

Article

Development of Bio-Electrochemical Reactor for Groundwater Denitrification: Effect of Electric Current and Water Hardness

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Abstract: Nitrate-nitrogen (NO₃-N) contaminating groundwater is an environmental issue in many areas, and is difficult to treat by simple processes. A bio-electrochemical reactor (BER) using copper wire and graphite plate was developed to purify the NO₃-N-contaminated groundwater. The low (of 10 mA) and high (of 20 mA) electric currents were applied to the BERs, and various influent hardness levels from 20 to 80 mg/L as CaCO₃ due to groundwater characteristics were supplied to clarify the total nitrogen removal efficiency and NO₃-N removal mechanisms. In the BER-10, the bio-electrochemical reactions caused 85% of total nitrogen to be removed through heterotrophic and autohydrogenotrophic denitrification in the suspended sludge and biofilm. However, the chemical deposit occurring at the cathode from water hardness affected the decreasing denitrification performance; 12.6% of Mg and 8.8% of Ca elements were observed in the biofilm. The enhancement of electrochemical reactions in the BER-20 caused integrating electrochemical and bio-electrochemical reactions; the NO₃-N was electrochemically reduced to NO₂-N, and it was further biologically reduced to N₂. A better total nitrogen removal of 95% was found; although, a larger deposit of Mg (22.8%) and Ca (10.8%) was observed. The relatively low dissolved H₂ in the BER-20 confirmed that the deposit affected the decreasing gaseous H₂ transfer and inhibition of autohydrogenotrophic denitrification in the suspended sludge. According to the microbial analysis, both heterotrophic and autohydrogenotrophic denitrification were obtained in the suspended sludge of both BERs; *Nocadia* (26.8%) was the most abundant genus in the BER-10, whereas *Flavobacterium* (27.1%) and *Nocadia* (25.0%) were the dominant genera in the BER-20.

Keywords: groundwater denitrification; nitrogen contamination; bio-electrochemical reactor; biofilm; hydrogen-driven bacteria



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1. Introduction

Groundwater is an important natural resource, and a main resource for drinking water. Groundwater usually contains less contaminants and concentrations than surface water because the water flows through porous layers of soil as natural filtration. However, the extensive use of nitrogen fertilizer in agriculture and the waste from animal farms cause nitrate-nitrogen (NO₃-N) contamination in the groundwater [1]. In rural areas of Yantai (China), the NO₃-N concentration in the groundwater ranged from 0.1 to 166.4 mg/L, and 63.3% of the monitoring points were over 11 mg/L of the standard level [2,3]. In addition, geological factors are another source for NO₃-N contamination; an NO₃-N concentration ranging 100 to 300 mg/L was observed in wells with depths between 60–120 m [4]. The

consumption of high $\text{NO}_3\text{-N}$ levels directly relates to birth defects in newborns and methemoglobinemia [3]. Due to the long-term effects to health and the environment from the $\text{NO}_3\text{-N}$ -contaminated water, applicable treatment technologies to purify the contaminated groundwater for safe drinking water is a critical and challenging issue.

Physical and chemical $\text{NO}_3\text{-N}$ treatment technologies have commonly been applied for groundwater, such as electrodialysis, reverse osmosis, and ion change [5,6]. However, the above processes produce concentrated brine, which needs further treatment. On the other hand, biological autotrophic denitrification can be an alternative option due to low organic carbon in groundwater. Among of several electron donors and denitrification technologies, a bio-electrochemical reactor (BER), a combined treatment process of heterotrophic and electrochemical autohydrogenotrophic denitrification, is increasingly used for removing $\text{NO}_3\text{-N}$ from drinking water resources [7,8]. Hydrogen (H_2) is electrochemically produced at the cathode, and is utilized as the electron donor. The sufficient H_2 supply, depending on the current intensity, is a key operating factor; the theoretical H_2 requirement is 0.357 mg to complete the reduction of $\text{NO}_3\text{-N}$ 1 mg [9]. In addition, the anodic oxidation directs carbon dioxide (CO_2) generation, which is necessary inorganic carbon source for autohydrogenotrophic denitrification, and plays a role in the acid–base balance in the system. Callegari et al. developed a two-stage bio-electrochemical system [10]; nitrite (NO_2^-) and nitrous oxide (N_2O) were observed in the first stage's effluent, and were successfully removed in the second stage. In addition, the $\text{NO}_3\text{-N}$ and hydrocarbon contaminants, including toluene, benzene, BTEX, and light PAHs, were successfully removed in BER due to the microbial metabolism with poised electrodes [11].

