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Stimulation impact of electric currents on heterotrophic denitrifying microbial viability and denitrification performance in high concentration nitrate-contaminated wastewater

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ABSTRACT

Electric current stimulation has been shown to have a positive influence on heterotrophic denitrifying microbial viability and has the potential to improve wastewater denitrification performance. This study investigated the effects of varying current densities on microbial activity and NO_3^- removal efficiency under heterotrophic conditions.NO_3^- removal rate was highest at an applied current density of 400 mA/m^2. However, the optimum removal efficiency of total inorganic nitrogen (TIN; 99%) was achieved when the current density was fixed at 200 mA/m^2. Accumulation of NH_4^+-N and NO_2^--N byproducts were also minimized at this current density. The activity of heterotrophic denitrifying microorganisms was much higher at both 200 and 400 mA/m^2. Moreover, the average adenosine-5′-triphosphate (ATP) content (an indicator of cell metabolism) at a current density of 1600 mA/m^2 was lower than that under no current, indicating heterotrophic denitrifying microbial activity can be inhibited at high current densities. Hence, direct electrical stimulation on the activity of heterotrophic denitrifying microorganisms in the developed system should be lower than 1600 mA/m^2. This study improves the understanding of electric current influence on heterotrophic denitrifying microorganisms and promotes the intelligent application of direct electrical stimulation on wastewater treatment processes.

Introduction

Discharges of nitrate (NO_3^-) in wastewater to receiving waters leads to eutrophication (Posadas et al., 2013), resulting in proliferation of algae and decreased dissolved oxygen (DO) concentrations, fish kills and a loss of biodiversity (Monteagudo et al., 2012). NO_3^- contamination of drinking water also poses a serious health risk to humans and animals (Chen et al., 2003). Numerous prior studies have shown that the heterotrophic biological denitrification can be used to remove NO_3^- from domestic, industrial and agricultural wastewater, groundwater, and urban and agricultural runoff (Boley et al., 2002; Zhao et al.,

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2011; Zhang et al., 2012; Tong et al., 2013, 2014). The carbon source and electron donor for heterotrophic denitrification can either be an externally supplied organic carbon source (such as methanol) (Cheng and Lin, 1993) or an internal organic carbon source provided by the wastewater. The use of an internal organic carbon source is preferred for treatment of wastewater containing high organic matter concentrations, such as pharmaceutical, aquaculture, or meat processing wastewater, to reduce chemical and aeration costs and also avoid carry over of excess organic matter into the effluent (Saremi et al., 2013; Zhang and Huang, 2015).

Recent studies have also shown that biofilm-electrode reactors (BERs) can be used for combined biological and electrochemical treatment of NO_3^--contaminated water. In this process, hydrogen (H_2) produced by electrolysis serves as an electron donor for hydrogenotrophic (H_2 oxidizing) denitrification (Prosnansky et al., 2002), $NaHCO_3$ or H_2CO_3 acts as inorganic carbon source (Mousavi et al., 2012), and bacteria from *Proteobacteria*, *Bacteroidetes*, Chloroflexi, and *Planctomycetes* were dominant members in the autotrophic denitrifying biofilms (Xiao et al., 2015). The following stoichiometric equation describes the process (Mousavi et al., 2012):

$$2.16NO_3^- + 7.24H_2 + 0.8CO_2 \rightarrow 0.16C_5H_7O_2N + N_2 + 5.6H_2O + 2.16OH^- \quad (1)$$

Advantages of biofilm-electrode denitrification are lower biomass production, no carry over of excess organic substrate into the product water (Ergas and Reuss, 2001) and no required external addition of H_2 (Tong et al., 2013). Its main disadvantages are a lower NO_3^- removal rate compared with heterotrophic denitrification (Tang et al., 2012) and high electrical energy consumption.

In order to improve the denitrification efficiency and decrease electrical energy consumption, a combined heterotrophic/biofilm-electrode autotrophic denitrification process was developed in the previous studies (Zhao et al., 2011; Tong et al., 2013, 2014). In this synergistic process, CO_2 generated by heterotrophic denitrification is used as a carbon source by hydrogenotrophic denitrifying bacteria. Moreover, the electric current in the synergistic reactor not only electrolyzes the water to produce hydrogen for autotrophic denitrification, but can also positively influence microbial activity (Zhang et al., 2014a). On the other hand, the use of high current in a bio-electrochemical reactor system has the potential to inhibit microbial activity and may even cause sterilization of the reactor contents (Okochi and Matsunaga, 1997).

