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Aerobic Denitrification Is Enhanced Using Biocathode of SMFC in Low-Organic Matter Wastewater

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Abstract: Nitrate (NO_3^-) in wastewater is a rising global threat to ecological and health safety. A sufficient carbon source, as the electron donor, is essential in the conventional biological denitrification process. It is not appropriate to add extra carbon sources into specific water bodies in terms of material cost and secondary pollution. Thus, innovative NO_3^- removal technologies that are independent of carbon sources, are urgently needed. This study constructed sediment microbial fuel cells (SMFCs) for aerobic denitrification in low-organic matter wastewater and explored the key factors affecting denitrification efficiencies. The SMFC treatments removed 72–91% NO_3^- through two main denitrifying stages which were driven by carbon sources (COD) and generated electrons, respectively. After COD was fully consumed, denitrification efficiencies were enhanced in SMFC treatments by 24–47% using the generated electrons within 3 days. In this stage, the NO_3^- removal efficiencies were positively correlated with external current intensities ($p < 0.05$). The improved denitrification efficiencies were attributed to two enriched phyla in the SMFC cathode. The dominant genera also demonstrated the heterotrophic denitrifying capacity of the SMFC biocathode. Furthermore, electrical characteristics could be used to monitor or regulate the denitrification process in the SMFC system. In conclusion, this study presents an innovative treatment strategy that is economical and eco-friendly compared with conventional physicochemical methods.

Keywords: heterotrophic denitrification; sediment microbial fuel cell; nitrate removal; microbial community

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1. Introduction

Nitrogen (N) is one of the most common elements existing in the natural environment. Owing to human activities, added N has an impact on the global N cycle by altering the composition, productivity, and other properties of many natural ecosystems. Moreover, N is more readily lost from ecosystems to stream water, groundwater, and the atmosphere than are most other essential elements [1]. Thus, N is one of the most important parameters for estimating the quality of water as well as ecological functions. The nitrogen released in surface water and subsurface aqueous environments is mainly in the form of domestic, agricultural, and industrial wastewater, resulting from fertilizer use, landfill leachates, and sewage wastewater discharge, etc. [2]. Since most released N in wastewater is ultimately converted to nitrate (NO_3^-) through biological systems [3], NO_3^- becomes one of the predominant nitrogenous species with high mobility and stability in the environment, especially in water bodies [4]. A series of ecological and health risks induced by NO_3^- have attracted wide attention [5]. For instance, high NO_3^- concentration could lead to

eutrophication [6], resulting in algal blooms in freshwater or estuaries [7]. High concentrations of NO_3^- and its reduction product, nitrite (NO_2^-), have been demonstrated to damage the quality and yield of aquaculture species and even to threaten human health via drinking these water sources [8].

For NO_3^- removal in wastewater, physicochemical methods and biological methods are widely studied in terms of treatment effect and cost. Physicochemical methods, such as reverse osmosis, ion-exchange, electrodialysis, and adsorption, are systematically optimized to remove NO_3^- [9]. The obvious advantage of physicochemical methods is the high efficiency and controllability in the treatment process. However, the high operational and material costs also hinder the widespread use of physicochemical methods [10]. Moreover, physicochemical methods are not suitable for water treatment in specific environments, such as aquaculture ponds and water source regions, to avoid potential disturbance to the ecological environment.

In contrast, biological methods for NO_3^- removal are considered to be low-cost, highly efficient, and eco-friendly [11]. Nevertheless, it is challenging to use biological methods to maintain the high performance of denitrifying bacteria due to the complexity and variations of microbial communities [12]. For high-strength NO_3^- removal in wastewater, biological denitrification suffers from low efficiency because of the significant nitrite accumulation that inhibits the activity of denitrifying bacteria [13]. In conventional biological denitrification, the carbon source is essential to maintain the proper carbon/nitrogen (C/N) ratio in order to provide sufficient electron donors in the denitrifying process [14]. Only the biologically available forms of carbon and nitrogen are effective, which creates particular requirements for the carbon source conditions for biological denitrification treatments [15]. Due to the insufficiency of carbon sources, extra carbon sources are commonly supplemented to sustain the denitrification [16], which increases the material cost. More importantly, secondary pollution will be caused if carbon is added to specific water bodies, including ponds, rivers, lakes, and oceans [17]. Even if the C/N ratio is theoretically sufficient, conventional technologies still require high internal recirculation from aerobic to anoxic conditions [18], and inevitably generate a large amount of sludge during the denitrification process [19]. Therefore, innovative biological technologies for NO_3^- removal, independent of carbon source, are urgently needed.

Microbial fuel cells (MFCs), a novel bioelectrochemical technology, were originally created to convert chemical energy into electrical energy by using exoelectrogens, and have been found capable of treating pollutants in recent decades [20]. The reduction and oxidation steps can be divided into two half-reactions in the anodic and cathodic compartments of an MFC, which allows the oxidation of substrates to occur in the anode, and the reduction to occur in the cathode. The electrons generated from substrate oxidation are transferred via the external circuit and used for the terminal electron acceptor (TEA) reduction in the cathode, such as the reduction of oxygen and NO_3^-. Clauwaert et al. [21] first used NO_3^- as TEA in the cathode of an MFC reactor, presenting the promising prospect of autotrophic denitrification. Thereafter, different types of MFCs have been investigated to perform NO_3^- removal in wastewater. Virdis et al. [22] used the cathode of a dual-chamber MFC to reduce NO_3^- to nitrogen gas at a rate of 0.41 $kg \cdot m^{-3} \cdot d^{-1}$. To enhance denitrification efficiency and reduce the operation/material costs of an MFC, different types of MFCs have been investigated for wastewater denitrification. Zhang et al. [23] developed a single-chamber MFC with a rotating cathode to passively supply oxygen and minimize the pump energy and obtained the removal of 92 and 82% of total nitrogen (TN) under a continuous feeding mode and batch mode, respectively. A stacked MFC system with five air-cathode MFC units connected in series achieved an 85% chemical oxygen demand (COD) and 94% TN removal under a hydraulic retention time of 2.5 h [24]. A larger-scale MFC was successfully developed for N removal with a 7.2 L volume [25]. Numerous studies of MFCs have been performed for N removal in wastewater. However, several difficulties need to be solved for the application of MFCs. For example, the material costs of exchange membranes and the added carbon sources limit the widespread application

of MFC in wastewater treatment. Hence, a membrane-less MFC, independent of added carbon sources, would be preferable in terms of economic reality.