Furthermore, groundwater usually contains calcium and magnesium ions, also known as water hardness, due to the presence of alkaline earths. The hardness concentration varied from 70 to 544 mg/L in India's groundwater, and the water samples were mostly categorized as moderately hard and hard [12]. The water hardness is non-toxic and has no harmful health effects; however, it causes unpleasant effects, such as scaling in equipment, and requiring more soap and detergents. Due to previous studies, the water hardness (e.g., Ca^{2+} , Mg^{2+}) was able to deposit on the cathode surface during electrolysis. This chemical deposition adversely affected the cathodic electrolysis, and directly impacted the microbial activity, leading to a decline in $\text{NO}_3\text{-N}$ removal by autohydrogenotrophic denitrification [13,14]. The electrochemical method was applied to remove the hardness. Approximately 63% of eliminated hardness was deposited on the cathode in the form of CaCO_3 and $\text{Mg}(\text{OH})_2$, [15] whereas the rest was removed through floc surface adsorption [16]. Recently, Zeng et al. realized that the toxicity of nanoparticles (e.g., graphene oxide) to ecology was increased by increasing the water hardness [17].

The main objective of this study was to develop a BER using simple electrodes of copper wire and graphite plate to purify the $\text{NO}_3\text{-N}$ -contaminated groundwater. The system was continuously operated under various electric currents and water hardness levels. The $\text{NO}_3\text{-N}$ removal efficiency and denitrification microbial community were compared and discussed.

2. Materials and Methods

2.1. Materials

Chemicals included sodium nitrate, NaNO_3 (99%, KemAus, Cherrybrook, Australia, CAS no. 7631-99-4); potassium dihydrogen phosphate, KH_2PO_4 (99%, KemAus, Cherrybrook, Australia, CAS no. 7778-77-0); calcium dichloride, CaCl_2 (97%, KemAus, Cherrybrook, Australia, CAS no. 10043-52-4); sodium acetate trihydrate, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ (99%, Sigma-Aldrich, St. Louis, MI, USA, CAS no. 6131-90-4); and magnesium sulfate, MgSO_4 (97%, Sigma-Aldrich, St. Louis, MI, USA, CAS no. 7487-88-9).

Copper wire and graphite plate were electrodes materials, and were purchased from local suppliers.

2.2. Sludge Acclimatisation and Influent Preparation

Seed sludge was collected from a wastewater treatment plant in Phitsanulok, Thailand. The seed sludge had mixed liquid suspended solids (MLSS) of 12 mg/L, and mixed liquid volatile suspended solids (MLVSS) of 9.5 g/L. A synthetic groundwater (influent) was prepared with the following chemicals: 0.121 g/L NaNO₃, 0.170 g/L CH₃COONa·3H₂O, and 0.018 g/L KH₂PO₄; its NO₃-N concentration was approximately 20 mg/L, and no NO₂-N and NH₄-N were detected in the influent. The CH₃COONa·3H₂O represented a dissolved organic carbon in the groundwater; the concentration was approximately 30 mg/L of TOC (total organic carbon) [18], and a small amount of KH₂PO₄ was a major nutrient for microorganisms. During the experiments, various amounts of MgSO₄ and CaCl₂ were added in the influent (see in Table 1). The latter two chemicals represented the hardness concentration, which increased stepwise from 20, 40, and 80 mg/L as CaCO₃. The dissolved oxygen (DO) concentration was around 5 mg/L in the influent, which is similar value to that of actual groundwater.

Table 1. Operating condition of bio-electrochemical reactors.

Reactor	Electric Current (mA)	Water Hardness (mg/L as CaCO ₃)	Chemicals Used in the Influent Preparation (g/L)	
			MgSO ₄	CaCl ₂
BER-10	10	0 *	0	0
		20 ± 1	0.05	0.03
		40 ± 1	0.10	0.06
		80 ± 2	0.20	0.11
BER-20	20	0 *	0	0
		20 ± 1	0.05	0.03
		40 ± 1	0.10	0.06
		80 ± 2	0.20	0.11

* Reactors' start-up.