Recently, the influence of direct current (DC) and cultivating voltage on microbial viability and denitrification performance has been widely studied. According to the previous studies, it could be found that the microbial activity would be increased as the DC or current density increase (Wei et al., 2011; Safari et al., 2013; Liu et al., 2015a) and higher cultivating voltage helped to economize the operating voltage (Liu et al., 2015b). Xiao et al. (2016) also found that higher current density also can improve the recovery efficiency of NO_3^--N. However, the above research works all were the basis of low C/N ratio and current density. There is a lack of information on the influence of relatively high current on heterotrophic denitrification performance and bacterial activity in high organic matter wastewater. In particular, there is no relevant report on the upper limit of positive electron stimulation on denitrifying microbial viability to reflect the microbial tolerance or the influence of electrostimulation on heterotrophic denitrification in wastewaters with high concentrations of organic matter.

Therefore, the overall goal of this research was to explore the stimulation impact of electric currents on heterotrophic denitrifying microbial viability and denitrification performance in the wastewater with high concentration nitrate. The specific objectives were to: (1) investigate the impact of electric current density on denitrification performance in laboratory-scale bioreactor with seven different electric current density (0, 50, 100, 200, 400, 800, and 1600 mA/m^2, respectively); (2) determine the optimum current density to enhance heterotrophic denitrification performance and microbial activity; and (3) determine the upper limit for direct electrical stimulation on the activity of heterotrophic denitrification.

1. Materials and methods

1.1. Microorganism acclimation

Mixed liquor suspended solids (MLSS) were collected from a pre-anoxic zone in the Qinghe Sewage Treatment Plant (Beijing, China). Heterotrophic denitrifying bacteria were acclimated in a 2 L culture bottle at room temperature (20 ± 2 °C) according to the previous study (Liu et al., 2015a). Briefly, $NaNO_3$ (7 mmol/L), KH_2PO_4 (0.3 mmol/L) and glucose (10 mmol/L) were added in the media as microbial acclimation nutrients and were replaced every 4 days. Denitrification performance was monitored in the beginning and end of each acclimation cycle. Acclimation of heterotrophic denitrifying bacteria was considered complete when the NO_3^- removal efficiency was stable (p-value <0.05).

1.2. Synthetic high organic matter wastewater

Synthetic wastewater was prepared based on the previous study (Tong et al., 2013) of microbial cultivation and contained (per L): 2.0 g KNO_3, 5.0 g $C_6H_5Na_3O_7$ (sodium citrate), 0.2 g $MgSO_4{\cdot}7H_2O$, 1.0 g K_2HPO_4, 1.0 g KH_2PO_4. The pH was adjusted to 7.2 using 1 M NaOH. The resulting chemical oxygen demand (COD) and NO_3^--N concentrations in the synthetic wastewater were approximately 3.75 g/L and 280 mg/L, respectively. The use of a heterotrophic inoculum and the high COD concentration of the synthetic wastewater was expected to select for heterotrophic over hydrogenotrophic denitrifying bacteria (Xiao et al., 2013), therefore autotrophic denitrification was assumed to be negligible in this study.

The freshly prepared synthetic wastewater was sterilized at 121 °C for 30 min using autoclave (SX-500, TOMY, Japan) and then deoxygenated by sparging with N_2 for 5 min before each experiment. Note that all chemical reagents used in the experiments were of analytical grade.

1.3. Experimental apparatus

Experiments were carried out in a laboratory-scale bio-electrochemical reactor system as shown in graphical abstract section. Briefly, the experimental apparatus consisted of a 1–L effective volume glass column bio-electrochemical reactor (inside diameter = 9 cm, height = 16 cm) sealed with a rubber stopper, a DC regulated power supply, and a constant temperature magnetic stirrer. In the bioreactor, the cathode was made of a spiral stainless-steel wire (diameter = 1 mm, length = 3 m), while the anode was a stainless-steel rod (diameter = 4 mm, length = 20 cm). The anode was fixed in the center of the cathode and the electrode spacing was 15 mm. No reference electrode was used in the bio-electrochemical reactor system according to the previous study (Liu et al., 2015a). A sampling point was located in the center of the reactor. The reactor was operated on a constant temperature magnetic stirrer (H01-1C, Chijiu, China) at the conditions of 30 °C and 200 r/min to ensure a constant temperature and homogeneous distribution of heterotrophic denitrifying bacteria in the reactor. A DC regulated power supply (PS-302D, Shenzhen, China; Current = 0–2 A; Voltage = 0–30 V) was used to provide a constant DC for the experiments.

800 mL synthetic high concentration nitrate-contaminated wastewater was added in each bio-electrochemical reactor and run at the special condition for 14 days as described in Section 1.4. New anode and cathode electrodes were used and fresh acclimated sludge were added in each condition.

1.4. Experimental procedures

1.4.1. Confirmation of electrochemical reduction fraction

Abiotic experiments were performed at current densities of 0, 50, 100, 200, 400, 800 and 1600 mA/m^2 to quantify the contribution of electrochemical NO_3^- reduction. Note that the applied current densities have been designed according to the exponential relationship to investigate larger DC range. Each experiment was performed at constant current density over 14 days in 1 L of the synthetic wastewater described above. Concentrations of NO_3^--N, NO_2^-N, and NH_4^+-N and pH were measured in triplicate at the beginning and end of the experiment. ATP concentrations, an indicator of cell metabolism (Wang et al., 2017) were measured every 24-h to verify that the systems remained abiotic throughout the experiment. If ATP concentration was detected higher than the method detection limit, the experiment was re-run.