Electron donor supply is another challenge for the large-scale application of conventional MFCs. In previous surveys, the substrate for anodic oxidation in MFCs mainly used soluble carbon sources [20], which restricted the potential for application in sensitive water bodies, such as water sources, and aquaculture regions with strict water quality requirements. It is of note that the addition of carbon sources to these water bodies would be strictly forbidden, though NO_3^- treatment is still urgently required to meet the strict water quality requirements. This highlights the difficulty of removing N in wastewater with low COD concentration via biological technologies. Fortunately, the sediment MFC (SMFC) configuration was designed for in situ treatment and allows the anode to utilize the organic contents in sediment without a separator to divide the anode and cathode [26]. For natural water bodies (such as inland lakes, rivers, and wetlands, etc.), the low organic matter concentration determines the shortage of carbon sources in the overlying water. The dissolved oxygen (DO) and anaerobic sediment together create a favorable redox gap for SMFC construction [27], making the SMFC a viable method for water treatment. Moreover, nutrients are passively supplemented during sedimentation from contaminated water, such as aquaculture ponds, to provide sustainable electron donors for SMFCs [28]. Additionally, the lack of buffer solution in these low-organic matter water bodies raises a new challenge for SMFCs in the water denitrification process. Therefore, it is of great interest to investigate the feasibility of constructing SMFCs for N removal in low-organic matter wastewater without a buffer solution. To the best of our knowledge, little research has addressed this purpose.

In this study, a series of SMFCs were successfully installed using aquaculture sediment for NO_3^- removal in wastewater. The synthetic wastewater composition was simplified to simulate real circumstances with low-organic matter and no buffered solution. The objective was to explore the feasibility and efficiency of denitrification by using an SMFC cathode in low-organic matter wastewater under aerobic conditions. Hence, SMFC treatments were applied to investigate the effect of generated electrons on denitrification in the cathode region. The results may inspire an innovative direction for in-situ wastewater treatment that is economical, eco-friendly, and independent of added nutrients. The SMFC performance was estimated in terms of power generation and NO_3^- removal. Additionally, the cathode microbial community structure was analyzed to explore the key species for NO_3^- removal in this system.

2. Materials and Methods

2.1. Sediment and Wastewater Preparation

Sediment was taken at depths of 0–10 cm below the sediment surface from a fish culture pond. Immediately after collection, the sediment was diluted, sieved (10 mesh), and stored in a PE bucket in the dark for sedimentation. The overlying water was carefully skimmed before the sediment was collected from the bottom of the bucket. The prepared sediment had a 68.9 $\pm$ 0.07% solid content and a 4.18 $\pm$ 0.05% organic matter content with pH of 6.8. The wastewater contained 10 mg·L^{-1} NO_3^-–N (potassium nitrate), 50 mg·L^{-1} COD (glucose), 12.5 mL·L^{-1} trace minerals (as described in Ref. [29]) without buffer solution or additional salinity.

2.2. SMFC Construction

The experimental SMFC was constructed as a membrane-less plexiglass cylinder with internal dimensions of 14 cm in diameter $\times$ 120 cm in height, and the total volume was 18.5 L (Figure 1). A small cylinder (10 cm in diameter $\times$ 30 cm in height) was embedded at the bottom of the larger one for anode installation into which 1 L of sediment was loaded. The experimental photos of the SMFC device are provided in the Supplementary File (Figure S1).

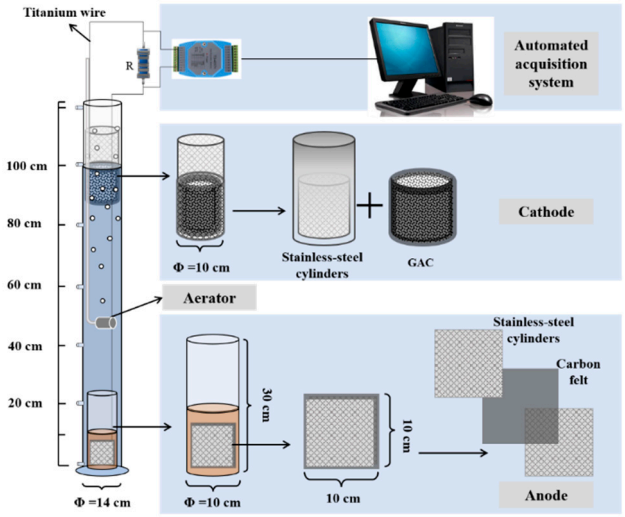


Figure 1. Schematic diagram of sediment microbial fuel cell.

Carbon felt (3 mm thickness) was used to manufacture SFMC anodes. It was first washed with pure water, then soaked in 1 M NaOH and 1 M HCl solution overnight, respectively. After being thoroughly washed and dried, the carbon felt was cut into 10 × 10 cm squares as anodes with effective surface areas of 100 cm^2. Carbon felt was inoculated with the anolyte from a constructed wetland microbial fuel cell (CW-MFC) [30] that had been continuously cultivated for more than 2 years. Then, each piece of carbon felt was sandwiched between two pieces of stainless-steel mesh to improve the mechanical strength of electrodes, and titanium wire with a diameter of 0.5 mm was sewn into the felt for electric circuit connection. The anode was vertically installed into the sediment in the small cylinder with a titanium wire connected to the external circuit.

Granular activated carbon (GAC, 2 mm in diameter with a specific area of 500–900 $m^2 \cdot g^{-1}$) and 20-mesh stainless-steel cylinders were used to compose the cathode. GAC was inoculated with the catholyte from the CW-MFC mentioned above. GAC was loaded between two stainless-steel cylinders (8 and 10 cm in diameter) to form a 1 cm thick layer biocathode of a 10 cm height, in the shape of a GAC cup, as shown in Figure 1. Titanium wire was sewn into the stainless-steel cylinders for external circuit connection.

The synthetic wastewater was gently injected into the large cylinder to form a 100 cm tall column with a total volume of 15.4 L (sediment included). The cathode was settled just below the water surface and connected to the anode via the external circuit. The microporous aerator and gas flow controller were used together to maintain DO of 2.0 for the SMFC cathode. Shade cloth was employed to enclose the whole large cylinder to avoid any impact of light source. All materials in this SMFC device have been used in different configurations of MFCs; the material durability would not be negatively affected for at least 2 years of operation. The total material cost of each SMFC device was 5.2 dollars including the anode, cathode, and external circuit (as seen in Table S1). There would be no further energy consumption and material cost in actual application.

2.3. Experimental Procedures

Five treatments were constructed for denitrification in low-organic matter wastewater in batch mode. Four of them belonged to the close-circuit (CC) group and one treatment belonged to the open-circuit (OC) group. In the CC group, the SMFC configuration was completed with resistors of 1000, 500, 200, and 100 Ω inserted in the external circuit and named T-1000, T-500, T-200, and T-100, respectively. The T-open treatment in the OC group employed the SMFC anode and cathode with a disconnected external circuit, aiming to distinguish the contribution of the material and bioelectricity to the denitrification process. The control group, without SMFC configuration, was used to assess natural attenuation and named NC. Before the experiment, five treatments had experienced a culturing period

for more than 150 d to enable microbes to fully grow and proliferate within the biocathode, and to obtain stable electrochemical performance of the anode and cathode. Sufficient batches of treatments also eliminated the interference of material adsorption on pollutant removal. All treatments were operated in triplicate at a constant ambient temperature of 25 ± 2 °C.

An automated acquisition system was used in the external circuit of each treatment to continuously record the output voltage. Water samples were collected at 20-, 60-, and 100-cm height in each treatment for COD and nitrogen concentration measurement. The GAC in T-open and T-100 treatments were sampled for microbial analysis.