2.3. Experimental Setup and Operation

A copper wire (40 cm length × 0.1 cm diameter) was spirally covered on a graphite plate electrode (7.5 cm length × 15 cm width × 1 cm height), and both materials were placed into cylindrical BERs containing 1.4 L of working volume. Approximately 100 mL of acclimatized sludge was added into the reactors. The first reactor (namely, BER-10) was supplied with 10 mA of electric current from a DC power source, whereas the higher electric current of 20 mA was supplied to another reactor (namely, BER-20). The influent was continuously fed to the BERs with flow rate of 4.2 L/day (hydraulic retention time was around 8 h). In order to maintain low in-situ DO, small plastic beads covered the top of BERs to avoid oxygen transfer across the air–water interface; the in-situ DO was less than 0.5 mg/L. A low speed of magnetic stirrer (~150 rpm) was used to maintain the homogeneous mixtures. The experiments were operated at room temperature (~25 °C) with uncontrolled pH (7.5–8.5). The schematic diagram of BERs is shown in Figure 1a.

2.4. Microbial Characterization

Microbial characterization was identified by using the sequence of 16S rDNA. Briefly, the genomic DNA of sludge in the BERs was extracted from 0.2 g of biomass using a Nucleospin® DNA stool (Macherey-nagel, Düren, Germany). The 16S rDNA region was amplified using two primers: Forward primer,

(5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3');

and reverse primer,

(5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3').

The purified PCR was checked for its quantification using NanoDrop, and was subjected to DNA sequencing via the Illumina MiSeq system by Macrogen, Inc. (Seoul, Korea).

Sequencing analysis was performed by BLASTN, available online at the National Centre for Biotechnology Information, NCBI.

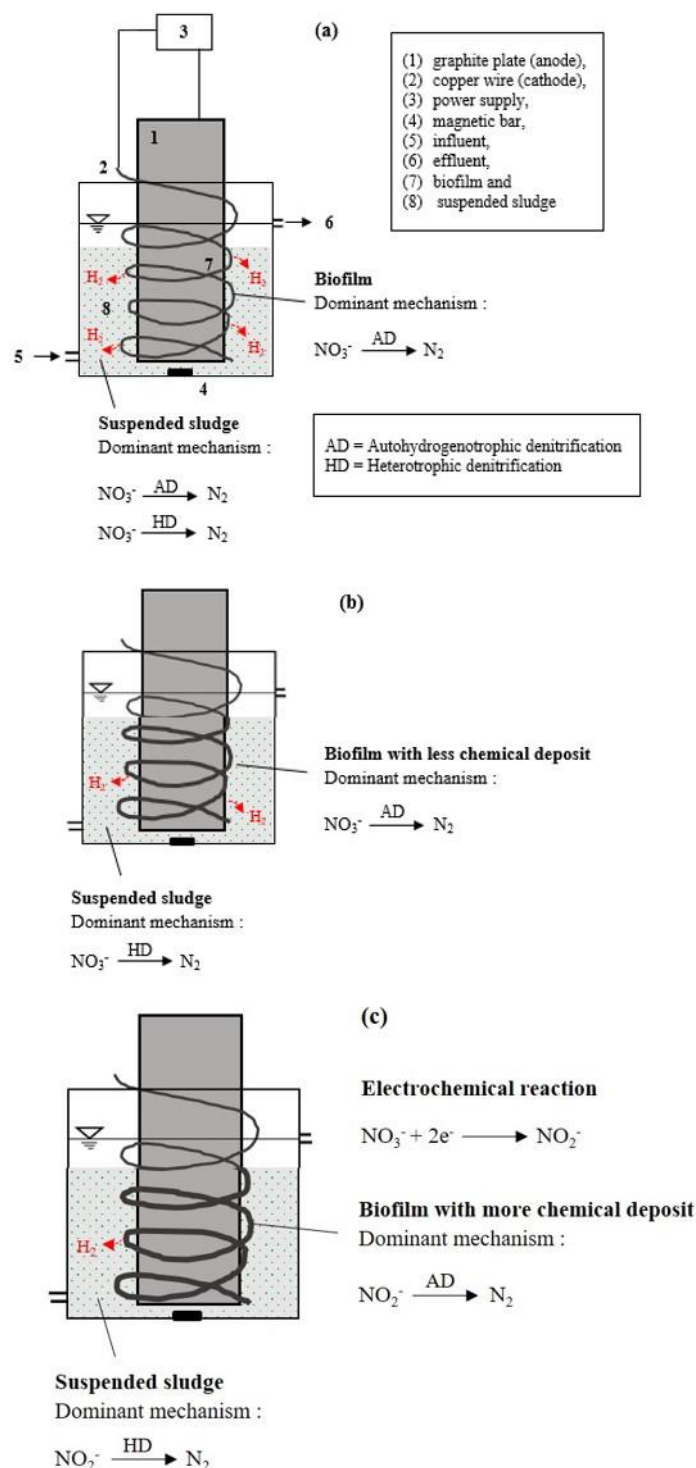


Figure 1. Suggested $\text{NO}_3\text{-N}$ removal mechanisms in the BERs under different conditions: (a) low electric current and low water hardness, (b) low electric current and high water hardness, and (c) high electric current.