1.4.2. Heterotrophic denitrification under different current densities

Bioreactor experiments were similar to the abiotic experiments described in Section 2.3.1, except that 10 mL of acclimated heterotrophic denitrifying bacteria (described in Section 2.1) were added to the reactor at the beginning of each experiment. Note that experiments were in duplicate at each condition. COD and pH were measured at the beginning and end of each experiment. NO_3^--N, NO_2^--N, NH_4^+-N, and ATP concentrations were measured every 24-h to quantify denitrification performance and microbial activity.

1.5. Analytical methods

NO_3^--N, NO_2^--N and NH_4^+-N were measured according to Standard Methods (APHA, 2012) (4500-NO_3^- C, 4500-NO_2^- B, and 4500-NH_3 F) using an ultraviolet spectrophotometer (DR 6000, HACH, the USA). Method detection limits (MDLs) for NO_3^--N, NO_2^--N and NH_4^+-N were 0.08, 0.003, and 0.025 mg/L, respectively. COD was measured using *Standard Method* 5220 D (MDL: 25 mg/L). pH was measured using a calibrated pH meter and probe (InLab Expert Pro, METTLER TOLEDO, Swiss; MDL: 0–14). ATP content was measured using an ATP analyzer (AF-100, TOA-DKK, Japan; MDL: 0.02 nmol/L).

2. Results and discussion

2.1. Electrochemical nitrate reduction

Results from tests of abiotic electrochemical reduction of NO_3^- at varying current densities are shown in Fig. 1. NO_3^- reduction efficiency gradually increased from 0 to 26.6% (80.1 mg NO_3^--N/L reduced) as current density increased from 0 to 1600 mA/m^2, with significant NO_3^--N removals observed at current densities between 400 and 1600 mA/m^2. Our previous study (Liu et al., 2015a) reported the electrochemical reduction on NO_3^- removal at the current densities between 0 and 400 mA/m^2 and they found the similar phenomenon that no NO_3^- was reduced at 50–200 mA/m^2, and a small amount of NO_3^- was removed at 250–400 mA/m^2. This might be due to the stainless-steel wire cathode utilization, which has been shown to have the NO_3^- reduction performance by Dash and Chaudhari (2005). They investigated electrochemical denitrification of simulated groundwater and reported a high selectivity for NO_3^- reduction to NH_4^+ using a Fe cathode, which was similar to the stainless-steel wire used in this study.

Similarly, Liu et al. (2015a) reported that the maximum NO_3^- removal efficiency was 10.3% at 400 mA/m^2 when applied current densities were between 0 and 400 mA/m^2 in a bio-electrochemical reactor. Zhao et al. (2012) also observed that the NO_3^- reduction efficiency increased to 5.7% by electrochemical catalysis and reduction in an intensified BER as the applied current increased to 500 mA.

Both NO_2^- and NH_4^+ accumulation increased with increasing current densities, as shown in Fig. 1. Maximum NO_2^--N accumulation concentration of 6.62 ± 0.05 mg/L and NH_4^+-N accumulation concentration of 23.19 ± 0.08 mg/L were both observed at an applied current density of 1600 mA/m^2. This can be explained through the electrochemical NO_3^- reduction using the stainless-steel electrode, where NO_2^- and NH_4^+ are the main by-products of electrochemical NO_3^- reduction (Brylev et al., 2007; Li et al., 2010).

Overall, abiotic electrochemical reduction can occur in the system and 10.6% of NO_3^- was reduced to NO_2^- and NH_4^+ at 1600 mA/m^2, which consumed 39.8% of NO_3^- electrochemical reduction. Fe cathodes, for example of stainless-steel in this study, have been proven to be relatively efficient promoters for NO_3^- electrochemical reduction (Li et al., 2010). At the cathode, NO_3^- are mainly reduced to NO_2^-, NH_4^+ and N_2, which is electrochemically inactive (Li et al., 2010). NH_4^+ and NO_2^- are the unfavorable reduction products, limiting the application of the electrochemical process for denitrification (Brylev et al., 2007). Hence, bio-electrochemical reactor was used for NO_3^- reduction in this study.