2.4. Analysis Methods

Output voltages were monitored at 12-h intervals using an automatic acquisition unit system (DAM-3057; Art Technology Co. Ltd., Beijing, China). The current was calculated according to Ohm's law. Electrode potential values were measured vs. the saturated calomel electrode.

Three water samples collected at different heights in each treatment were determined according to standard methods [31]. The mean values of water quality parameters were used for pollutant removal calculation.

The GAC samples of the T-open and T-100 were collected and immediately delivered for microbial analysis based on PCR amplification of the 16S rRNA V3–V4 regions. Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA).

Statistical analyses were conducted using SPSS 19.0 (SPSS, Chicago, IL, USA). Differences in parameters were evaluated using a paired *t*-test, and $p < 0.05$ indicated a statistically significant difference. The Pearson correlation coefficient was used for correlation analysis significant at the 0.05 level.

3. Results and Discussion

3.1. Electrochemical Performance of the SMFCs

For the OC group, the open-circuit voltage reached above 770 mV and remained in a long-term stable phase for the entire experimental period, as seen in Figure 2a, indicating a significant difference in the potential conditions between the anode and the cathode in the open circuit. Differing from the OC group, all four SMFC treatments of the CC group went through a slow decline period. As seen in Figure 2a, the output voltage of T-1000 decreased from 230 down to 161 mV with an average output voltage of 180 mV. The average output voltages of T-500, T-200, and T-100 were comparatively lower because of the lower external resistance at 108, 48, and 24 mV, respectively. The mean current values in the external circuit of T-1000, T-500, T-200, and T-100 were 0.181, 0.217, 0.240, and 0.238 mA, respectively (Table 1). Although the dispersion of different SMFC currents was distinguished in the first 5 d, the currents, thereafter, decreased and stabilized within 0.15–0.20 mA in all four SMFC treatments.

Traditional biological technologies for energy recovery from wastewater could utilize the accumulated feedstock for biorefining, for example, cultivated *Spirulina* is widely reported as a biodiesel source [32]. In contrast to this, the power generation in SMFC mainly relied on the anode microbes directly converting the chemical energy of organic substrates in the sediment into the output electricity [26]. Based on our previous work in the start-up period, the organic substrate was capable of supporting SMFC anode activity for a month without undermining its performance (seen in Figure S2). Hence, the gradual output voltage decline in the CC group was attributed to the deterioration of biocathode performance. Since the initial COD concentration was merely 50 $mg \cdot L^{-1}$, rapid COD consumption displayed an obvious voltage decline in the first 5 d before a stable phase under sterile COD conditions in the catholyte. In addition, electrode polarization occurred in the CC group; SMFC treatments of the CC group obtained anode potential values ranging from −180 to −50 mV (vs. the saturated calomel electrode), as seen in Figure 2b,

while the OC group was maintained at ~300 mV. In the CC group, lower external resistance led to more significant polarization in the SMFC system; as a result, the mean cathode potential values of T-1000, T-500, T-200, and T-100 were −68, −127, −154, and −164 mV, respectively. The characteristics of the cathode potential and the external current indicated the reduction rate of TEA in the cathode region, including the denitrification process performed by the SMFC cathode in this study. The electrochemical performance, in turn, affected the denitrification efficiency as well as the microbial community structure. One limitation of the current experiment was that the utilization of generated bioelectricity in the biocathode region might be replaced by using input electricity; it would be interesting to analyze the different impacts on the microbial activities with endogenous and exogenous electron supplies. The comparison between the CC and OC groups will be further analyzed to determine the mechanism of cathode denitrification in SMFC.

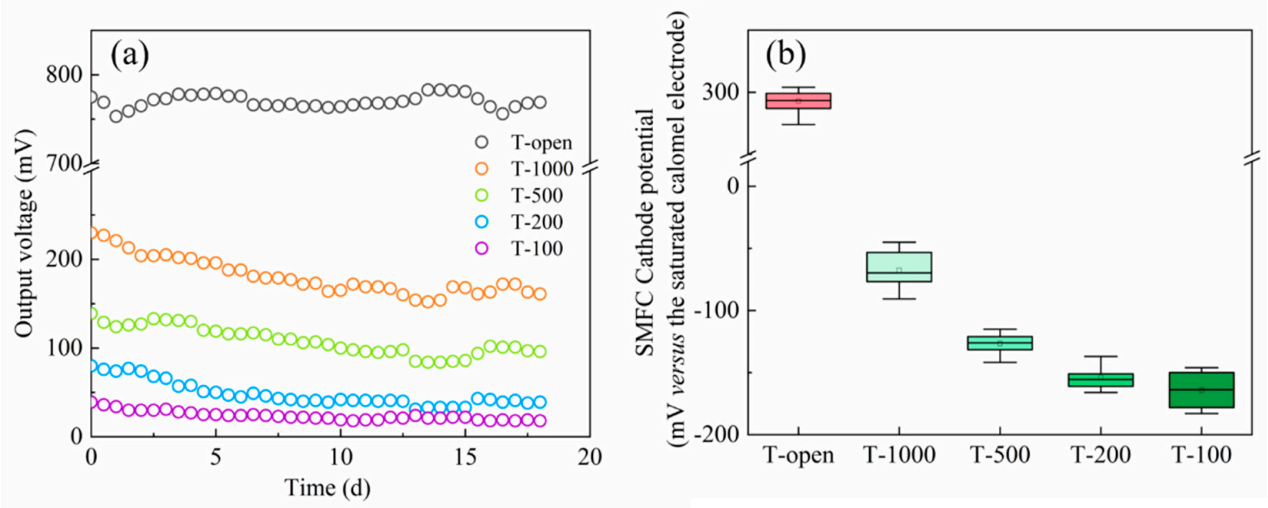


Figure 2. Dynamic changes in SMFC output voltage (**a**) and cathode potential vs. the saturated calomel electrode (**b**).

Table 1. Electrochemical performance of different treatments over the entire experimental period.

Samples	Voltage (mV)	Current (mA)	Quantity of Electric Charge (C)	Power Density ($mW \cdot m^{-3}$)
T-open	770	-	-	-
T-1000	181	0.181	281	186.7
T-500	108	0.217	337	134.1
T-200	48	0.240	372	65.5
T-100	24	0.238	369	32.3

3.2. Denitrification Efficiency

3.2.1. COD Removal

The initial COD concentration (51.6 $mg \cdot L^{-1}$), as provided by adding glucose, provided an initial C/N ratio of 5:1. It took NC 5 d to achieve 90% COD removal at a rate of 9.3 $mg \cdot L^{-1} \cdot d^{-1}$, as shown in Figure 3. The absence of cathode configuration restrained the COD consumption in NC. For all five treatments, it took only 2 d to achieve 90% COD removal because the major structure of the cathode consisted of well-inoculated GAC. Enriched microbes were capable of COD removal owing to the acclimation in the pre-experimental culturing period. Hence, all five treatments presented similar COD removal efficiencies (88.6–89.8%) in the first 2 d. Previous research using MFCs for enhanced denitrification achieved effective COD removal for nitrate reduction, as summarized by Nguyen and Babel [33]. This research generally exhibited more than 85% COD removal for different kinds of carbon sources of different initial concentrations.

