مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۰۹۱۹۷۵۵۶۴۲۴ - (۰۲۱) ۸۸۶۷۱۶۷۶
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

C/N ratio and carbon source effect on water denitrification by pure culture of Hyphomicrobium denitrifican

Khakbaz Varkani, Maryam, Nosrati, Mohsen, Shojaossadati, Seyed Abbas

Biotechnology Group, Chemical Engineering Department, Tarbiat Modares University, P.O. Box 14115-143, Tehran, Iran

Abstract

Pure culture of *Hyphomicrobium denitrifican* DSM 1869 was investigated for biological denitrification. The bacterium was cultured in an optimized mineral salt medium with acetic acid and methanol as carbon sources in 100-milliliter flasks. The initial concentrations of nitrate were 100, 250 and 400 mg NO_3-N/l and carbon to nitrogen ratios were 1.2, 2.6 and 4.0. The results show that increasing carbon to nitrogen ratio from 1.2 to 2.6, consequences in higher nitrate conversions. The maximum specific denitrification rate (2.53 mg/l.h) was obtained for $(NO_3\text{-}N)_0$ concentration of 400 mg/l, carbon to nitrogen ratio of 2.6 and acetic acid as the sole carbon source. However, increasing carbon to nitrogen ratio from 2.6 to 4.0 resulted in the decrease of specific denitrification rate. Although removal of NO_3-N was seen by using methanol as the sole carbon source, it was not very suitable for *Hyphomicrobium denitrifican* to decrease high NO_3-N concentrations, and acetic acid was a better alternative. The results showed that *Hyphomicrobium denitrifican* DSM 1869 is a good alternative for activated sludge, which is widely used in water denitrification processes. Nitrite accumulation was seen in experiments with low carbon to nitrogen ratios and the ones that methanol was used as the sole carbon source.

Keywords*:* Heterotrophic biodenitrification, pure culture, carbon source, C/N ratio, groundwater

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۰۹۱۹۷۵۵۶۴۲۴ - (۰۲۱) ۸۸۶۷۱۶۷۶
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

1. Introduction

In many countries, groundwater is used as a drinking water supply, and high nitrate concentrations present a potential risk to public health, particular to infants [1, 2]. Major sources of nitrate in groundwater supplies are wastewater, fertilizers, and livestock farming [3-7]. According to U.S. Environmental Protection Agency and European Community the permissible concentration of nitrates in drinking water is 44.3 and 50 mg NO_3^-/lit, respectively; although the recommended levels of nitrate is 25 mg NO_3^-/lit according to European Community [8-10]. Because ingestion of high levels of nitrate may cause negative effects on human health, efficient and economic removal processes are needed [10, 11]. Several methods with different performance and cost levels are available in the drinking water denitrification. Ion exchange, reverse osmosis and biological denitrification are commonly used methods [12-14]. By biological denitrification, nitrate in the water is converted into gaseous nitrogen through a number of steps. Known as nitrate respiration, the reaction sequence of this process is shown in equation 1 [9, 15, 16].

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (1)$$

Biological denitrification is an alternative for nitrate removal due to lower operating and capital cost when compared to physical-chemical processes. Denitrifiers belong to a biochemically and taxonomically diverse group of facultative anaerobes and occur commonly in nature soil and water. Approximately 60 types of bacteria, mainly heterotrophs, are capable of using nitrogen oxides (nitrate and nitrite) as electron acceptors and produce mainly N_2 as reduction product [17-19]. Biological denitrification of drinking water with heterotrophic microorganisms has been widely applied because of its high efficiency and low cost. However, residual carbon sources from such processes may cause problems in drinking water treatment [20-22]. Being more toxic than nitrate, nitrite can further react with secondary amines in acidic conditions to form carcinogenic nitrosamines. Many studies have shown that a direct relationship exists between nitrosamine and human cancers. It has been reported that the reduction of nitrate to nitrite was greatly influenced by nitrate-reducing bacteria such as *Micrococcus spp.*, *Vibrio spp.*, *Staphylococcus carnosus* and *Escherichia coli*. This was due to the production of nitrate reductase during the growth of these microorganisms [9, 21-24]. In this study, it is aimed to investigate effects of different parameters on the biodenitrification using *Hyphomicrobium denitrifican* DSM 1869. These parameters are nitrate concentration, carbon source including methanol and acetic acid and C/N ratio. Nitrate and nitrite concentration, pH and COD were investigated during the experiments.

2. Material and Methods

2.1. Growth medium and microorganism

Mineral salt medium (MSM) used for enrichment of *Hyphomicrobium denitrifican* contained methanol 0.6% (v/v), NH_4SO_4 1750 mg/lit, $MgSO_4.7H_2O$ 100 mg/lit, $FeSO_4.7H_2O$ 20 mg/lit, $Na_2HPO_4.H_2O$ 6140 mg/lit, KH_2PO_4 680 mg/lit, $ZnSO_4.7H_2O$ 1.5 mg/lit, $CaCl_2.2H_2O$ 20 mg/lit, $CoCl_2.6\ H_2O$ 0.6 mg/lit, $CuSO_4.5H_2O$ 0.04 mg/lit, $MnSO_4.5H_2O$ 5 mg/lit, $Na_2MoO_4.2H_2O$ 0.04

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ – ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۰۹۱۹۷۵۵۶۴۲۴ – (۰۲۱) ۸۸۶۷۱۶۷۶
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

mg/lit and H_3BO_3 0.2 mg/lit. The pH was 7.0 and all the chemicals used for denitrification experiments were of analytical grade.