Moreover, ATP contents were all below the MDL in the abiotic experiments, indicating there were no denitrifying bacteria present during these experiments. After 14 days of treatment, the pH in the effluent increased to 9.17 at an applied current

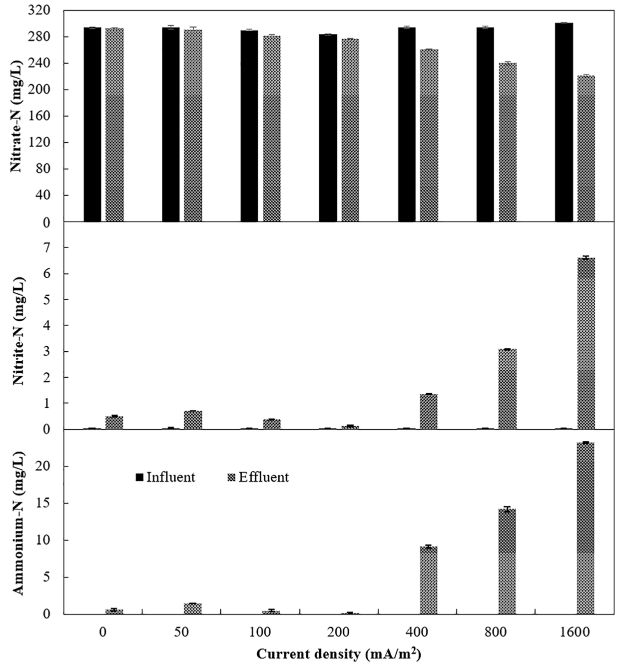

Fig. 1 – NO_3^--N reduction as well as NO_2^--N and NH_4^+-N accumulation in electrochemical reduction progress under different current densities.

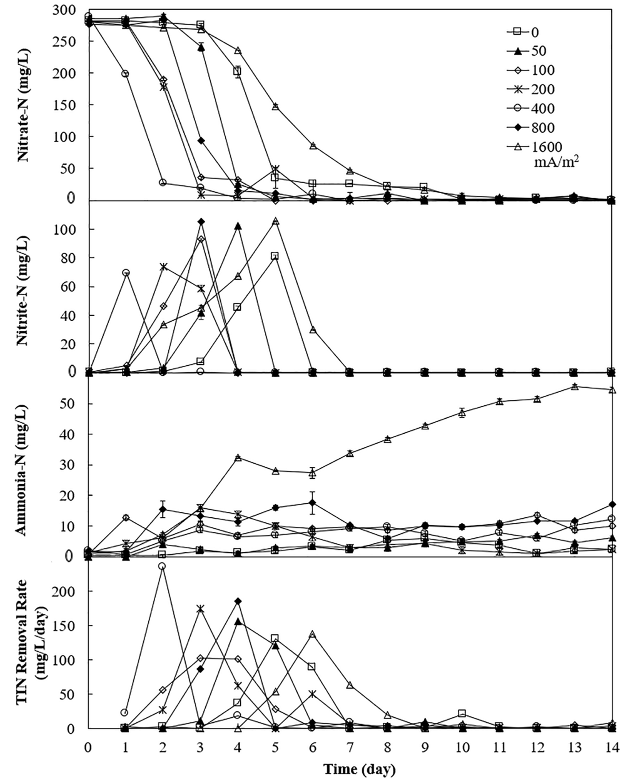

Fig. 2 – NO_3^--N removal as well as NO_2^--N and NH_4^+-N accumulation versus time in a bioreactor under different current densities.

density of 1600 mA/m^2, which was the condition that led to the highest pH increase. The pH increase may have been due to the generation of hydroxyl ions (OH^-) during electrochemical reduction of NO_3^- (Li et al., 2010). Similarly, Liu et al. (2015a) investigated the pH increase in a bio-electrochemical reactor and found that the pH increased from 7.23 to 9.23 at 250 mA/m^2 and to 11.14 at 400 mA/m^2. However, unlike their study, the pH increase was not significant in our research (only from 7.20 to 8.31 at 400 mA/m^2), most likely due to buffering provided by K_2HPO_4 and KH_2PO_4 in the synthetic wastewater.

2.2. Heterotrophic denitrification under varying current densities

Heterotrophic denitrification using sodium citrate as carbon source was described as below:

$$18NO_3^- + 5C_6H_5Na_3O_7 + 18H^+ \rightarrow 9\,N_2 + 15NaOH + 30CO_2 + 14H_2O \quad (2)$$

Based on Eq. (2), for every 1.0 mol NO_3^--N removed, 0.28 mol sodium citrate would be consumed and 0.83 mol OH^- as alkalinity are produced. In this study, the mole ratio between sodium citrate and NO_3^--N was 0.68, which was much higher than 0.28 for heterotrophic denitrification in Eq. (2). Hence, carbon source was overdose.

2.2.1. Nitrate reduction

NO_3^--N, NO_2^--N and NH_4^+-N concentrations over time at varying current densities in the bioreactor are presented in Fig. 2. NO_3^- reduction rates increased as the applied current density increasing from 0 to 400 mA/m^2, and then decreased with increasing current density from 400 to 1600 mA/m^2. NO_3^- removal rate was enhanced at current densities of 50, 100, 200, 400, and 800 mA/m^2, when compared with the results under the condition of no applied current. Denitrification inhibition was only observed at the highest current density of 1600 mA/m^2, where NO_3^- removal rate significantly lower than that without DC.