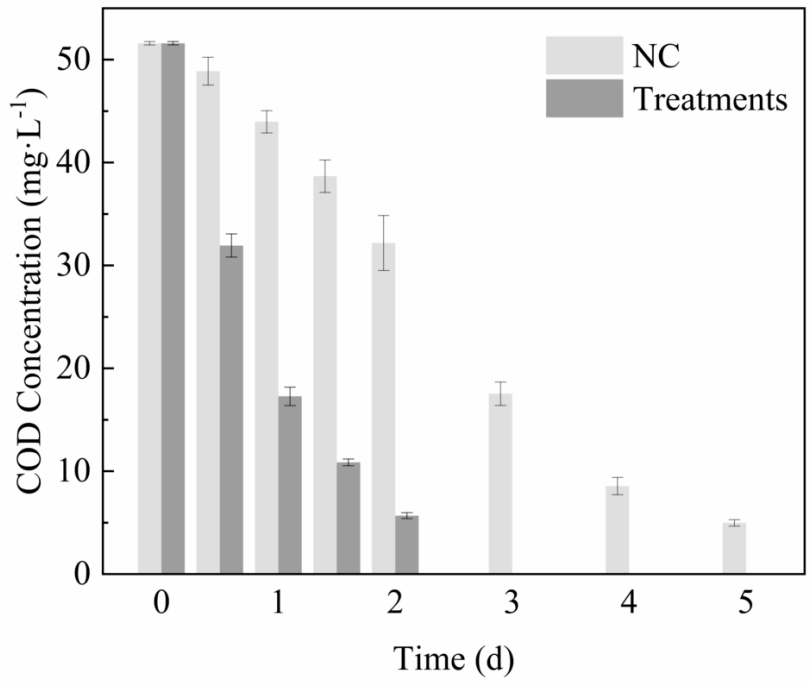


Figure 3. Changes of COD concentrations of NC and treatments.

In biological denitrification, organic matter could maintain the metabolism of microbes and supply electrons for denitrification [3]. In low-organic matter wastewater, denitrifying bacteria have to compete for the limited carbon source with other aerobic bacteria [29], and the DO participates in the electron competition in the water. In other words, fewer electron donors and more electron acceptors inhibit biological denitrification in low-organic matter wastewater. Previous researchers had already noticed the shortage of electron donors in biological treatments, and taken advantage of MFCs to reduce the C/N ratio requirement compared to conventional technologies [33]. In this study, no extra carbon source was added for denitrification purposes. Instead, the SMFC configuration was employed to execute efficient biological denitrification in low-organic matter wastewater—the SMFC integrated the oxidation of organic matter in sediment and the reduction of NO_3^- in the overlying water. Once the COD was almost consumed, the enhancement of NO_3^- removal in the SMFC treatments began to occur. However, in the current study, the COD in wastewater was mainly composed of glucose which is a biologically available nutrient for the SMFC biocathode. The C and N elements in actual wastewater would be in the forms of complex compounds, and that would make it more difficult to use or remove the C and N in real-world applications.

3.2.2. Nitrogen Removal

At the beginning of this experiment, the initial TN concentration in the wastewater consisted entirely of NO_3^- (potassium nitrate); thus, the TN removal was mainly dependent on NO_3^- removal. According to the measured concentrations of NO_3^- and TN, NO_3^- accounted for more than 97% of the TN in each sample. Therefore, the dynamic changes of the NO_3^-–N concentration directly reflected the denitrification efficiencies of each treatment.

As shown in Figure 4a, the NO_3^- removal in NC indicated the efficiency of natural attenuation in wastewater. NC achieved 28.1 and 48.1% NO_3^- removal at 2 and 5 d, respectively, by biological denitrification using the dissolved carbon source. Thereafter, until 10 d, further denitrification could barely continue in NC—only 2.04 $mg \cdot L^{-1}$ (4.8%) of the remaining NO_3^- was removed during this period with sterile COD (<5.0 $mg \cdot L^{-1}$) in wastewater.

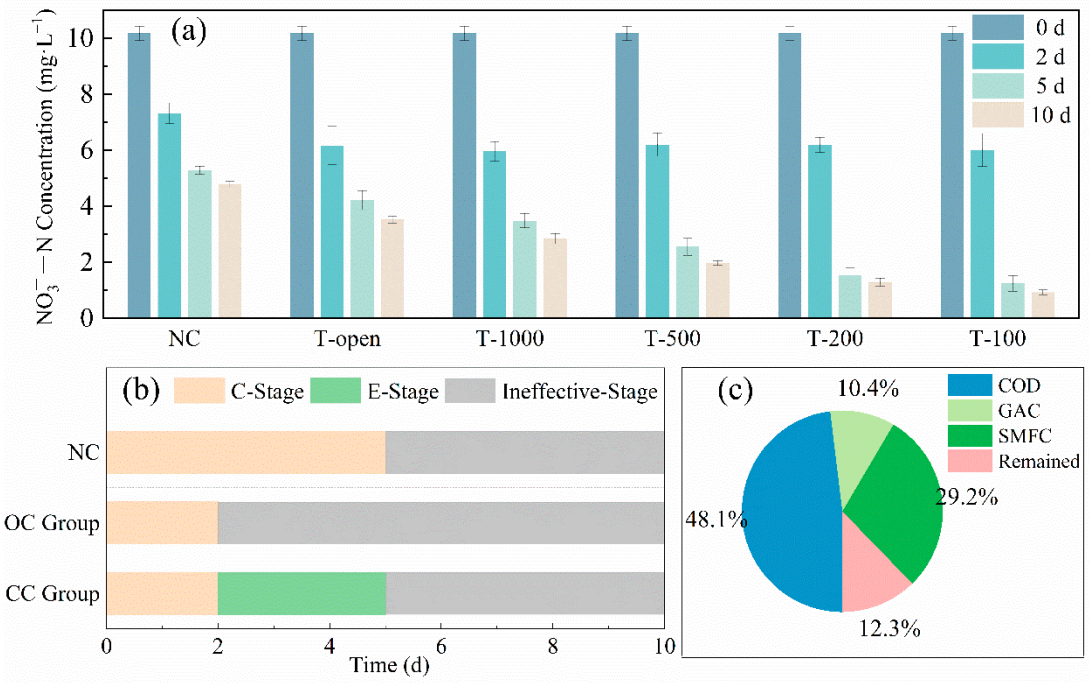


Figure 4. Changes of NO_3^- concentration of NC and treatments on 0, 2, 5, 10 d (**a**). Different denitrification stages of NC, CO group, and CC group (**b**). Contributions of different influencing factors to NO_3^- removal of T-100 on 5 d (**c**).

