Najm et al., 2017). Increments in the algal biomass concentration also resulted in concomitant increase in the DO concentration which could contribute to enhancement of the nitrification at the algal–bacterial biocathode. However, the nitrification was weakened, probably due to excessive consumption of DO for organic carbon degradation. Cathode potential and TOC was also monitored which can help to provide additional indication of effect of glucose addition on nitrogen removal in the algal–bacterial biocathode. As shown in Fig. 7E, the cathode potential decreased faster during light period in the cathode with addition of glucose than that in the cathode without addition of glucose which can be attributed to reduced DO supply to the cathodic biofilm resulting from organic carbon oxidation by aerobic heterotrophs (Huang et al., 2011). This result provided further evidence that deterioration of NH_4–N removal after addition of organic carbon was due to DO consumption by microbial carbon oxidation which decreased NH_4–N nitrification rate. However, it can be noted that the cathode potential was more negative in the biocathode with addition of glucose than that in the biocathode without addition of glucose during the dark periods. Low cathode potential was in favor of the NO_x-N removal since the nitrate and nitrite reduction rate generally positively correlated with the negativity of the cathode potential (Pous et al., 2015). Although the concentration of TOC decreased rapidly at the beginning of the experiment in the biocathode with addition of glucose but it was maintained at a much higher value until the end of the experiment as compared to the biocathode without addition of glucose (Fig. 7F). This is mainly attributed to the high concentration algal biomass production due to heterotrophic growth of alga in the presence of high concentration of organic carbon source which cause significant increase in photosynthetically produced dissolved organic carbon (Zhuang et al., 2016). These dissolved organic carbons can be utilized by denitrifying bacteria for enhancing the removal of NO_x-N.

3.4. Proposed pathways for nitrogen removal in the algal-bacterial biocathode

The pathways for the removal of nitrogen in the algal-bacterial biocathode with daily light/dark cycle were proposed based on the nitrogen removal data and bacterial community data (Fig. 8). In

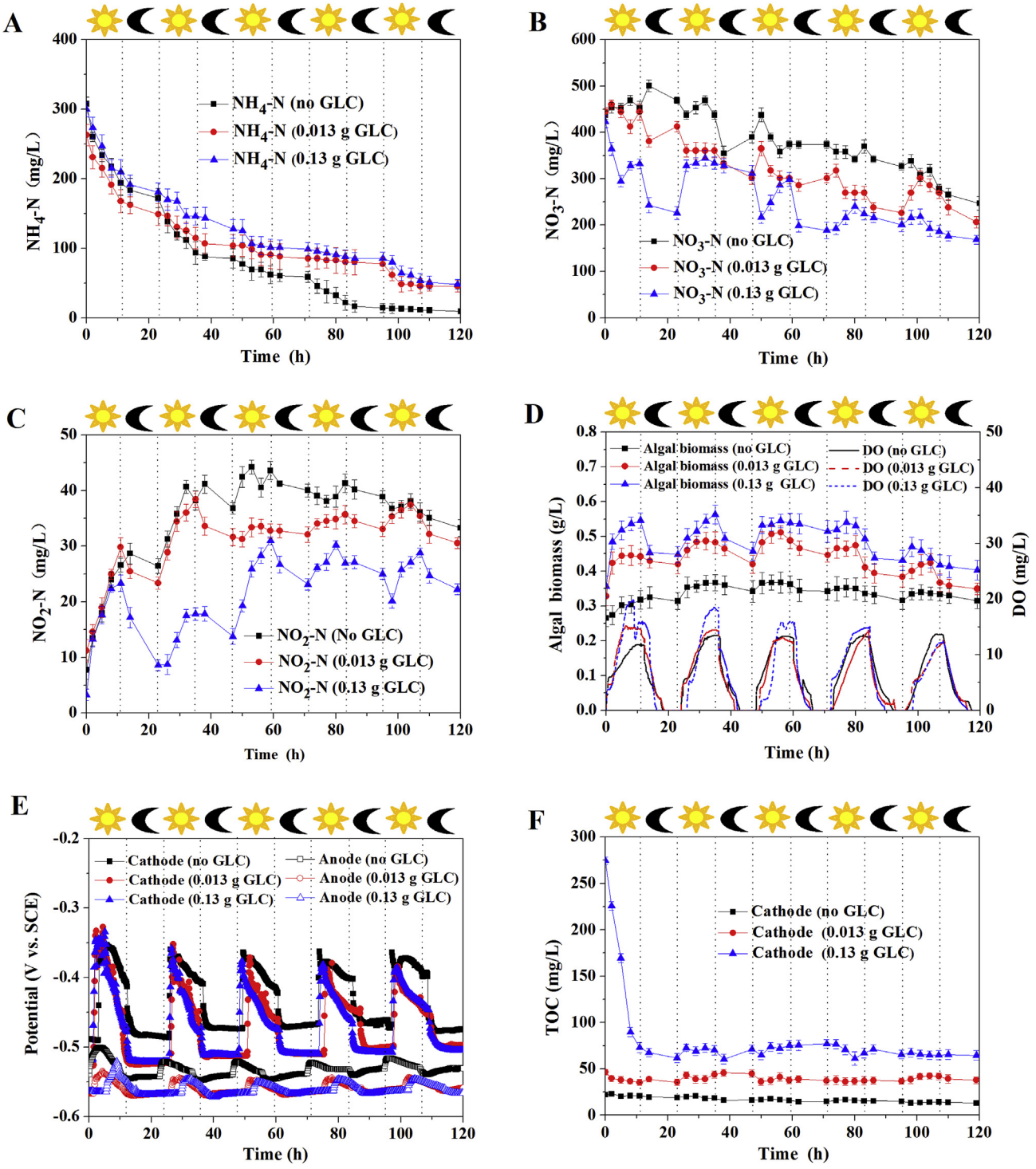

Fig. 7. Comparison of removal of NH_4–N (A), NO_3–N (B) and NO_2–N (C) and change of algal biomass and DO (D), electrode potential (E) and DO in the algal–bacterial biocathode under addition of different concentration of glucose.