At the optimum current density of 400 mA/m^2, NO_3^--N decreased from 288.34 ± 2.04 to 26.93 ± 0.59 mg/L over the first two days of the experiment, while 6 days were required to remove a similar amount of NO_3^--N when no current was applied. During the 2nd day, the amount of NO_3^--N reduction in 400 mA/m^2 microcosms was 109 times that in no current microcosms. Appropriate electrical stimulation would greatly improve the denitrification performance. In addition, no lag phase was observed at an applied current density of 400 mA/m^2. This might be due to that the lag phase was shorter than 24 h of our sampling interval time. However, it still can indicate that an additional advantage of electrical stimulation at an optimal current density was to shorten the acclimation phase requirement for heterotrophic denitrifying microorganisms, which has also been reported by Liu et al. (2017) according to the electro-stimulation study on the low C/N NO_3^--contaminated groundwater.

The occurrence of peak NO_2^- accumulation was observed firstly at the optimal current density of 400 mA/m^2, while the accumulation peak was delayed at both higher and lower applied current densities (Fig. 2). NO_2^--N accumulation followed the trends in NO_3^- reduction, most likely because heterotrophic denitrification is a two-step processes, in which NO_2^- is an intermediate byproduct (Soares, 2000; Park et al., 2008). Some microbial species, such as *Aeromonas hydrophila*, *Comamonas acidovorans*, *Pseudomonas (mendocina)*, and *Shewanella putrefaciens* (Liessens et al., 1992), can only reduce NO_3^- to NO_2^-. Liu et al. (2015a) investigated electrostimulation on the denitrification performance and also found that NO_2^--N accumulation increased and then decreased. Hence, NO_2^--N accumulation in the bioreactor indicates that the rate of NO_2^- production exceeded the rate of NO_2^- reduction. Moreover, the occurrence of NO_2^- accumulation peak also can reflect the bioreactor in the condition of highest NO_3^- removal from the side.

Fig. 2 also shows the variations in the time requirement for complete NO_2^- degradation at varying applied current densities. Only 2 days was required for complete NO_2^--N degradation at a current density of 400 mA/m^2, compared with 6 days when no current was applied. When the applied current density was higher than 400 mA/m^2, the required time increased. These results indicated that second-step of heterotrophic denitrification ($NO_2^- \rightarrow N_2$) could be enhanced by applying DC at an appropriate current density. Wu et al. (2016) also reported the similar phenomenon, where NO_2^- reduction would 1.8-fold enhance as the 1 V electron stimulation applied. On the other hand, the longest time requirement of 7 days was observed at 1600 mA/m^2, showing the similar conclusion to the result of NO_3^- that denitrification inhibition occurred. Hence, a current density of 1600 mA/m^2 was too high to inhibit the heterotrophic denitrifier activity in this study.

NH_4^+ accumulation at varying current densities is also shown in Fig. 2. NH_4^+-N accumulation under any applied current was higher than that without electrical energy. NH_4^+ may be produced by both electrochemical NO_3^- reduction through the stainless-steel cathode (Liu et al., 2015a) and/or the dissimilatory NO_3^- reduction to NH_4^+ (DNRA) process (Zhang et al., 2014b). Prior studies have shown that the DNRA process is favored by higher electron donor/acceptor ratios (i.e., a higher C/N will lead to more NH_4^+ accumulation) (Zhang et al., 2012). In this study, COD/N ratio was high enough to produce some NH_4^+ byproduct through DNRA. When no current was applied, the NH_4^+-N concentration was maintained between 0.4 and 6.0 mg/L throughout the 14 days experimental period only due to DNRA process. On the other hand, when the current densities were changed between 0 and 800 mA/m^2, NH_4^+-N concentration accumulations were always lower than 20 mg/L (<7% NO_3^- was transferred to NH_4^+). However, when the bioreactor run at the current density of 1600 mA/m^2, NH_4^+-N concentration increased up to 55.55 mg/L over time. Compared with the abiotic experiments presented previously in Section 2.1, it could be known that the maximum NH_4^+-N accumulation from NO_3^- electrochemical reduction would be gained when the applied current density was the highest of 1600 mA/m^2.

During the abiotic experiments, it was found that electrochemical NO_3^- reduction increases as the current density increased from 0 to 1600 mA/m^2. However, it could be seen that the accumulation of NH_4^+-N in the bioreactor decreased when the applied current density was fixed at 100 or 200 mA/m^2. This phenomenon was only can be explained from the hypothesis of electrical inhibition for DNRA process. When the applied current density was higher than 100 mA/m^2, the DNRA process was weakened. Hence, the DNRA process would decline further when the current density was higher than 200 mA/m^2. However, when the applied current density was high enough, such as 1600 mA/m^2, the final NH_4^+-N accumulation concentration still increased even DNRA process is very weak, owing to the substantial enhancement of NO_3^- electrochemical reduction as applied current density increasing.

