Autohydrogenotrophic denitrification by a bioelectrochemical process: A viability study

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ABSTRACT

In this study, viability of the autotrophic bacteria was investigated in a denitrification process. Bench-scale bioelectrochemical denitrification with separated chambers reactor were applied for nitrate reduction from synthetic wastewater. The influences of current density, retention time, mixing on viability of autotrophic denitrifying bacteria were investigated in the bioelectrochemical reactor (BER). It was found that by increasing the current density up to 8 mA/cm^2, nitrate reduction rate was increased. At higher current density (24 - 32 mA/cm^2), denitrification rate due to excess of hydrogen gas on cathode surface and accumulation of nitrite, was decreased. Low current density (<16 mA/cm^2) has not had a significant effect on viability of denitrifying bacteria. Mixing of the solution reduced the adverse effects of electric current on bacteria and enhanced the denitrification rate which was mainly due to prevention of bacteria localization, increasing the contact of hydrogen and bacteria, and delay in membrane fouling. The viability of cultivable bacteria has not been significantly influenced by running time.

Key words: Nitrate; autotrophic; Bioelectrochemical; Viability; Reduction; Wastewater

INTRODUCTION

Due to extensive usage of nitrogenous fertilizers and discharge of industrial and domestic wastewater, nitrate contamination of water is becoming a main environmental concern. High levels of nitrate in drinking water cause serious health problems such as methemoglobinemia in infants and gastric cancer [1, 2]. Because of such health problems, nitrate removal from water is urgent and has been a hot topic over the recent years. Therefore, maximum concentration level of nitrate in drinking water has been set 10 mg NO_3^-/l by World Health Organization and Environmental Protection Agency [3, 4]. Various technologies such as the ion exchange [5], reverse osmosis [6], electrocatalytic [7], adsorption [8], electrodialysis [9] and biological process [10], have been used to eliminate nitrate ion from water and wastewater. Nevertheless, these methods have several drawbacks such as high installation and maintenance costs, difficult operation, brine production, membrane fouling, further treatment, slow process and carbon source requirement [3, 11]. A large number of investigators thus have focused on the reduction of nitrate by the electrochemical process usually because of its efficiency, very low sludge production, small area occupation and facile operation [12-14]. Electrochemical processes, however, have some weaknesses such as high consumption of electrical energy. Therefore, integration of electrochemical and biological processes as bioelectrochemical systems has been recommended to overcome the potential problems [15, 16]. Denitrifying bacteria are classified into two main groups of heterotrophic and autotrophic. Organic carbon compounds are the main carbon and energy source of heterotrophic bacteria [17], while inorganic carbons such as carbon dioxide or bicarbonate can be used as the carbon source of autotrophic bacteria. On the other hand, autotrophic bacteria make use of hydrogen sulfide, ferrous iron, and hydrogen as the energy source [17, 18]. Evasion of poisoning effect of some organic carbon, low biomass build-up and less sludge production are some of the advantages of autotrophic over heterotrophic denitrification. Such advantages will result in reduction of reactor clogging and easier post-treatment [3, 17]. Bioelectrochemical systems which have been recently developed are considered as a clean technology for water and wastewater treatment [19]. A bioelectrochemical system, when used for nitrate reduction from water and wastewater, is known as a bioelectrochemical denitrification process [20]. In bioelectrochemical denitrification, autotrophic denitrifying microorganisms make use of hydrogen generated at the cathode by the

electrolysis of water as an electron donor to reduce nitrate into nitrogen gas. The reaction mechanisms of nitrate reduction by hydrogen gas in bioelectrochemistry systems are:

