

Autohydrogenotrophic denitrification by a bioelectrochemical process: A viability study

Mahdi Safari¹, Abbas Rezaee^{*1}, Bitia Ayati², Ahmad Jonidi Jafari³

1) Department of Environmental Health, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

2) Department of Environmental Engineering, Faculty of Civil & Environmental Engineering, Tarbiat Modares University, Tehran, Iran

3) Department of Environmental Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

*Author for Correspondence: Email: rezaee@modares.ac.ir

Received 17 Sep. 2013; Revised: 1 Nov. 2013, Accepted: 13 Dec. 2013

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/cm², nitrate reduction rate was increased. At higher current density (24 - 32 mA/cm²), denitrification rate due to excess of hydrogen gas on cathode surface and accumulation of nitrite, was decreased. Low current density (<16 mA/cm²) 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 NO₃/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

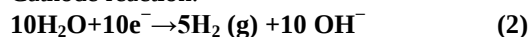
weaknesses such as high consumption of electrical energy. Therefore, integration of electrochemical and biological processes as bioelectrochemical systems has been recommended to overcome the potential problems [15, 16]. Denitrifying bacteria are classified into two main groups of heterotrophic and autotrophic. 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:

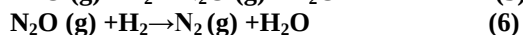
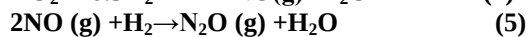
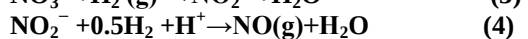
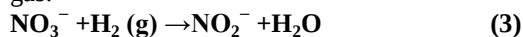
Anode reaction:



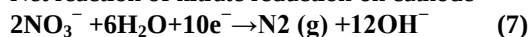
Cathode reaction:



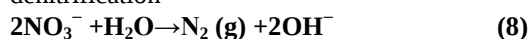
Sequential reactions of nitrate reduction to nitrogen gas:



Net reaction of nitrate reduction on cathode



Overall reaction of bioelectrochemical denitrification



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/cm² 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 14 mA/cm²) 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 KH₂PO₄, 1.0 g L⁻¹ K₂HPO₄·12H₂O, 0.5 g L⁻¹ NaCl, 2.0 g L⁻¹ NaHCO₃, 0.1 g L⁻¹ MgSO₄·7H₂O, 0.01 g L⁻¹ CaCl₂ [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.

Bioelectrochemical 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.

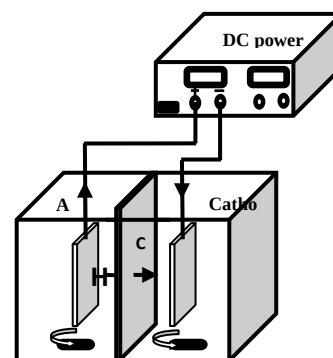


Fig. 1: Schematic diagram of the bioelectrochemical reactor

The plexiglass reactor consists of two chambers separated by a cation exchange membrane (50 cm², 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, H₂O₂ (30 % V/V), 0.5 M H₂SO₄, and then deionized water; each step was conducted for 1 h. To prevent membrane swelling when placed in the Bioelectrochemical 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 cm² were used as cathode and anode electrodes, respectively. Prior to using the carbon felt in the reactor, it was immersed 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 NO₃⁻ and isolated autotrophic denitrifies bacteria. Current densities ranging from 2 to 32 mA/cm² were applied by DC power supply. The carbon dioxide (CO₂) 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.

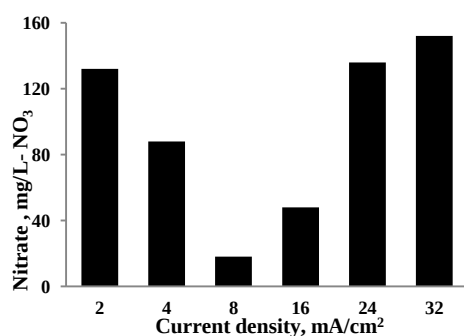


Fig. 2: Effect of current density on bioelectrochemical nitrate reduction ($\text{NO}_3^- = 200 \text{ mg L}^{-1} \text{ NO}_3^-$, Time=8h)

As shown in this figure, by increasing the current density from 2 to 8 mA/cm², nitrate concentration was decreased from 200 to 18 mg/l - NO₃⁻ after 8h running time. The optimum current density for maximum nitrate reduction in BER was 8 mA/cm². 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 NO₃⁻ were studied for 8 h r time (Fig. 3).