A summary of NO_3^--N removal, NO_2^--N accumulation and NH_4^+-N accumulation, at different applied current densities for both abiotic experiments and bioreactor experiments is shown in Table 1, where heterotrophic denitrification under different current densities was gained through electrochemical reduction and heterotrophic denitrification minus electrochemical reduction. As shown in Table 1, no NO_2^- accumulation was observed from only heterotrophic denitrification. It could be also found that a complex reaction process occurs in the bioreactor when conditions favoring heterotrophic denitrification and DC were applied simultaneously. When the current density increased from 0 to 1600 mA/m^2, the increasing proportion of NO_3^- removal from electrochemical reduction leads to NO_3^- removal from heterotrophic denitrification process decreasing from 280 to 200 mg NO_3^--N /L. Compared to the report by Liu et al. (2015a), it could be found that higher applied current density for positive stimulation not only leads to higher denitrification rate, but also promotes more electrochemical NO_3^- reduction to reduce the heterotrophic denitrification load. Hence, higher optimum current density enhanced heterotrophic denitrification from both improve reaction rate and decrease load.

2.2.2. Total inorganic nitrogen (TIN) removal

Final concentrations of inorganic nitrogen species and TIN removal efficiency are summarized in Table 2. The removal efficiency of TIN was 99.10% at a current density of 200 mA/m^2, when NH_4^+-N and NO_2^--N byproduct accumulations were only 2.49 and 0.03 mg/L, respectively. When the applied current density was 400 mA/m^2, even though the NO_3^- reduction rate was the highest, the NH_4^+-N accumulation (12.11 ± 0.31 mg/L, Table 2) was also much higher than that of 200 mA/m^2.

TIN removal rates (mg/L/day) at different current densities during the 14 days experiment are also shown in Fig. 2. The TIN removal rate changed over time and showed different trends at different current intensities. When the current density was fixed at 400 mA/m^2, the fastest increase of TIN removal rate was observed. The rate increased up to 235.76 mg/L/day and 81.2% of TIN was removed by the second day. The average TIN removal rate was the lowest at 1600 mA/m^2, which was even lower than that of under no current condition.

2.3. Effect of current densities on heterotrophic denitrifying microbial activity

Samples were collected every 24 h for ATP content analysis and the results are shown in Fig. 3. ATP concentrations are always detected as an indicator of cell metabolism (Wang et al., 2017) and were used as a proxy for heterotrophic denitrifying microbial activity in this study. Meanwhile, most bacteria were wrapped in the sludge (Liu et al., 2015a)

Table 1 – Summary of NO_3^- reduction and NO_2^- and NH_4^+ accumulation in different reaction types under different current densities.

Current density (mA/m^2)	Electrochemical reduction			Electrochemical reduction and heterotrophic denitrification			Heterotrophic denitrification [a]		
	NO_3^--N removal (mg/L)	NO_2^--N accumulation (mg/L)	NH_4^+-N accumulation (mg/L)	NO_3^--N removal (mg/L)	NO_2^--N accumulation (mg/L)	NH_4^+-N accumulation (mg/L)	NO_3^--N removal (mg/L)	NO_2^--N accumulation (mg/L)	NH_4^+-N accumulation (mg/L)
0	1.36	0.47	0.58	281.53	0.24	2.49	280	– [b]	2
50	3.75	0.68	1.47	283.92	0.14	6.23	280	–	5
100	8.52	0.36	0.45	281.53	0.28	9.97	273	–	10
200	6.48	0.11	0.09	279.82	0.03	2.49	273	–	2
400	33.06	1.34	9.08	279.14	0.05	12.11	246	–	3
800	53.51	3.08	14.16	276.07	0.07	17.09	223	–	3
1600	80.10	6.61	23.19	280.85	0.11	54.49	201	–	31

[a] Heterotrophic denitrification was gained through: Electrochemical reduction and heterotrophic denitrification - Electrochemical reduction. Due to the calculation, the number of significant figures was decreased.
[b] No calculated accumulation.

together with synthetic wastewater under the condition of fully mixing, hence the ATP content in the wastewater could reflect the heterotrophic denitrifying microbial activity in the bio-electrochemical reactor. A declining trend in the activities of heterotrophic denitrifying microorganisms was observed in the late stage of each experiment, owing to the nutrient consumption. Even so, the final ATP content in the bioreactor were all between 130.4 and 160.8 nmol/L at current densities of 100, 200, and 400 mA/m^2, which was significantly higher than that of between 13.3 and 68.2 nmol/L at 0, 50, 800, and 1600 mA/m^2. It can be considered that the activity of heterotrophic denitrifying microorganisms kept at the higher level when the appropriate current densities were applied.