the algal-bacterial biocathode, the alga plays a crucial role in removing of nitrogen, not only because it can direct assimilation of nitrogen through production of algal biomass but also because the photosynthesis and respiration of alga induced by daily light/dark cycle can create an alternating aerobic/anaerobic conditions which can provide heterogeneous niches to sustain diverse bacterial communities for various nitrogen species removal. Bacterial community analysis revealed the co-existence of nitrifiers, aerobic and anaerobic denitrifiers, and autotrophic denitrifiers in the algal-bacterial biocathode that enable alternating (or simultaneous) nitrification and denitrification nitrogen removal process occur in the algal-bacterial biocathode under daily light/dark cycle. During light period, photosynthesis of alga produces oxygen which can be utilized by aerobic nitrifiers to conversion of NH_4–N to NO_2–N (Equation (1)) and further to NO_3–N (Equation (2)) while simultaneously releasing dissolved organic matters (glucose) as organic

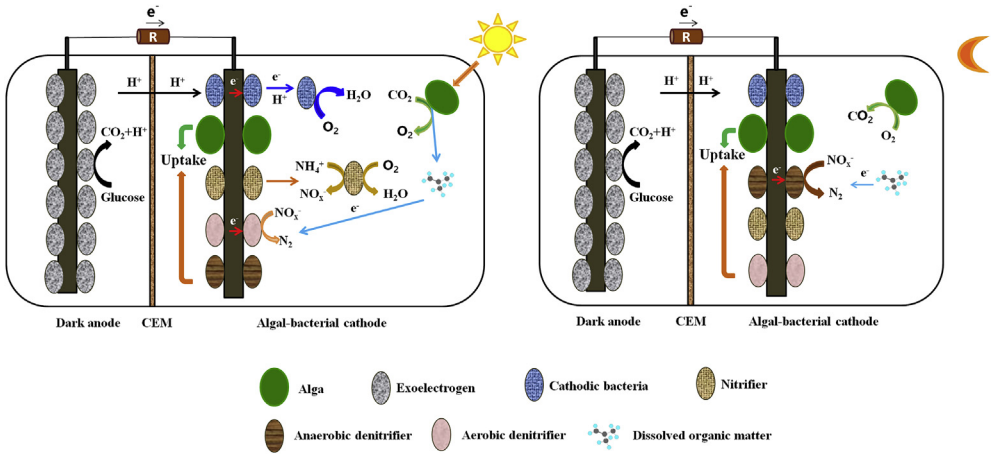

Fig. 8. Proposed pathways for nitrogen removal in the algal–bacterial biocathode under daily light/dark cycle.

carbon source for aerobic denitrifiers to reduce NO_3–N (Equation (3)). During dark period, respiration of alga can consume dissolved oxygen and help maintain an anaerobic environment in the algal-bacterial biocathode. The anaerobic denitrifiers couple the oxidation of photosynthetically produced dissolved organic matters (glucose) to the reduction of NO_3–N (Equation (3)). In addition, the autotrophic denitrifiers available on the cathode are able to respire with electrode as the electron donor and NO_x-N as the terminal electron acceptor (Equation (4)). Although the specific microbial reactions, which were responsible for the nitrogen removal, were different depending on light and dark regimes, however, the concurrence of these reactions during light period could not be excluded due to the coexistence of aerobic, anoxic and anaerobic zones (inside and back of the electrode without illumination) within the biocathode.

$$2\ NH_4^+ + 3O_2\ 2\ NO_2^- + 4\ H^+ + 2H_2O \quad (1)$$

$$2\ NO_2^- + O_2\ 2\ NO_3^- \quad (2)$$

$$5C_6H_{12}O_6 + 24\ NO_3^-\ 12\ N_2 + 30\ CO_2 + 18H_2O + 24\ OH^- \quad (3)$$

$$2\ NO_3^- + 12\ H^+ + 10\ e^-\ N_2 + 6H_2O \quad (4)$$

4. Conclusion

Self-sustained power generation in the PBES was achieved under day/night cycle by alternately using photosynthetic dissolved oxygen and nitrate/nitrite as electron acceptors in the biocathode.

High concentration of nitrogen removal in the algal-bacterial biocathode was achieved by combined processes of bioelectrochemical reduction and algal-bacterial interactions during power generation, including nitrifying using the photosynthetic oxygen, bioelectrochemical denitrification using the cathode as sole electron donor, heterotrophic denitrification using the photosynthetically produced dissolved organic matters as carbon source and algal-bacterial uptake.

Accelerated nitrogen removal and improved cathode performance were achieved at high concentration of nitrogen and phosphate buffer due to enhanced photosynthetic oxygen releases and algal-bacterial interactions for nitrogen transformation. Addition of external organic carbon has negative effect on NH_4–N removal and cathode performance due to reduced oxygen supply for nitrification and cathode oxygen reduction resulting from oxygen consumption by aerobic carbon oxidation whereas it enhanced NO_x-N removal, mainly attributed to the continuous release of high concentration of photosynthetically produced dissolved organic matters as carbon source for heterotrophic denitrification.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.01.191.

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