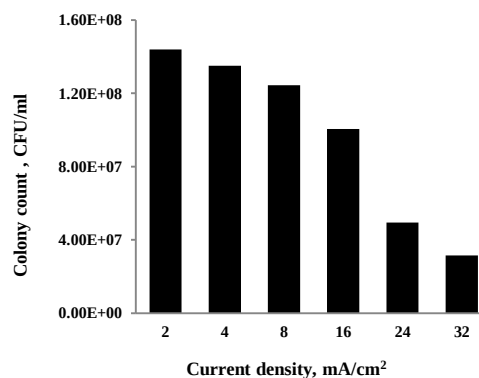


Fig.3: Effect of current density on colony count of denitrifying bacteria in BER($\text{NO}_3^- = 200 \text{ mg L}^{-1} \text{ NO}_3^-$, Time=8h)

In general, it has been observed that bacteria viability decreased by increasing the current density. Low current density (2 and 4 mA/cm²) has not had a significant effect on viability of denitrifying bacteria. While with the higher current densities (16, 24 and 32 mA/cm²) 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/cm², with minimum lethal effects. Wei *et al.* (2011) showed that by application of a current density from 3.7 to 24.7 A/m², 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.

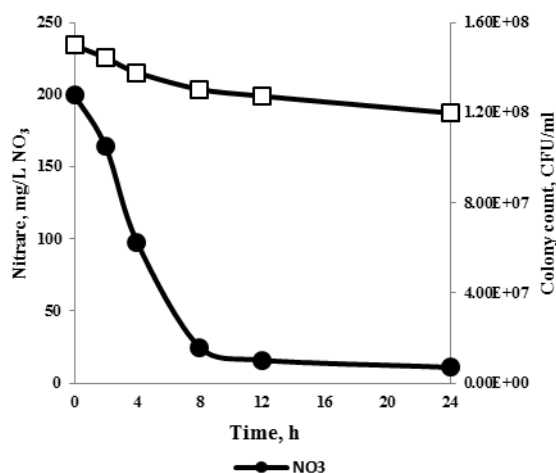


Fig. 4: Influence of running time on nitrate reduction rate and viability of denitrifying bacteria in BER (Initial NO₃⁻=200 mg L⁻¹ NO₃⁻, Initial bacteria= 3×10⁸, Current density=8 mA/cm²)

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/cm² current density was displayed in Fig. 6.

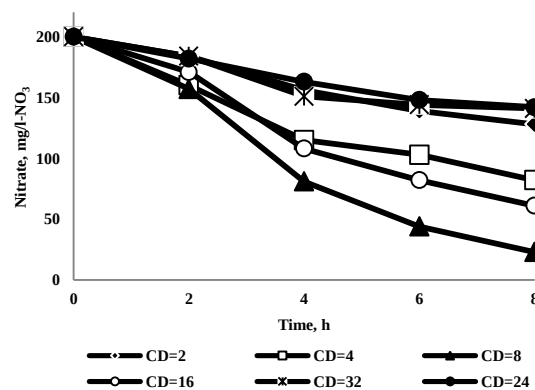


Fig. 5: Simultaneous effects of current density and running time on nitrate reduction