Fig. 3 also illustrates the average ATP concentration change over time in the bioreactor under different applied current densities. It could be seen that the ATP content within 14 days increased when the applied current density rose from 0 to 400 mA/m^2, showing that proper application of the DC field could improve biomass concentration (Ibeid et al., 2013). Zhang et al. (2014a) also reported that weak electrical stimulation from microbial fuel cells could improve 1.5-fold ATP of heterotrophic denitrifying bacteria. The average ATP content decreased when the current density further increased to higher than 400 mA/m^2. Importantly, the average ATP content at the current density of 1600 mA/m^2 was lower than that of 0 mA/m^2, showing that heterotrophic denitrifying microbial activity was inhibited. Similar regular to NO_3^- removal rate was presented in Section 2.2.1. The negative effect of DC was observed at a relative higher current density (Jackman et al., 1999), which was 1600 mA/m^2 in this study. Hence, in order to enhance the denitrification performance, the applied current density should be under 1600 mA/m^2 (upper limit of applied current density). The detailed critical value of high current density (between 800 and 1600 mA/m^2) for heterotrophic denitrification performance and microbial activity will be fully investigated in the future study. Diao et al. (2004) investigated the bactericidal action of electrochemical disinfection, also reporting that the current intensity for the electrolytic stimulation process was normally kept at a lower level to avoid the side-effect on microorganisms at high current density. Moreover, the average ATP content and variation tendency within 14 days in the bioreactor at the applied current density of 200 mA/m^2 was equal to that of 400 mA/m^2. Hence, 200–400 mA/m^2 applied current density were beneficial to heterotrophic denitrifying microorganisms.

Moreover, the average ATP contents in the bioreactor were all increased during the first 2 days no matter at any applied current densities (Fig. 3). Compared the first 2 days ATP concentrations, it could be seen that the growth rate of average ATP contents was: (1) much higher than that of no applied current when the current density was fixed at 200 or 400 mA/m^2; (2) whereas all lower than that of on applied current at other applied current densities of 50, 100, 800 and 1600 mA/m^2. Hence, the bioreactor showed the fastest bacteria acclimation at the current densities of 200 and 400 mA/m^2. For example, during the 2nd day, NO_3^--N removal efficiency was achieved at 90.66% in the 400 mA/m^2 microcosms and ATP content was 5 times higher than that in no current microcosms, in which only 2–3 mg/L NO_3^--N was removed.

Table 2 – Nitrogen species concentration and TIN removal efficiency at the end of each set of experiments.

Current density (mA/m^2)	NO_3^--N (mg/L)	NO_2^--N (mg/L)	NH_4^+-N (mg/L)	TIN (mg/L)	Removal Efficiency of TIN (%)
0	BDL[a]	0.24 ± 0.02	2.49 ± 0.31	2.73	99.03
50	2.04 ± 0.02	0.14 ± 0.02	6.23 ± 1.11	8.42	97.06
100	BDL	0.28 ± 0.05	9.97 ± 0.62	10.25	96.37
200	BDL	0.03 ± 0.02	2.49 ± 0.82	2.52	99.10
400	0.68 ± 0.02	0.05 ± 0.02	12.11 ± 0.31	12.84	95.58
800	BDL	0.07 ± 0.00	17.09 ± 0.53	17.17	93.82
1600	BDL	0.11 ± 0.05	54.49 ± 0.93	54.60	80.63

BDL: below detection limit (0.08 mg/L for NO_3^--N).

Liu et al. (2015a) used the similar bio-electrochemical reactor to investigate the effect of electro-stimulation on the activity of heterotrophic denitrifying bacteria with methanol as the organic substrate. The authors confirmed that denitrification activity could be enhanced by electro-stimulation, with the highest ATP level obtained at an applied current density of 200 mA/m^2. The higher optimal current density (400 mA/m^2) observed in this study may indicate that the microorganisms had greater viability when a higher organic substrate concentration (COD of 525 ± 15 mg/L in this study) was available. Furthermore, methanol has been shown to be toxic to some denitrifying microorganisms (Tong et al., 2014), which might be another reason to explain the lower optimum and adaption conditions gained by Liu et al. (2015a).

Wei et al. (2011) investigated the influence of electric current on bacterial viability in wastewater treatment and found that the live cell dropped by 29% at DC of 24.7 A/m^2. They also discovered that cells on the cathode surface exhibited the highest death rate, whereas bacteria outside the space between electrodes were the lowest affected, and suggested that sufficient mixing should be used to avoid localized inactivation of bacterial cells. In this study, the all-time mixing was used to reduce the cell death on the cathode. Hence, the higher endurance of electrical stimulation was received. Even the current density was up to 1600 mA/m^2, the heterotrophic denitrifying bacteria still had the certain activity until the bioreactor operation to 11 days.