2.5. Analytical Method

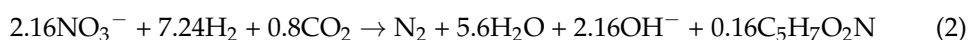
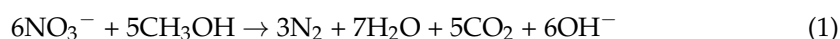
The $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$, and water hardness concentrations in the influent and effluent were measured by an ultraviolet spectrophotometric screening method (4500- $\text{NO}_3^- \text{ B}$), colorimetric method (4500- $\text{NO}_2^- \text{ B}$), phenate method (4500- $\text{NH}_3 \text{ F}$), and EDTA titrimetric

method (2340 C). The concentrations of MLSS and MLVSS were determined according to the standard method (2540). The dissolved organic carbon was analyzed by a total organic carbon (TOC) analyzer (Analytikjena, multi N/C 2100s). The DO and pH were measured by a DO meter (CyberScan DO 110 Model) and pH meter (Eutech Instruments). The denitrification performance was determined by total nitrogen removal and $\text{NO}_3\text{-N}$ removal efficiencies. The available elements at the cathode were analyzed by a scanning electron microscope and energy dispersive x-ray spectrometer (SEM-EDS, Leo1455VP).

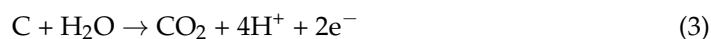
3. Results and Discussions

In order to investigate the effect of water hardness at low and high electric currents on the denitrification performance, the influent containing various hardness concentrations, from 20 to 80 mg/L as CaCO_3 , was fed to the BER-10 and BER-20. When starting the reactors (feeding soft water containing 20 mg/L of $\text{NO}_3\text{-N}$ and no hardness), the biofilm formations at the copper wire were observed. The total nitrogen and $\text{NO}_3\text{-N}$ removal efficiencies were continuously increasing and reached stable values of ~85%. This represented the steady state in which the microorganisms responsible for heterotrophic and autohydrogenotrophic denitrification were in abundance. The theoretical equations of heterotrophic and autohydrogenotrophic denitrification are suggested in Equations (1) and (2), respectively [11]. The organic carbon (i.e., CH_3OH) consumption in the heterotrophic denitrification was from dissolved organic carbon in the influent and lysed bacteria [19]. On the other hand, the anodic and cathodic reactions generated gaseous CO_2 and H_2 (see Equations (3) and (4)), which were essential substances for autohydrogenotrophic denitrification.

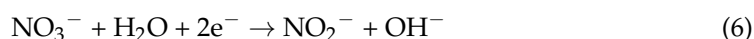
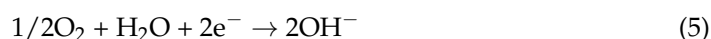
Biological denitrification reactions:



Electrochemical reaction at the anode:



Electrochemical reactions at the cathode:



3.1. Performance at Low Electric Current

The denitrification performance of BER-10 when increasing the hardness concentrations is presented in Table 2. At the 20 mg/L as CaCO_3 of water hardness, the total nitrogen removal efficiency was 85%, whereas a higher $\text{NO}_3\text{-N}$ removal of 92% was observed. The effluent $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$, and TOC were 1.5, 1.3, 0.1, and 10 mg/L, respectively. The consumption of organic carbon to remove $\text{NO}_3\text{-N}$ in this reactor was lower than that in the conventional heterotrophic denitrification [20]. The existing biofilm at the cathode also supported the occurrence of autohydrogenotrophic denitrification in the reactor [21]. The combination of heterotrophic and autohydrogenotrophic denitrification for $\text{NO}_3\text{-N}$ removal in the BER-10 is suggested in Figure 1a. The autohydrogenotrophic denitrification was the dominant mechanism occurring in the biofilm attaching at the copper wire, where gaseous H_2 was directly generating from water hydrolysis. Due to the gaseous H_2 transfer, the sufficient dissolved H_2 was observed in the reactor (of 1.0–1.2 mg/L), and this caused the autohydrogenotrophic denitrification to also occur in the suspended sludge. Due to the existing organic carbon and anaerobic condition in the reactor, the heterotrophic denitrification was another $\text{NO}_3\text{-N}$ removal mechanism, which happened in the suspended

sludge. Therefore, in order to purify $\text{NO}_3\text{-N}$ groundwater containing low hardness using the BER-10, the $\text{NO}_3\text{-N}$ was biologically reduced to N_2 via either heterotrophic or autohydrogenotrophic denitrification in the suspended sludge and biofilm.