In all five treatments, the NO_3^- removal efficiencies were higher compared with NC. Among them, further enhanced NO_3^- removal was achieved in the SMFC treatments where less external resistance coexisted with a higher removal rate. On the one hand, the existence of the GAC cathode provided sufficient biomass to effect microbial metabolism including denitrification. On the other hand, the external current continued transferring electrons to the SMFC cathode for autotrophic denitrification. In the absence of sufficient COD in wastewater, the advantage of the SMFC configuration gradually emerged via biocathode denitrification by using electrons generated from the anode half-reaction. For convenience of analysis, the denitrification process could be divided into the following two stages: a COD-driven stage (C-stage) and an electron-driven stage (E-stage) according to two dominant electron donors. The first 5 days were regarded as the C-stage for NC, and the first 2 days for the OC and CC groups. Then 2 to 5 d was the E-stage of the CC group including T-1000, T-500, T-200, and T-100. Ineffective denitrification periods of the NC, OC, and CC groups are also highlighted in Figure 4b.

The different NO_3^- removal efficiencies between OC and NC demonstrated the denitrification capacity of the cultured microbial community enriched in biocathode. By comparison, the NO_3^- removal efficiencies of T-open were 11.2, 10.4, and 12.5 percentage points higher than that of NC in 2, 5, and 10 days, respectively, as shown in Figure 4a. This fact verified the improvement of biomass on denitrification capacity. The electrochemical effect on denitrification needed further analysis between the OC and CC groups. Both OC and CC groups achieved similar NO_3^- removal efficiencies (39.1–41.4%) in the C-stage, approximately 10 percentage points higher than NC at the same time of operation. However, NC realized 48.1% NO_3^- removal over the entire C-stage, overwhelming the other two treatment groups. In the OC and CC groups, as long as the COD was sufficient within 2 d (C-stage), the biocathodes displayed an advantage in denitrification by using COD as the electron donor, resulting in faster NO_3^- removal. After the COD was rapidly consumed, further denitrification was continuously sustained by using electrons generated in the CC group in E-stage. During this stage, NO_3^- removal was improved in the four SMFC treatments compared with T-open, confirming the effectiveness of denitrification using electrons transferred via the external circuit. In previous research, Clauwaert, Rabaey, Aelterman, Schamphelaire, Pham, Boeckx, Boon and Verstraete [21] first reported that MFC devices

can integrate the nitrogen removal process into the system through biological and bioelectrochemical reactions and obtained 0.146 kg NO_3^-–N m^{-3} net cathodic compartment. Within the CC group, higher currents in T-100 and T-200 led to more NO_3^- removal in E-stage of 4.77 mg·L^{-1} (46.9%) and 4.67 mg·L^{-1} (45.9%), respectively. In contrast, the lower current in T-1000 and T-500 only obtained 2.48 mg·L^{-1} (24.3%) and 3.64 mg·L^{-1} (35.8%) NO_3^- removal in E-stage. Taking T-100 as an example, most NO_3^- removal occurred in 5 d, several factors contributing to the total NO_3^- removal of 87.7%. By calculating the different values of NO_3^- concentrations in NC, T-open, and T-100 on 5 d, it was found that the initial COD could contribute 48.1% to total NO_3^- removal (as shown in Figure 4c), and microbes in GAC enhanced the removal efficiency by 10.4%. With the effect of electric current, another 29.2% NO_3^- was removed using generated electrons in SMFC.

After two rapid denitrifying stages (C-stage and E-stage), NO_3^- removal was inhibited, especially in T-200 and T-100, as the lower NO_3^- concentration had lost its competitiveness for electrons with constant DO at 2.0 mg·L^{-1}. Additionally, gradually decreased power generation after 5 d was observed through the decreased current in the SMFC, which significantly hindered NO_3^- reduction. Moreover, from the view of the entire treatment period, the optimal NO_3^- removal efficiency of the SMFC reached 90.8%; the remaining NO_3^- was only 0.93 mg·L^{-1} within 10 d. Zhang et al. [34] had obtained 92% TN removal in a two-chamber MFC with carbon felt as the cathode and anion exchange membrane as the separator. Ren et al. [35] observed 96% nitrate reduction in an air-cathode MFC without membrane. The NO_3^- removal efficiencies in the current experiment corresponded with the recent research [33]. However, in terms of the net nitrogen removal amount, the autotrophic denitrification in the current experiment was much lower compared to previous studies [36]. This fact is attributed to two main reasons—the relatively lower concentration of NO_3^-, and the larger scale of the SMFC devices. In this experiment, an SMFC device of 1.0-m effective height was constructed to elongate the distance of the anode and cathode to a realistic extent, as in conditions such as ponds and wetlands. The lower concentrations of COD and nitrogen in overlying water were also set according to these conditions. Two factors restricted the power generation in this SMFC, decreasing the reduction reaction rates for NO_3^-, however, high removal efficiency was still obtained in SMFC treatments.

3.3. Correlation Analysis

In an MFC system for NO_3^- removal, autotrophic denitrification plays an essential role in NO_3^- reduction by using the generated electrons via the external circuit [33]. The SMFC configuration separates the anode and cathode into solid and liquid phases and minimizes the interference of organic and oxygen diffusion. To identify the key factor influencing autotrophic denitrification, the correlation between electrochemical performance and NO_3^- removal was assessed. According to the dynamic changes in the NO_3^- concentrations, the SMFC exhibited no significant removal improvement during the C-stage because the conventional denitrification dominated most of the NO_3^- removal using COD as the electron donor. In the E-stage, gradient removal effects were observed in T-1000, T-500, T-200, and T-100. The relationship between the electrical characteristics and NO_3^- removal efficiencies required further investigation in this period. Therefore, Pearson's correlation coefficient was used to explore the correlation between these two variables. Three electrochemical parameters—output voltage, current, and power density—were calculated using mean values of the E-stage, and the removed NO_3^- was calculated using a decreased NO_3^- concentration accordingly.

During the E-stage of denitrification, the Pearson correlation coefficient between the removed NO_3^- and electric current was 0.965, i.e., a strong positive correlation. In contrast, the output voltage and power density were both negatively correlated with NO_3^- removal (−0.990 and −0.943). The output voltage presents the potential gap between two electrodes, and the power density presents the electricity used in the external circuit. Theoretically, once the external resistance changes, the voltage and power density would not remain unchanged, especially in fuel cell devices, a phenomenon described as electrode

polarization. However, instead of output voltage and power density, the external current directly determines the quantity of electrons that is used for the TEA reduction in the SMFC cathode. The results confirmed that the quantity of electrons via the external circuit was the determining factor in autotrophic denitrification, regardless of the output electric energy converted from chemical energy.

As mentioned above, the condition of T-100 is most favorable for denitrification in the SMFC cathode. However, it is worth noting that, in this study, the organic content in sediment was sufficient to sustain the anode half-reaction steadily, making the cathodic reaction the rate-limiting factor in the buffer-free circumstance. Once the cathode volume drastically increases, the oxidation rate in the anode region might be incapable of sustaining the electron generation, thereby reducing the total denitrification efficiency of SMFCs. Therefore, the enlarged cathode area or higher area ratio (cathode/anode) in SMFCs would be a significant factor for high load wastewater treatment, especially for industrial application purposes.