The change of COD in the end among different runs also could reflect the microbial activity, because the rest of COD showed the organic carbon source consumption. Profiles of COD concentration at the end of each microcosm under different current densities are shown in Fig. 4. COD remaining concentration decreased rapidly as the current density increasing from 0 to 200 mA/m^2. Ibeid et al. (2013) investigated the modification of activated sludge properties by current application, and reported that the application of a DC field could improve the degradation rate of organic colloids. They also found that electro-osmosis, electrophoreses and electro-migration, the motion of particles and the physical–chemical interactions between solids and the liquid were different at higher current density, which might impact COD removal performance. In addition, She et al. (2006) investigated electrolytic stimulation of *Enterobacter dissolvens* using a DC and glucose as sole carbon source, finding that organic carbon source (glucose) consumption increased 1.5-fold under weak electric field and the best stimulating effects in terms of cell growth and the dehydrogenase activity were obtained at a DC of 10 mA. The authors also reported that the application of a DC also led to accelerated cell death during the later stationary phase due to the presence of anodic intermediates including H_2O_2, OH· and O_2.

The concentrations of COD were almost equal at the current densities of 200 and 400 mA/m^2. In general, the current density increase would cause more COD consumption in electrochemical reaction. However, the microbial demand of organic matter reduced as the increase of current density between 200 and 400 mA/m^2, causing the phenomenon of COD consumption in total unchanged.

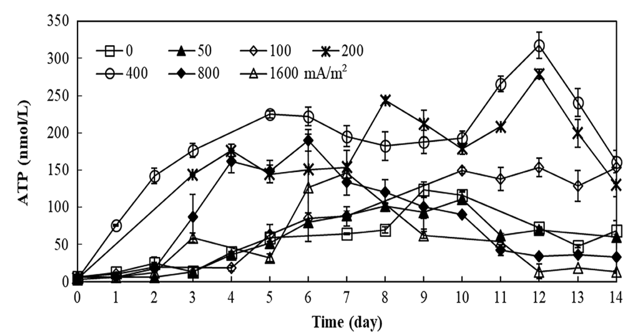

Fig. 3 – Heterotrophic denitrifying bacteria activity under different current densities.

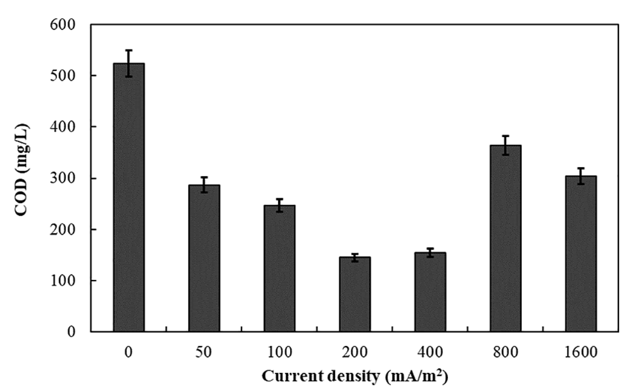

Fig. 4 – The end concentrations of COD under different current densities.

Moreover, the concentration of COD in the end increased when the current density was higher than 400 mA/m^2. It could also be found that the COD consumption was proportional to the TIN removal efficiency (Table 2) when the applied current density was changed between 0 and 800 mA/m^2. However, COD showed a little decreased at 1600 mA/m^2, due to the electrochemical oxidation under such high current density. Li et al. (2005) studied the reaction pathways and mechanisms of the electrochemical degradation of phenol, a kind of organic matter, on different electrodes, finding that electrochemical role could oxide the organic matter under current. Moreno-Casillas et al. (2007) investigated electrocoagulation mechanism for COD removal, also reporting that Fe electrodes could get a better results of COD removal.

In summary, electric current stimulation had the influence on heterotrophic denitrification microbial viability and NO_3^- removal efficiency. Both heterotrophic denitrification microbial activity and NO_3^- reduction efficiency increased firstly and then decrease as the applied current densities increasing from 0 to 1600 mA/m^2. The average ATP content at the current density of 1600 mA/m^2 was lower than that at 0 mA/m^2, showing the inhibition of heterotrophic denitrifying microbial activity. Further study will be needed to investigated the detailed critical value of high current density microbial activity together with the in-depth research on the mechanism of denitrifying bacteria electrical stimulation.

3. Conclusions

Electrical stimulation affects heterotrophic denitrifying microbial activity. NO_3^- removal rate and microbial activity were the highest at 400 mA/m^2 and no microbial lag phase was observed at this applied current densities. However, the highest TIN removal efficiency of 99% was achieved at 200 mA/m^2. Microbial activity and denitrification performance are inhibited at 1600 mA/m^2. Therefore, electric current stimulation had the influence on heterotrophic denitrification microbial viability and NO_3^- removal efficiency in the wastewater treatment, providing a better understanding of electrical stimulation on heterotrophic denitrifying microorganisms.

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