Table 2. Average nitrogen concentration and total nitrogen removal efficiency in the BER-10.

Water Hardness Condition (mg/L as CaCO_3)	Influent $\text{NO}_3\text{-N}$ (mg/L)	Effluent (mg/L)			Efficiency (%)	
		$\text{NO}_3\text{-N}$	$\text{NO}_2\text{-N}$	$\text{NH}_4\text{-N}$	Total Nitrogen Removal	$\text{NO}_3\text{-N}$ Removal
20 ± 1	20 ± 0.5	1.5 ± 0.3	1.3 ± 0.2	0.0 ± 0.1	85.6 ± 1.7	92.1 ± 1.7
40 ± 1	20 ± 0.5	3.8 ± 0.5	2.5 ± 0.5	0.0 ± 0.0	67.1 ± 1.8	80.3 ± 2.3
80 ± 2	20 ± 0.5	3.6 ± 0.3	2.3 ± 0.2	0.0 ± 0.0	68.4 ± 1.7	80.2 ± 2.0

At the moderate water hardness of 40 mg/L as CaCO_3 , the decrease in removal efficiencies was observed in the BER-10: approximately 67% of total nitrogen and 80% of $\text{NO}_3\text{-N}$ (Figure 1b). Similarly, the removal efficiency of water hardness also decreased from 37% (at 20 mg/L of CaCO_3) to 12%, as shown in Figure 2. The SEM-EDS analysis revealed that both Mg and Ca elements of 16.8% and 8.1%, respectively, were detected in the biofilm (Figures 3a and S1). This is because chemical reactions occurred to precipitate the aqueous Mg and Ca ions in the influent and deposit as solid phases (i.e., CaHPO_4 , CaCO_3 , MgCO_3) at the cathode [22]. The chemical deposit of Ca and Mg negatively affected the reduction of dissolved H_2 and the activity of the autohydrogenotrophic denitrification in the suspended sludge. However, the autohydrogenotrophic denitrification was still dominant and occurred in the biofilm. The total nitrogen and $\text{NO}_3\text{-N}$ removal efficiencies were kept stable at the high water hardness of 80 mg/L as CaCO_3 . During the moderate and high water hardness, the heterotrophic denitrification became the key $\text{NO}_3\text{-N}$ removal mechanism in the suspended sludge, as supported by the larger consumption of organic carbon and less TOC concentration in the effluent (~ 5 mg/L). The possible mechanisms are proposed in Figure 1b.

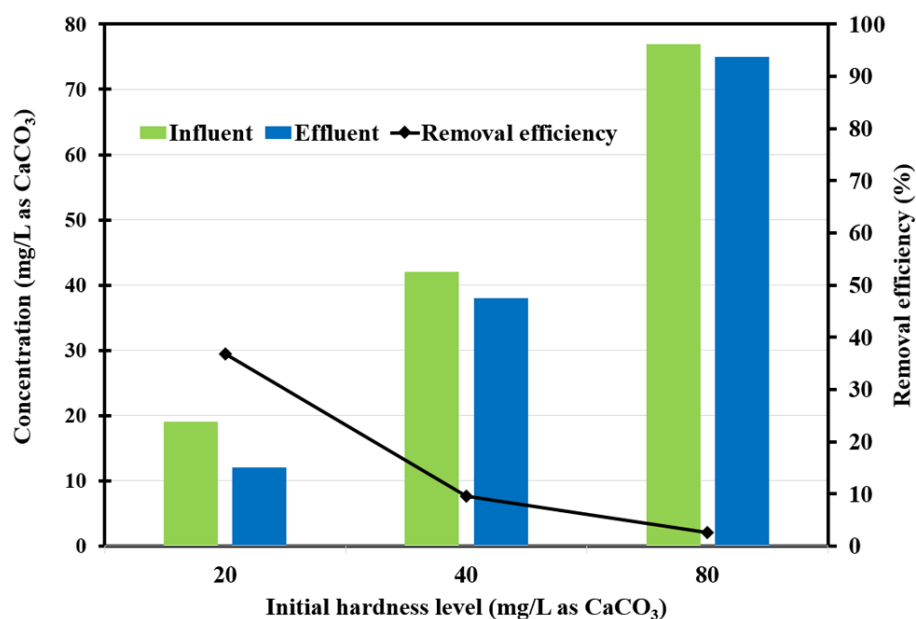


Figure 2. Water hardness concentration and removal efficiency.