Anode reaction:

$$5H_2O \rightarrow 2.5O_2\ (g) + 10\ H^+ + 10e^- \quad (1)$$

Cathode reaction:

$$10H_2O + 10e^- \rightarrow 5H_2\ (g) + 10\ OH^- \quad (2)$$

Sequential reactions of nitrate reduction to nitrogen gas:

$$NO_3^- + H_2\ (g) \rightarrow NO_2^- + H_2O \quad (3)$$

$$NO_2^- + 0.5H_2 + H^+ \rightarrow NO(g) + H_2O \quad (4)$$

$$2NO\ (g) + H_2 \rightarrow N_2O\ (g) + H_2O \quad (5)$$

$$N_2O\ (g) + H_2 \rightarrow N_2\ (g) + H_2O \quad (6)$$

Net reaction of nitrate reduction on cathode

$$2NO_3^- + 6H_2O + 10e^- \rightarrow N2\ (g) + 12OH^- \quad (7)$$

Overall reaction of bioelectrochemical denitrification

$$2NO_3^- + H_2O \rightarrow N_2\ (g) + 2OH^- \quad (8)$$

Bioelectrochemical reduction of nitrate has been reported in the previous studies. Clauwaert *et al*.[21] was applied a divided bio-electrochemical cell for reduction of nitrate from synthetic wastewater. Nitrate removal efficiency was achieved to 74% at current density of 24.3 mA/cm^2 and pH level of 7.2. Tiehm *et al.* (2009) studied the influence of electrical current on viability of vinyl chloride (VC) degrading microorganisms and reported that electrical current (0.04 to 14mAcm^{-2}) has had inhibiting effects on microorganisms after 4 h exposure [11]. To the best of our knowledge and based on the literature, there is no previous report on the viability of denitrifying bacteria in a bioelectrochemical process. Hence, the objective of present study was to investigate viability of the autotrophic bacteria in the denitrification process. To evaluate the process, the viability of autotrophic denitrifying bacteria was studied as the function of different operational parameters, including current density, retention time and mixing.

MATERIALS AND METHODS

Isolation of autotrophic denitrifying bacteria

The autotrophic denitrifying bacteria were isolated from activated sludge derivative of a municipal wastewater treatment plant (Tehran, Iran). The sludge was added to a 1 liter flask containing culture media, sodium nitrate as nitrogen source and phosphate buffer. The culture media was prepared by dissolving 0.3 g L^{-1} KH_2PO_4, 1.0 g L^{-1} $K_2HPO_4.12H_2O$, 0.5 g L^{-1} NaCl, 2.0 g L^{-1} $NaHCO_3$, 0.1 g L^{-1} $MgSO_4.7H_2O$, 0.01 g L^{-1} $CaCl_2$ [22]. Nitrogen gas was sparged in the flask for generating the anoxic condition. Anoxic reactor operated over 3 month at room temperature (25 °C) in batch mode. The bacterial activity, anoxic condition and pH were adjusted during the reactor operation [23]. Bacterial inoculates (consortium of autotrophic denitrifying bacteria) were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard.

Bioelecterochmical reactor construction and operation

Bench-scale bioelectrochemical denitrification with separated chambers reactor were applied for nitrate reduction from synthetic wastewater. Fig. 1 shows a schematic flow diagram of the experiments.