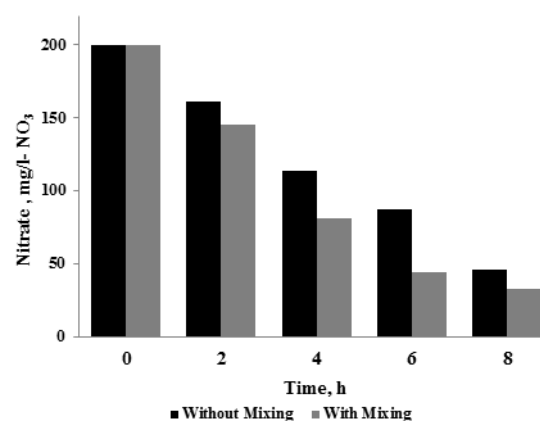


Fig. 6: Effects of reactor mixing on nitrate reduction rate in BER (Initial NO₃⁻=200 mg L⁻¹ NO₃⁻, Current density=8 mA/cm²)

The denitrification rate was slightly improved when reactor solutions were mixed at 100 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/cm², 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.

ACKNOWLEDGMENTS

The authors are grateful for financial support provided by Tarbiat Modares University.

REFERENCES

- [1] Choi JH, Shin WS, Choi SJ, Kim YH. Reductive denitrification using zero-valent iron and bimetallic iron, *Environ Technol.* 2009;30(9): 939-46
- [2] Mook WT, Arouaa MK, Chakrabartia MH, Lowc CTJ, Aravindd PV, Brandon NP. The application of nano-crystalline PbO_2 as an anode for the simultaneous bio-electrochemical denitrification and organic matter removal in an up-flow undivided reactor, *Electrochim. Acta.* 2013;94: 327-35
- [3] Ghafari Sh, Hasan M, Aroua MK. Bio-electrochemical removal of nitrate from water and wastewater-A review. *Bioresour. Technol.* 2008;99(10): 3965-74
- [4] Mook WT, Chakrabarti MH, Aroua M K, Khan GMA, Ali BS, Islam MS, Abu Hassan M A. Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. *Desalination*, 2012; 285: 1-13
- [5] Song H, Yao Z, Wang M, Wang J, Zhu Z, Li A. Effect of dissolved organic matter on nitrate-nitrogen removal by anion exchange resin and kinetics studies. *J. Environ. Sci.* 2013; 25(1): 105-13
- [6] Richards L A, Vuachere M, Schafer AI. Impact of pH on the removal of fluoride, nitrate and boron by nanofiltration/reverse osmosis. *Desalination*, 2010; 261(3): 331-37
- [7] Isoda N, Yokoyama H, Nojiri M, Suzuki S, Yamaguchi K. Electro reduction of nitrite to nitrogen oxide by a copper-containing nitrite reductase model complex incorporated into collagen film, *Bioelectrochem.* 2010; 77(2): 82-88
- [8] Rezaee A, Godini H, Jorfi S. Nitrate removal from aqueous solution using $MgCl_2$ impregnated activated carbon, *Environ. Eng. Manag. J.* 2010; 9(3): 449-52
- [9] Midaoui EI, Elhannouni AF, Taby M, Chay L, Menkouchi MA, Echihabi L, Hafsi M. Optimization of nitrate removal operation from ground water by electrodialysis. *Seper. Purif. Technol.* 2002; 29(3): 235- 41
- [10] Bernat K, Wojnowska-Baryła I. Carbon source in aerobic denitrification, *Biochem. Eng. J.* 2007; 36(2): 116-22
- [11] Wan D, Liu H, Qu J , Lei P. Bio-electrochemical denitrification by a novel proton-exchange membrane electrodialysis system—a batch mode study, *J. Chem. Technol. Biotechnol.* 2011; 85(11): 1540-46
- [12] Li M, Feng C, Zhang Z, Shen Z, Sugiura N. Electrochemical reduction of nitrate using various anodes and a Cu/Zn cathode. *Electrochem. Commun.* 2009; 11(10): 853-56
- [13] Zhang Y, Angelidaki I. Bioelectrode-based approach for enhancing nitrate and nitrite removal and electricity generation from eutrophic lakes. *Water Res.* 2012; 46(19): 6445-53
- [14] Mattarozzi L, Cattarin S, Comisso N, Guerriero P, Musiani M, Vazquez-Gomez L, Verlato E. Electrochemical reduction of nitrate and nitrite in alkaline media at CuNi alloy electrodes. *Electrochim. Acta*, 2013;89: 488-96
- [15] Morris JM, Fallgren PH, Jin S. Enhanced denitrification through microbial and steel fuel-cell generated electron transport. *Chem. Eng. J.* 2009; 153(1-3): 37-42
- [16] Mousavi S, Ibrahim S, Aroua MK, Ghafari S. Development of nitrate elimination by autohydrogenotrophic bacteria in bio-electrochemical reactors – A review. *Biochem Eng. J.* 2012; 67(15): 251-64
- [17] Rijn JV, Tal Y, Schreier HJ. Denitrification in recirculating systems: theory and applications. *Aquacult. Eng.* 2006; 34(3): 364-76
- [18] Wan D, Liu H, Qu J, Lei P, Xiao S, Hou Y. Using the combined bioelectrochemical and sulfur autotrophic denitrification system for groundwater denitrification. *Bioresour. Technol.* 2009; 100(1): 142-48
- [19] Behera M, Jana PS, More TT, Ghangreka MM. Rice mill wastewater treatment in microbial fuel cells fabricated using proton exchange membrane and earthen pot at different pH, *Bioelectrochem.* 2010; 79(2): 228-33
- [20] Cho JS, Park JY, Yoo Y. Novel three-dimensional bioelectrode for mediatorless bioelectrochemical denitrification. *J. Biotechnol. Let.* 2008; 30(9): 1917-20
- [21] Clauwaert, P, Desloover J, Shea C, Nerenberg R, Boon N, Verstraete W, Enhanced nitrogen removal in bio-electrochemical systems by pH control. *Biotechnology Letters*, 2009; 31(10): 1537-43
- [22] Zhan G, Zhang L, Li D, Su W, Tao Y, Qian J. Autotrophic nitrogen removal from ammonium at low applied voltage in a single-compartment microbial electrolysis cell. *Bioresour. Technol.* 2012; 116: 271-77