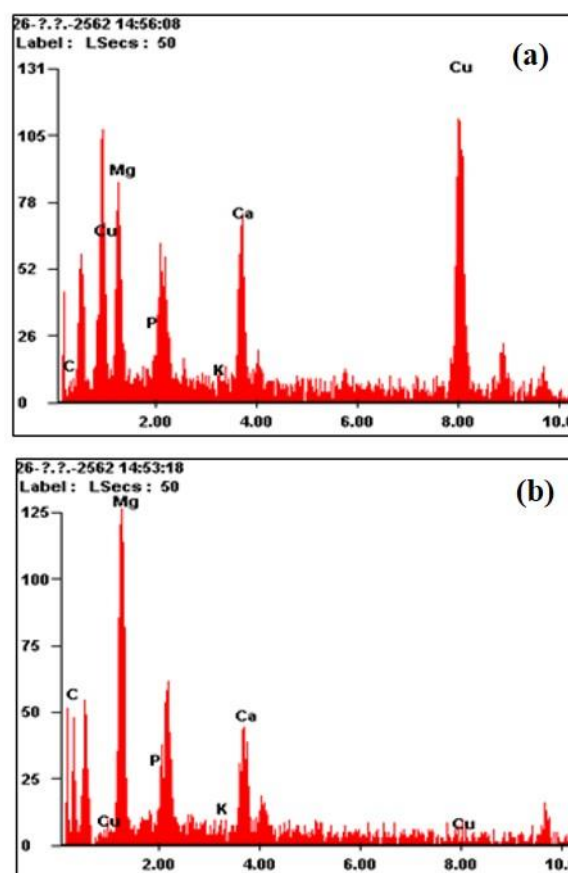


Figure 3. SEM-EDS analysis in the biofilm: (a) BER-10 and (b) BER-20.

3.2. Performance at High Electric Current

In this experiment, a high electric current of 20 mA was supplied to the BER, and the denitrification performance was evaluated under various water hardness concentrations. From Table 3, the total nitrogen and $\text{NO}_3\text{-N}$ removal efficiencies achieved the stable values of 92% and 94%, respectively, at 20 mg/L as CaCO_3 of water hardness. The performance of BER-20 was kept consistent (of 93–95%) at higher water hardness concentrations of 40 and 80 mg/L. The effluent $\text{NO}_2\text{-N}$ was relatively low of <0.5 mg/L, whereas the effluent TOC was approximately 10 mg/L during the three stages of water hardness. The results were different from the previous BER-10, in which its efficiencies were decreased by the operating period due to the chemical deposit of Ca and Mg ions. The possible mechanisms for $\text{NO}_3\text{-N}$ removal in the BER-20 are illustrated in Figure 1c. At a high electric current, the electrochemical reactions, including the reduction of O_2 to OH^- and NO_3^- to NO_2^- , were enhanced at the cathode (Equations (5) and (6)) [21]. The reduction of O_2 and the disappearance of $\text{NO}_3\text{-N}$ may lead to H_2 evolution at the copper wire [22]. Later, the intermediate $\text{NO}_2\text{-N}$ was utilized through heterotrophic and autohydrogenotrophic denitrification. In addition, the autohydrogenotrophic denitrification possibly mainly occurred in the biofilm, which was close to the H_2 generation source. On the other hand, the suspended sludge was playing a role of heterotrophic denitrification, because the dissolved H_2 was limited (approximately <0.5 mg/L). The available elements in the biofilm were further determined by SEM-EDS: 22.8 wt% of Mg and 10.8 wt% of Ca (Figures 3b and S2). The greater contents of Mg and Ca compared to that in the BER-10 was explained as the high electric current enhancing the electrochemical reactions; however, it also increased the chemical deposit in the biofilm. The achievement of overall $\text{NO}_3\text{-N}$ removal using the BER-20 was because the $\text{NO}_3\text{-N}$ was partially reduced to $\text{NO}_2\text{-N}$ through electrochemical

reactions, and the $\text{NO}_2\text{-N}$ was further biologically reduced to N_2 by the cooperation of heterotroph and autohydrogenotroph denitrification in either biofilm or suspended sludge.

Table 3. Average nitrogen concentration and total nitrogen removal efficiency in the BER-20.