Overall, this analysis has informed a new perspective on the denitrification process and provided a valid strategy for NO_3^- removal in low-organic matter wastewater. Based on the data obtained in this study, an evaluation model would be necessary for application in real-world water bodies in order to estimate the effective region and treatment efficiency of each SMFC device, as well as the distribution of SMFCs in a broad expanse of water [37]. Moreover, because the electric current in the SMFC external circuit was closely related to NO_3^- removal efficiency, electrical signals could be used to monitor or regulate the denitrification process, which could optimize project management.

3.4. Microbial Community Analysis

3.4.1. Microbial α-Diversity

Significantly enhanced NO_3^- removal was observed in T-100 compared with T-open. Therefore, two GAC samples were collected at the end of E-stage from T-open and T-100 for microbiological analysis (16S rRNA gene sequencing), aiming to illustrate the effect of the SMFC current on the cathode microbial community structure. Microbial species diversity was estimated by calculating the α-diversity, based on the indexes of Shannon, Simpson, ACE, and Chao1 (Table 2).

Table 2. Species diversity in SMFC cathode.

Sample	Shannon	Simpson	Ace	Chao1	Coverage
T-open	4.78	0.0211	1022	1020.6	0.990725
T-100	4.14	0.0421	763	759.0	0.993125

The lower Shannon index (4.14 vs. 4.78) and higher Simpson index (0.0421 vs. 0.0211) of T-100 demonstrated the lower community diversity, indicating that the electrochemical characteristics contributed to the enrichment of specific bacteria in the SMFC cathode. The lower Ace index (763 vs. 1022) and Chao1 index (759.0 vs. 1020.6) indicated less community richness in T-100. The external current and potential value of the SMFC cathode were the main differences between T-open and T-100. Owing to these features of the biocathode, the microbial community of T-100 acclimated to effect bioelectrochemical activity and denitrification function. Hence, dominant species might be more abundant in T-100.

3.4.2. Taxonomic Analysis

The relative abundances of T-open and T-100 were compared at the phylum and genus levels in Figure 5a. The dominant phyla—Proteobacteria and Actinobacteriota—together accounted for 71.6 and 80.3% of the relative abundances in the two samples, respectively. The relative abundance of Proteobacteria in the T-100 cathode was 53.1%, higher than that in T-open (50.9%). Similarly, the relative abundance of Actinobacteriota in T-100 reached 27.3%, about 1.3 times the relative abundance in T-open. The difference

between the two treatments referred to the connected external circuit, with which the SMFC configuration could be accomplished. Proteobacteria, the most dominant species, were commonly observed in the electrode of MFC, as summarized by Yang and Chen [27], especially β-Proteobacteria. The higher relative abundance of Proteobacteria resulted from the acclimation in close-circuit SMFC, and ensured the half-reaction of the cathode [33]. As reported, Actinobacteriota played essential roles in the process of denitrification [38], and was increased by a higher C/N ratio [39]. In this study, the presence of Proteobacteria and Actinobacteriota effected the simultaneous removal of COD and NO_3^- in wastewater, and the SMFC configuration was conducive to the richness of denitrifying bacteria to facilitate the denitrification process, as seen in T-100.

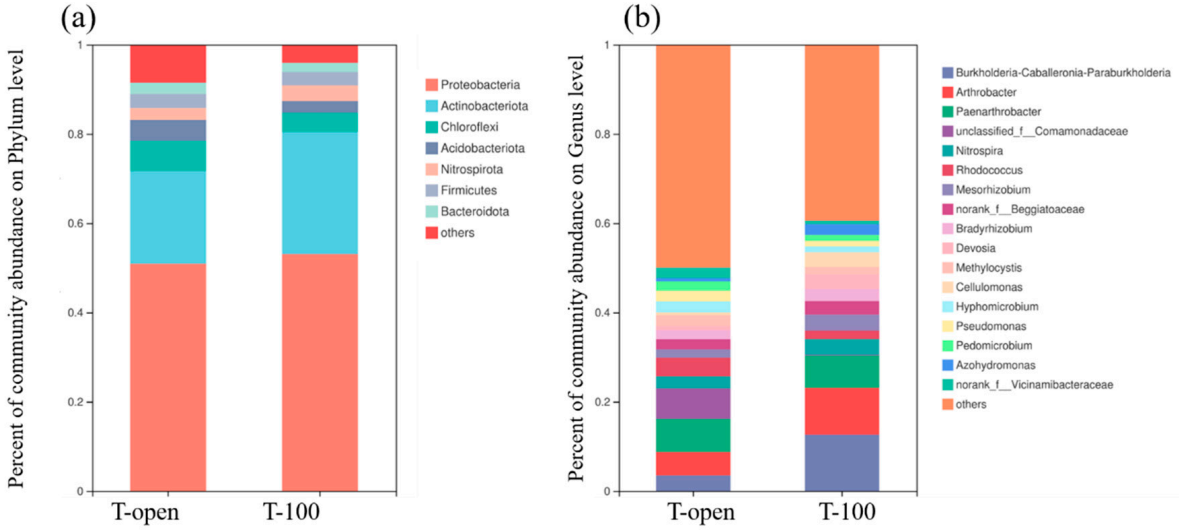


Figure 5. Microbial community composition at the phylum (**a**) and genus (**b**) levels.

Microbial community composition at the genus level is shown in Figure 5b. *Burkholderia*, *Caballeronia*, and *Paraburkholderia*, belonging to β-Proteobacteria, have been widely reported as nitrogen fixers [40–42]. These dominant species gained 12.6% relative abundance in T-100, much more than the 3.5% in T-open. This species enrichment might result from the appearance of nitrogen gas (N_2) in the cathode region. From this point of view, denitrification should be sustained in the SMFC cathode, gently producing N_2. The electric current in T-100 accelerated the heterotrophic denitrifying function. This result also corresponds with the rapid NO_3^- removal efficiency during E-stage. *Arthrobacter*, another dominant genus, was reported in the effective transformation of ammonium, hydroxylamine, nitrate, and nitrite nitrogen [43]. Additionally, Zhang et al. [44] have demonstrated the efficient heterotrophic nitrification-aerobic denitrification of *Arthrobacter* and achieved 95% nitrogen removal in both mariculture and domestic wastewater. The wastewater treated in this study maintained a steady DO of 2.0, selecting denitrifying bacteria that adapt to aerobic conditions. Hence, *Arthrobacter* was enriched in both T-open and T-100 for its aerobic denitrification capacity. The T-open cathode contained 5.2% *Arthrobacter* in the absence of external current; a higher relative abundance (10.5%) in T-100 reflected the microbial-community acclimation towards robust denitrification function. The increased relative abundance of three nitrogen fixers and denitrifying bacteria explained the denitrifying capacity and high efficiency of NO_3^- removal in the SMFC cathode. Thus, it has been shown to be valid for NO_3^- removal by SMFCs in low-organic matter wastewater under aerobic conditions. Further relevant research could seek to clarify the molecular biological mechanisms of autotrophic denitrification in SMFCs by inoculating the typical bacteria onto the bioelectrodes. By analyzing the effect of denitrification and the changes in metabolic pathways, the correlation between bioelectrochemistry and denitrification could be well elaborated.