- [23] Zhao Y, Zhang B, Feng C, Huang F, Zhang P, Zhang Z, Yang Y, Sugiura N. Behavior of autotrophic denitrification and heterotrophic denitrification in an intensified biofilm-electrode reactor for nitrate-contaminated drinking water treatment. *Bioresour. Technol.* 2012; 107: 159-65
- [24] Kondaveeti S, Min B. Nitrate reduction with biotic and abiotic cathodes at various cell voltages in bioelectrochemical denitrification system. *Bioprocess Biosyst. Eng.* 2013; 36(2): 231-38
- [25] APHA, AWW, WPCF, Standard Methods for the Examination of Water and Wastewater, 21th ed. American Public Health Association, Washington, DC, USA; 2005
- [26] Grommen R, Verhaege M, Verstraete M. Removal of nitrate in aquaria by means of electrochemically generated hydrogen gas as electron donor for biological denitrification. *Aquaculture Eng.* 2006; 34(1): 33-39
- [27] Wang H, Qu J. Combined bioelectrochemical and sulfur autotrophic denitrification for drinking water treatment. *Water Res.* 2003; 37(15): 3767-75
- [28] Zhan G, Zhang L, Li D, Su W, Tao Y, Qian J. Autotrophic nitrogen removal from ammonium at low applied voltage in a single-compartment microbial electrolysis cell. *Bioresour. Technol.* 2012; 116: 271-77
- [29] Zhou M, Fu W, Gu H, Lei L. Nitrate removal from groundwater by a novel three-dimensional electrode biofilm reactor. *Electrochimi. Acta*, 2007; 52(19): 6052-59
- [30] Islam S, Suidan MT. Electrolytic denitrification: Long term performance and effect of current intensity. *Water Res.* 1998; 32(2): 528-36
- [31] Wei V, Elektorowicz M, Oleszkiewicz JA. Influence of electric current on bacterial viability in wastewater treatment. *Water Res.* 2011; 45(16): 5058-62
- [32] Tiehm A, Lohner ST, Augenstein T. Effects of direct electric current and electrode reactions on vinyl chloride degrading microorganisms. *Electrochimi. Acta*, 2009; 54(12): 3453-59