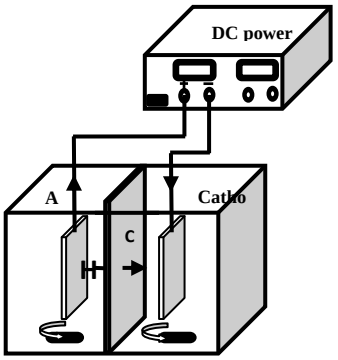

Fig. 1: Schematic diagram of the bioelecterochmical reactor

The plexiglass reactor consists of two chambers separated by a cation exchange membrane (50 cm^2, nafion 117, USA). The reactor consisted of an anode and a cathode chamber with the working volume of 2000 ml. The membrane pretreatment was carried out sequentially by boiling in deionized water, H_2O_2 (30 % V/V), 0.5 M H_2SO_4, and then deionized water; each step was conducted for 1 h. To prevent membrane swelling when placed in the Bioelectorhemical system, membranes were stored in deionized water prior to being used [24]. Carbon felt (Alfa Aesar Co., 3.18mm thick, 99.0%, USA) and stainless steel with effective area of 40 cm^2 were used as cathode and anode electrodes, respectively. Prior to using the carbon felt in the reactor, it was immerged into 0.1 M HCl for 24 h and then washed for several times by deionized water. The electrical energy was provided with a DC power supply (Atten, APS3005S-3D, China). The mixing was carried out using a magnetic stirring bar (ATL-4200, Anytech Co., Korea). Both anode and cathode chambers of BER were fed by the same culture media in anoxic condition. Cathode chamber was fed by 200 mg/l NO_3^- and isolated autotrophic denitrifies bacteria. Current densities ranging from 2 to32 mA/cm^2 were applied by DC power supply. The carbon dioxide (CO_2) was sparged to the cathode chamber to keep the pH between 7.0 and 8.0. The electrodes were connected to the DC power supply, and BER operate over 24 h. Nitrate concentration and colony count were determined at the end of each run.

Analysis

The nitrate concentration was determined according to standard methods for the examination of water and wastewater [25]. Before each spectrophotometric determination of nitrate, the samples were centrifuged for 15min at 4000 rpm to remove the insoluble particles (cells, other media components) from the supernatant. 1ml HCl 1N was then added to 1 ml of supernatant and the mixture was diluted to a constant volume of 50 ml. The light absorbance of samples was read against redistilled water at 220nm on a UV–vis-spectrophotometer (UV Ray, china). In order to avoid the interference from the organic matter, the absorbance of samples was also measured at 275 nm. Viability of cultivable denitrifying bacteria was monitored following the plate count procedure in the isolation media. The pH and ORP values were determined by a portable instrument (Oaklon, Malaysia).

RESULTS AND DISCUSSION

Various investigations have been done to reduce nitrate by bioelectrochemical processes [7, 20, 24].The important operating factors that have influences on viability of cultivable bacteria in a bioelectrochemical nitrate reduction process are electrical current, running time and mixing [3].

Effect of Current density

In bio-electrochemical denitrification systems electrical current plays a critical role as it enhances nitrate reduction rate in hydrogenotrophic denitrifying bacteria. The effect of different current densities on nitrate reduction using BER is shown in Fig.2.

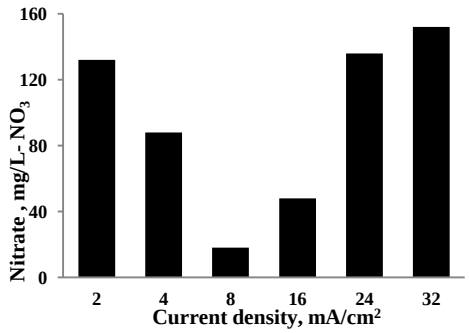

Fig. 2: Effect of current density on bioelectrochemical nitrate reduction (NO_3^-=200 mg L^{-1} NO_3^-, Time=8h)

As shown in this figure, by increasing the current density from 2 to 8 mA/cm^2, nitrate concentration was decreased from 200 to 18 mg/l - NO_3^- after 8h running time. The optimum current density for maximum nitrate reduction in BER was 8 mA/cm^2. Hydrogen production at the cathode is influenced by current density [4, 11]. Hydrogen gas, as an electron donor, is ideal for hydrogenotrophic nitrate reducing bacteria [11, 26]. Therefore, increasing the current density contributes to higher production of hydrogen gas, which enhances reduction of nitrate at the cathode [27]. Zhan *et al.* (2012) reported that by increasing electrical current from 4.4 to 14 mA, the nitrogen removal efficiency increased from 70.3% to 92.6% in a microbial electrolysis cell [28]. Zhao *et al.* (2007) investigated the autotrophic denitrification in a biofilm electrode reactor and showed that at HRT of 8 h, the nitrate removal efficiency was gradually enhanced when the electrical current increased from 5 to 500 mA [29]. Wan *et al.* (2011) studied nitrate reduction in a batch bioelectrochemical system and reported that the electrical current between 30 and 100 mA did not have any impact on nitrate reduction rate [11]. When the electrical current was higher than 30 mA, cathode chamber was saturated by hydrogen gas, and the hydrogenotrophic denitrification was not influenced by hydrogen gas as an electron donor [11]. Islam and Suidan (1998) showed that at electrical current higher than 60 and 100 mA, hydrogen gas inhibits denitrification rate [30]. Whereas, applying 20 mA current, resulted in nitrate removal efficiency of 90%. It seems that lethal influence of electrical current on denitrifying bacteria is important for decreasing denitrification rate at a higher current density. The viability of denitrifying bacteria in presence of different current density and 200 mg L^{-1} NO_3 were studied for 8 h r time (Fig. 3).