4. Conclusions

SMFCs were successfully installed using a GAC cathode for aerobic denitrification in low-organic matter wastewater. The COD content supported biological denitrification with an initial C/N of 5.0; about 40% NO_3^- could be removed in this way. Once COD was fully consumed, the generated electrons via the external circuit of SMFC maintained autotrophic denitrification in the cathode region; 24–47% NO_3^- could be removed with different currents in the SMFC external circuit within 3 days. Total NO_3^- removal in SMFC treatments could reach 72–91% in 10 d. The electric current enriched the Proteobacteria and Actinobacteriota phyla in the SMFC cathode, demonstrating substantial bioelectrochemical activity and denitrification capacity. The higher relative abundance of *Burkholderia*, *Caballeronia*, *Paraburkholderia*, and *Arthrobacter* in the SMFC cathode also demonstrated the heterotrophic denitrifying capacity. The NO_3^- removal efficiencies were positively correlated with the external current intensities ($p < 0.05$) in the SMFC system. Hence, electrical signals could be used to monitor or regulate the denitrification process in SMFCs, thus making use of SMFCs a promising strategy for nitrogen treatment in low-organic matter wastewater. This study has presented an economical and eco-friendly strategy for wastewater treatment, especially for heterotrophic denitrification. Based on the results of this study, further studies are recommended to focus on the practical application method for real wastewater of different characteristics.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13243512/s1, Figure S1: The photos of SMFC devices: the plexiglass cylinders (a,b); the GAC cathode (c), Figure S2: The output voltages of different treatments in culturing period, Table S1: Material cost analysis of each SMFC device.

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References

1. Abbas, S.Z.; Rafatullah, M. Recent advances in soil microbial fuel cells for soil contaminants remediation. *Chemosphere* **2021**, *272*, 129691. [CrossRef] [PubMed]
2. APHA; Awwa; WEF. *Standard Methods for Examination of Water and Waste Water*, 22th ed.; American Public Health Association; American Water Works Association; Water Environment Federation: Washington, DC, USA, 2012.
3. Barwal, A.; Chaudhary, R. To study the performance of biocarriers in moving bed biofilm reactor (MBBR) technology and kinetics of biofilm for retrofitting the existing aerobic treatment systems: A review. *Rev. Environ. Sci. Bio/Technol.* **2014**, *13*, 285–299. [CrossRef]
4. Bhatnagar, A.; Sillanpää, M. A review of emerging adsorbents for nitrate removal from water. *Chem. Eng. J.* **2011**, *168*, 493–504. [CrossRef]
5. Bulgarelli, D.; Rott, M.; Schlaeppi, K.; Van Themaat, E.V.L.; Ahmadinejad, N.; Assenza, F.; Rauf, P.; Huettel, B.; Reinhardt, R.; Schmelzer, E.; et al. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* **2012**, *488*, 91–95. [CrossRef] [PubMed]
6. Akirgöz, M.; Bayrakdar, A.; Çalli, B. How do the influent COD/Nitrogen and internal recirculation ratios affect the oxidation ditch type pre-anoxic landfill leachate treatment? *J. Environ. Manag.* **2021**, *278*, 111598. [CrossRef]
7. Clauwaert, P.; Rabaey, K.; Aelterman, P.; De Schamphelaire, L.; Pham, T.H.; Boeckx, P.; Boon, N.; Verstraete, W. Biological Denitrification in Microbial Fuel Cells. *Environ. Sci. Technol.* **2007**, *41*, 3354–3360. [CrossRef]
8. Doskočil, R.; Škapa, S.; Olšová, P. Success evaluation model for project management. *M. Ekon. Manag.* **2016**, *19*, 167–185. [CrossRef]
9. Fang, Z.; Cao, X.; Li, X.; Wang, H.; Li, X. Biorefractory wastewater degradation in the cathode of constructed wetland-microbial fuel cell and the study of the electrode performance. *Int. Biodeterior. Biodegrad.* **2018**, *129*, 1–9. [CrossRef]