Water Hardness Condition (mg/L as CaCO_3)	Influent $\text{NO}_3\text{-N}$ (mg/L)	Effluent (mg/L)			Efficiency (%)	
		$\text{NO}_3\text{-N}$	$\text{NO}_2\text{-N}$	$\text{NH}_4\text{-N}$	Total Nitrogen Removal	$\text{NO}_3\text{-N}$ Removal
20 ± 1	20 ± 0.5	1.2 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	92.6 ± 0.8	94.1 ± 1.2
40 ± 1	20 ± 0.5	1.2 ± 0.4	0.1 ± 0.1	0.0 ± 0.0	93.6 ± 2.3	94.0 ± 2.3
80 ± 2	20 ± 0.5	0.9 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	95.0 ± 1.4	95.3 ± 1.4

In the literature, the effect of current density on electrolytic denitrification has been demonstrated. Islam and Suidan [23] revealed that the optimal electric current was 20 mA; the lower electric currents affected less of the $\text{NO}_3\text{-N}$ reduction, whereas H_2 inhibition and charge-induced repulsion were found at higher electric currents. The excessive H_2 in biofilm negatively affected the denitrification performance. Zhao et al. [14] reported that the optimal condition for cooperating heterotrophic and autohydrogenotrophic denitrification in the BER was 40 mA of electric current and 0.75 of C/N; over 97% of $\text{NO}_3\text{-N}$ was removed, and organic carbon was completely consumed in the effluent. In addition, a high $\text{NO}_2\text{-N}$ accumulation was observed in the effluent when the electric current was 40 mA and 100 mA. Furthermore, the denitrification rate around the cathode was enhanced by increasing the electric currents; the maximal rate was 9.77 mg TN/ g MLSS/h at 40 mA, which was 55% higher than that in the control bioreactor (no electric current) [24].

3.3. Bacterial Community of BERs

The suspended sludge in the BER-10 and BER-20 was further identified as a bacterial community at phylum and genus levels by 16S rDNA gene amplicon sequencing (Figure 4). Proteobacteria, which is Gram-positive bacteria and complete denitrifiers [25], was the dominant phylum in the BER-10, accounting for 64.9%; the rest were classified as Actinobacteria (26.8%), Bacteroidetes (4.4%), and Verrucomicrobia (4.0%). The bacterial community in the BER-20 also consisted of the above four phyla, each accounting for 32.3% of Proteobacteria, 28.8% of Bacteroidetes, 25.0% of Actinobacteria, and 14.0% of Verrucomicrobia. These bacterial phyla played a dominate role of $\text{NO}_3\text{-N}$ removal under aerobic and anaerobic conditions [26].

Nocadia, *Pseudomonas* and *Thermonas* were relatively abundant genera in the BER-10: about 26.8%, 17.9%, and 10.7%, respectively. *Nocadia* was defined as hydrogen-oxidizing bacteria, and is able to utilize H_2 as the electron donor and O_2 as the electron acceptor [27]. *Pseudomonas* and *Thermonas* were known as typical active denitrifiers when organic carbon was available; however, these genera were suggested in the main hydrogenotrophic denitrifiers community [28]. *Hydrogenophaga* was commonly found in the denitrification system, and, after carrying out hydrogenotrophic denitrification, was higher in the BER-10 (2.7%) than that in the BER-20 (1.9%). In addition, some bacterial genera were only found in the BER-10, including *Acinetobacter* (6.0%), *Limobacter* (5.6%), *Pedobacter* (2.5%), and *Brevundimonas* (1.6%). *Acinetobacter* was observed in the pure culture of the hydrogenotrophic denitrification system, which was accumulating $\text{NO}_2\text{-N}$ [29]. On the other hand, *Flavobacterium* (27.1%) and *Brevifollis* (14%) became more abundant in the BER-20. *Flavobacterium* was capable of using a nitrogenous compound for anaerobic respiration, with a high $\text{NO}_2\text{-N}$ denitrification rate of 275.35 mg/L-h and a high specific removal rate of 51.80 mg/g-h [30]. In addition, *Flavobacterium* was able to grow in an aeration condition ($\text{DO} > 5$ mg/L) [31]. *Brevifollis* belonged to the Verrucomicrobia phylum, and was found in an intermittent aeration biofilm reactor for high-performance simultaneous nitrification and denitrification [32]. The coexistence of heterotrophic and hydrogenotrophic denitrifiers and multiple functional bacteria, such as anaerobic denitrifiers (*Flavobacterium*), aerobic

denitrifiers (*Comamonas*), and hydrogenotrophic denitrifiers (*Thermomonas*), coexisted in the previous BER [33].