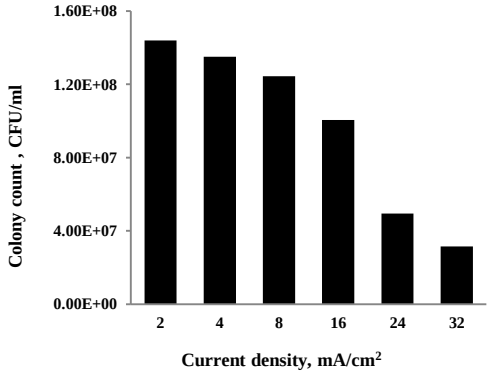

Fig.3: Effect of current density on colony count of denitrifying bacteria in BER(NO_3=200 mg L^{-1} NO_3^-, Time=8h)

In general, it has been observed that bacteria viability decreased by increasing the current density. Low current density (2 and 4 mA/cm^2) has not had a significant effect on viability of denitrifying bacteria. While with the higher current densities (16, 24 and 32 mA/cm^2) the viability of bacteria was strongly decreased. The nitrate reduction rate was decreased by increasing the current density, thus, it can be concluded that the decrease in the removal efficiency of nitrate is associated with reduced denitrifying bacteria viability. Regarding the optimum denitrification,

denitrifying bacteria can grow and reduce the nitrate in a current density of lower than 16 mA/cm^2, with minimum lethal effects. Wei *et al.* (2011) showed that by application of a current density from 3.7 to 24.7 A/m^2, the bacterial viability in wastewater over 4 h was reduced from 97 to 81% respectively [31].

Effect of running time

Fig. 4 shows the effect of running time on nitrate reduction and viability of denitrifying bacteria in BER. By increasing the time, denitrification rate was gradually increased.

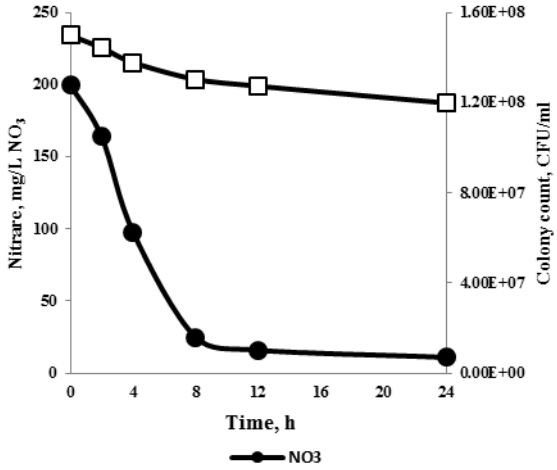

Fig. 4: Influence of running time on nitrate reduction rate and viability of denitrifying bacteria in BER (Initial NO_3^- =200 mg L^{-1} NO_3^-, Initial bacteria= $3\times10^{8,}$ Current density=8 mA/cm^2)

Zhou *et al.* (2007) has reported that in a three dimensional BER, by increasing time from 4 to 8 h, nitrate removal efficiency was boosted from 78 to 99% [29]. Simultaneous effects of current density and running time on nitrate reduction is shown in Fig. 5.