10. Ghafari, S.; Hasan, M.; Aroua, M.K. Bio-electrochemical removal of nitrate from water and wastewater—A review. *Bioresour. Technol.* **2008**, *99*, 3965–3974. [CrossRef]
11. He, T.; Xie, D.; Ni, J.; Li, Z.; Li, Z. Characteristics of nitrogen transformation and intracellular nitrite accumulation by the hypothermia bacterium Arthrobacter arilaitensis. *Sci. Total. Environ.* **2019**, *701*, 134730. [CrossRef]
12. Henry, M.; Gasco, L.; Piccolo, G.; Fountoulaki, E. Review on the use of insects in the diet of farmed fish: Past and future. *Anim. Feed. Sci. Technol.* **2015**, *203*, 1–22. [CrossRef]
13. Isiuku, B.O.; Enyoh, C.E. Pollution and health risks assessment of nitrate and phosphate concentrations in water bodies in South Eastern, Nigeria. *Environ. Adv.* **2020**, *2*, 100018. [CrossRef]
14. Jensen, V.B.; Darby, J.L.; Seidel, C.; Gorman, C. Nitrate in Potable Water Supplies: Alternative Management Strategies. *Crit. Rev. Environ. Sci. Technol.* **2014**, *44*, 2203–2286. [CrossRef]
15. Jin, L.; Zhang, G.; Tian, H. Current state of sewage treatment in China. *Water Res.* **2014**, *66*, 85–98. [CrossRef]
16. Mohan, T.K.; Nancharaiah, Y.; Venugopalan, V.; Sai, P.S. Effect of C/N ratio on denitrification of high-strength nitrate wastewater in anoxic granular sludge sequencing batch reactors. *Ecol. Eng.* **2016**, *91*, 441–448. [CrossRef]
17. Lai, X.; Zhao, Y.; Pan, F.; Yang, B.; Wang, H.; Wang, S.; He, F. Enhanced optimal removal of nitrogen and organics from intermittently aerated vertical flow constructed wetlands: Relative COD/N ratios and microbial responses. *Chemosphere* **2019**, *244*, 125556. [CrossRef] [PubMed]
18. Lardi, M.; Liu, Y.; Purtschert, G.; Pessi, G.; de Campos, S.B. Transcriptome Analysis of Paraburkholderia phymatum under Nitrogen Starvation and during Symbiosis with Phaseolus Vulgaris. *Genes* **2017**, *8*, 389. [CrossRef] [PubMed]
19. Lasagna, M.; De Luca, D.A.; Franchino, E. Nitrate contamination of groundwater in the western Po Plain (Italy): The effects of groundwater and surface water interactions. *Environ. Earth Sci.* **2016**, *75*, 240. [CrossRef]
20. Lewis, J.W.M.; Wurtsbaugh, W.A.; Paerl, H.W. Rationale for Control of Anthropogenic Nitrogen and Phosphorus to Reduce Eutrophication of Inland Waters. *Environ. Sci. Technol.* **2011**, *45*, 10300–10305. [CrossRef]
21. Lv, Y.; Wang, Y.; Ren, Y.; Li, X.; Wang, X.; Li, J. Effect of anaerobic sludge on the bioelectricity generation enhancement of bufferless single-chamber microbial fuel cells. *Bioelectrochemistry* **2019**, *131*, 107387. [CrossRef] [PubMed]
22. Maroušek, J.; Maroušková, A. Economic Considerations on Nutrient Utilization in Wastewater Management. *Energies* **2021**, *14*, 3468. [CrossRef]
23. Maroušek, J.; Strunecký, O.; Kolář, L.; Vochozka, M.; Kopecky, M.; Maroušková, A.; Batt, J.; Poliak, M.; Šoch, M.; Bartoš, P.; et al. Advances in nutrient management make it possible to accelerate biogas production and thus improve the economy of food waste processing. *Energy Sour. Part A Recover. Util. Environ. Eff.* **2020**, 1–10. [CrossRef]
24. Munoz-Cupa, C.; Hu, Y.; Xu, C.; Bassi, A. An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production. *Sci. Total. Environ.* **2020**, *754*, 142429. [CrossRef]
25. Mutchamua, H.H.G.; Bolsan, A.C.; Bonatto, C.; Chini, A.; Venturin, B.; Hollas, C.E.; Bonassa, G.; Antes, F.G.; Treichel, H.; Di Luccio, M.; et al. Sludge management in lagoons: The role of denitrification as a function of carbon biodegradation. *Bioresour. Technol. Rep.* **2021**, *15*, 100802. [CrossRef]
26. Nguyen, H.D.; Babel, S. Insights on microbial fuel cells for sustainable biological nitrogen removal from wastewater: A review. *Environ. Res.* **2021**, *204*, 112095. [CrossRef]
27. Palanisamy, G.; Jung, H.-Y.; Sadhasivam, T.; Kurkuri, M.D.; Kim, S.C.; Roh, S.-H. A comprehensive review on microbial fuel cell technologies: Processes, utilization, and advanced developments in electrodes and membranes. *J. Clean. Prod.* **2019**, *221*, 598–621. [CrossRef]
28. Park, Y.; Park, S.; Nguyen, V.K.; Yu, J.; Torres, C.I.; Rittmann, B.E.; Lee, T. Complete nitrogen removal by simultaneous nitrification and denitrification in flat-panel air-cathode microbial fuel cells treating domestic wastewater. *Chem. Eng. J.* **2017**, *316*, 673–679. [CrossRef]
29. Peng, L.; Liu, Y.; Gao, S.-H.; Chen, X.; Ni, B.-J. Evaluating simultaneous chromate and nitrate reduction during microbial denitrification processes. *Water Res.* **2016**, *89*, 1–8. [CrossRef]
30. Pereira, J.H.; Reis, A.C.; Homem, V.; Silva, J.A.; Alves, A.; Borges, M.T.; Boaventura, R.A.; Vilar, V.J.; Nunes, O.C. Solar photocatalytic oxidation of recalcitrant natural metabolic by-products of amoxicillin biodegradation. *Water Res.* **2014**, *65*, 307–320. [CrossRef]
31. Puri, A.; Padda, K.P.; Chanway, C.P. Evaluating lodgepole pine endophytes for their ability to fix nitrogen and support tree growth under nitrogen-limited conditions. *Plant Soil* **2020**, *455*, 271–287. [CrossRef]
32. Rahimi, S.; Modin, O.; Mijakovic, I. Technologies for biological removal and recovery of nitrogen from wastewater. *Biotechnol. Adv.* **2020**, *43*, 107570. [CrossRef]
33. Reis, V.M.; Estrada-De Los Santos, P.; Tenorio-Salgado, S.; Vogel, J.; Stoffels, M.; Guyon, S.; Mavingui, P.; Baldani, V.L.D.; Schmid, M.; Baldani, J.I.; et al. *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. *Int. J. Syst. Evolut. Microbiol.* **2004**, *54*, 2155–2162. [CrossRef]
34. Ren, Y.; Lv, Y.; Wang, Y.; Li, X. Effect of heterotrophic anodic denitrification on anolyte pH control and bioelectricity generation enhancement of bufferless microbial fuel cells. *Chemosphere* **2020**, *257*, 127251. [CrossRef]
35. Sun, H.; Xu, S.; Zhuang, G.; Zhuang, X. Performance and recent improvement in microbial fuel cells for simultaneous carbon and nitrogen removal: A review. *J. Environ. Sci.* **2016**, *39*, 242–248. [CrossRef]

36. Virdis, B.; Rabaey, K.; Yuan, Z.; Keller, J. Microbial fuel cells for simultaneous carbon and nitrogen removal. *Water Res.* **2008**, *42*, 3013–3024. [CrossRef]
37. Vitousek, P.; Hättenschwiler, S.; Olander, L.; Allison, S. Nitrogen and Nature. *Ambio* **2002**, *31*, 97–101. [CrossRef] [PubMed]
38. Yang, X.; Chen, S. Microorganisms in sediment microbial fuel cells: Ecological niche, microbial response, and environmental function. *Sci. Total. Environ.* **2020**, *756*, 144145. [CrossRef]
39. Yu, C.-P.; Liang, Z.; Das, A.; Hu, Z. Nitrogen removal from wastewater using membrane aerated microbial fuel cell techniques. *Water Res.* **2011**, *45*, 1157–1164. [CrossRef]
40. Zhang, F.; Ma, C.; Huang, X.; Liu, J.; Lu, L.; Peng, K.; Li, S. Research progress in solid carbon source–based denitrification technologies for different target water bodies. *Sci. Total. Environ.* **2021**, *782*, 146669. [CrossRef] [PubMed]
41. Zhang, G.; Zhang, H.; Zhang, C.; Zhang, G.; Yang, F.; Yuan, G.-E.; Gao, F. Simultaneous nitrogen and carbon removal in a single chamber microbial fuel cell with a rotating biocathode. *Process. Biochem.* **2013**, *48*, 893–900. [CrossRef]
42. Zhang, M.; Pan, L.; Liu, L.; Su, C.; Dou, L.; Su, Z.; He, Z. Phosphorus and nitrogen removal by a novel phosphate-accumulating organism, Arthrobacter sp. HHEP5 capable of heterotrophic nitrification-aerobic denitrification: Safety assessment, removal characterization, mechanism exploration and wastewater treatment. *Bioresour. Technol.* **2020**, *312*, 123633. [CrossRef]
43. Zhang, Q.; Qian, H.; Xu, P.; Li, W.; Feng, W.; Liu, R. Effect of hydrogeological conditions on groundwater nitrate pollution and human health risk assessment of nitrate in Jiaokou Irrigation District. *J. Clean. Prod.* **2021**, *298*, 126783. [CrossRef]
44. Zhang, Y.; Xu, Q.; Huang, G.; Zhang, L.; Liu, Y. Effect of dissolved oxygen concentration on nitrogen removal and electricity generation in self pH-buffer microbial fuel cell. *Int. J. Hydrogen Energy* **2020**, *45*, 34099–34109. [CrossRef]