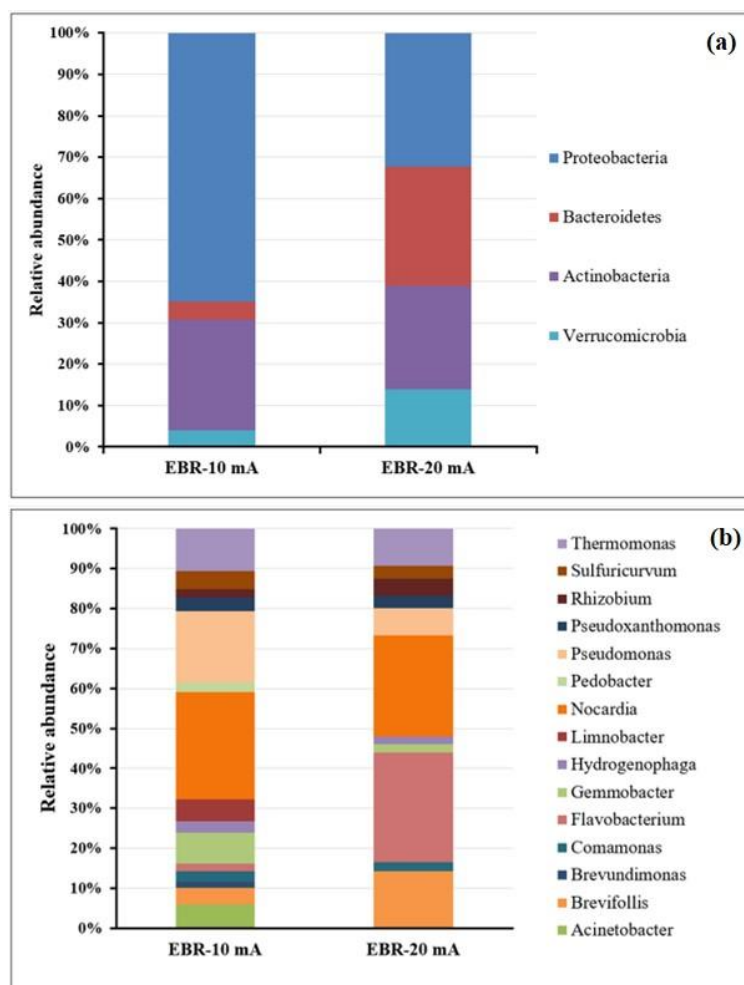


Figure 4. Abundance of bacterial community at (a) phylum and (b) genus levels in the suspended sludge.

4. Conclusions

The bio-electrochemical reactor (BER) developed in this study was a simple, economical, and feasible system to purify the $\text{NO}_3\text{-N}$ -contaminated groundwater. The applied electric current and existing hardness in the groundwater significantly influenced the total nitrogen removal efficiency and $\text{NO}_3\text{-N}$ removal mechanisms. The moderate and high hardness, ranging from 40 to 80 mg/L as CaCO_3 , caused a chemical deposit at the cathode and biofilm, which decreased the autohydrogenotrophic denitrification in the suspended sludge, and, consequently, declined the total nitrogen removal efficiency in the BER-10 (applying 10 mA of electric current). The BER-10 obtained a total nitrogen removal efficiency of 67% at the high hardness level. However, an increase in the electric current of 20 mA encouraged the enhanced electrochemical reactions; $\text{NO}_3\text{-N}$ was partially reduced to $\text{NO}_2\text{-N}$. Later, the $\text{NO}_2\text{-N}$ was denitrified to gaseous N_2 by either heterotroph or autohydrogenotroph. This phenomenon resulted in a consistent total nitrogen removal efficiency of 95% in the BER-20; although the higher Mg and Ca elements were deposited at the cathode. In addition, the combination of heterotrophic and autohydrogenotrophic denitrification, such as *Nocardia*, *Pseudomonas*, *Flavobacterium*, and *Hydrogenophaga*, were found in the BER-10 and BER-20. The difference in microbial diversity was due to different operating conditions and dominant biological $\text{NO}_3\text{-N}$ removal mechanisms. Furthermore, the long-term denitrification performance and electrode durability should be studied, as well as its actual groundwater implementation. The groundwater characteristics, including

other ions and contaminants, possibly causes synergy or inhibition effects to denitrification performance, $\text{NO}_3\text{-N}$ removal mechanisms, and the dominant bacterial community.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su14159454/s1>, Figure S1. SEM-EDS analysis in the biofilm of BER-10; Figure S2. SEM-EDS analysis in the biofilm of BER-20.

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