By increasing the current density and time, denitrification rate increased. At the running time of 0-2h and 6-8h nitrate reduction rate was slower than running time of 2-6h. At the beginning of the process, due to high concentration of nitrate, the reduction rate was slow. Also, at the end of the process, denitrification rate was rapid, mainly due to the fact that the accumulation of nitrate reduction by-products (nitrite, ammonia) was reduced. It has been reported that higher current intensity and long running time, excess of hydrogen caused nitrite accumulation in solution that led to inhabitation of the process [29].

Effect of Mixing

The influence of reactor mixing on nitrate reduction rate during 8h running time and application of 8 mA/cm^2 current density was displayed in Fig. 6.

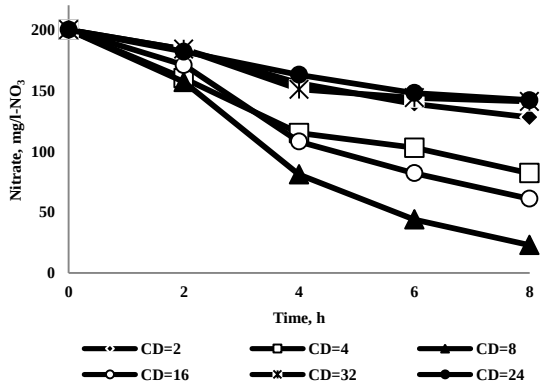

Fig. 5: Simultaneous effects of current density and running time on nitrate reduction

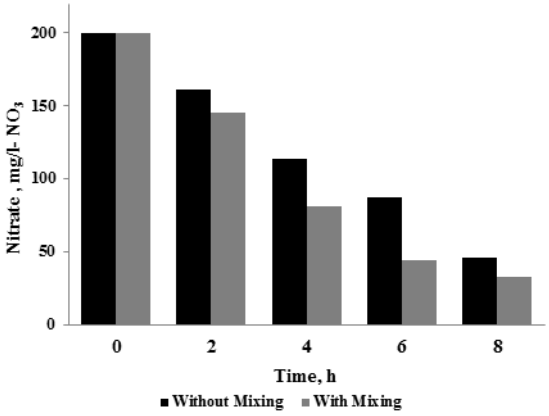

Fig. 6: Effects of reactor mixing on nitrate reduction rate in BER (Initial NO_3^-=200 mg L^{-1} NO_3^-, Current density=8 mA/cm^2)

The denitrification rate was slightly improved when reactor solutions were mixed at100 rpm during the experiments. The viabilities of denitrifying bacteria were increased by mixing the BER content. It appears that, proper mixing of the BER content can reduce the adverse effects of electric current on denitrifying bacteria. Thus, bacteria viability and denitrification rate was increased. In integrated biological and electrotechnology, adequate mixing, could increase the dispersion and diffusion of bacteria, which prevented bacterial localization and inactivation in the reactor [32]. In addition, mixing of the solution made more bacteria reaching to the surface of the cathode which enabled them to make use of hydrogen gas as electron donor and enhanced hydrogenotrophic denitrification efficiency. Also, membrane fouling and current loss between the electrodes were reduced by mixing.

CONCLUSIONS

The viability and nitrate reduction of autotrophic denitrifying bacteria were investigated in bench-scale separated BER chamber. Increasing the current density up to 8 mA/cm^2, because of higher

production of hydrogen gas at the cathode surface, enhanced the reduction rate of nitrate in BER. While at higher current density, denitrification rate due to inhabitation effects of excess of hydrogen gas on the cathode surface was decreased. At longer running time, denitrification rate was enhanced. Mixing of the solution due to prevention of bacterial localization, increasing the contact of hydrogen gas and bacteria, and membrane fouling delay could reduce the adverse effects of electric current on bacteria and enhanced the viability.

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