Hyphomicrobium denitrifican DSM 1869 was used for biodenitrification tests. After subculture at least three times in the MSM mentioned above at 30 ºC and 150 rpm in shaker incubator, the culture was made on plates containing MSM and agar, and maintained at 4ºC in refrigerator.

2.2. Artificial raw water and carbon source

The synthetic contaminated water was prepared from distilled water using potassium nitrate (KNO_3) as the contaminant. Nitrate concentrations were 100, 250 and 400 mg/lit. Methanol and acetic acid were used as carbon source and their concentrations were adjusted to give C/N weight ratios of 1.2, 2.6 and 4 by fixing the amount of KNO_3 (sole nitrogen source) and carbon source according to Table 1.

Table 1. Operational conditions in flasks

	Nitrate Conc. (mg/lit)	C/N Ratio	Carbon Source
Run 1	100	1.2	acetic acid
Run 2	250	1.2	acetic acid
Run 3	400	1.2	acetic acid
Run 4	100	2.6	acetic acid
Run 5	250	2.6	acetic acid
Run 6	400	2.6	acetic acid
Run 7	100	4	acetic acid
Run 8	250	4	acetic acid
Run 9	400	4	acetic acid
Run 10	100	1.2	methanol
Run 11	250	1.2	methanol
Run 12	400	1.2	methanol
Run 13	100	2.6	methanol
Run 14	250	2.6	methanol
Run 15	400	2.6	methanol
Run 16	100	4	methanol
Run 17	250	4	methanol
Run 18	400	4	methanol

2.3. Experimental set-up

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ – ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۸۸۶۷۱۶۷۶ (۰۲۱) – ۰۹۱۹۷۵۵۶۴۲۴
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

The experimental system used in the denitrification tests was 100 milliliter flasks. The anoxic condition was maintained by passing nitrogen gas (N_2) through the flasks. Mixing in the flasks was obtained by a magnetic mixer, which operated with a fixed speed of 100 rpm. Flasks were sealed with rubber plugs to maintain anoxic condition, and N_2 gas generated in reactions was discharged through exhaust pipes installed in rubber plugs.

2.4. Analytical methods

Samples were collected at fixed time intervals from the flasks and were centrifuged at 8000 rpm for 15 minutes. NO_3-N and NO_2-N concentrations were analyzed by ultraviolet spectrophotometric methods using a spectrophotometer of type CARY 100 Conc at 220 and 543 nm wavelengths, respectively. Ultraviolet spectrophotometric screening method and colorimetric method suggested in the Standard Methods for the Examination of Water and Wastewater were followed in the analysis of NO_3-N and NO_2-N, respectively [25]. Chemical oxygen demand (COD) was measured according to the closed reflux, colorimetric method that was suggested in the Standard Methods for the Examination of Water and Wastewater. 2.5 milliliter of the sample was treated with 1.5 milliliter digestion solution (which was a mixture of 10.216 g/L potassium dichromate, 167 milliliter concentrated sulphuric acid and 33.3 g/L mercuric sulphate) and 3.5 milliliter concentrated sulphuric acid, and digested at 150 °C for two hours using a thermo reactor (Hach model: DRB200). After cooling, the absorbance of the samples was read at 600 nm using a spectrophotometer (CARY 100 Conc). The concentration was determined with the aid of a calibration curve. Samples were centrifuged and filtered before analysis [25]. All chemicals used were of analytical grade and obtained from major retailers.

3. Results and Discussions

3.1. Influence of C/N ratio on biodenitrification

The C/N ratio is a key factor influencing the efficiency of denitrification [18]. Denitrification tests were carried out in order to determine the optimum C/N (w/w) ratio for different initial NO_3-N and carbon source concentrations. Biodenitrification relies on heterotrophic bacteria that require an organic carbon source. Acetic acid and methanol were used as sole carbon source and the C/N ratios used were 1.2, 2.6 and 4. Variations of the specific denitrification rate (SDR) with C/N ratios are shown in Figure 1.

$$SDR = \frac{(NO_3 - N)_0 - (NO_3 - N)_T}{T} \qquad (2)$$

where SDR is specific denitrification rate (mg/lit.hr), $(NO_3\text{-}N)_0$ is the initial NO_3-N concentration (mg/lit) and $(NO_3\text{-}N)_T$ is concentration of NO_3-N (mg/lit) after T hours. As shown in Figure 1, the denitrification efficiency depended on both the C/N ratio and carbon source. A varying degree of nitrate reduction from 10.5% to 78.8% was found. When using acetic acid as a carbon source, the maximum specific denitrification rate is 2.53 mg/lit.hr for $(NO_3\text{-}N)_0$=400 mg/lit

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ – ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۸۸۶۷۱۶۷۶ (۰۲۱) – ۰۹۱۹۷۵۵۶۴۲۴
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

and C/N ratio=2.6. It is seen from Figure 1 that by increasing the C/N ratio from 1.2 to 2.6, the specific denitrification rate increases for different $(NO_3\text{-}N)_0$ concentrations. However, by increasing the C/N ratio from 2.6 to 4.0, sometimes there is a drop in removal efficiency. It demonstrates that influent NO_3-N concentration is not the only cause of performance decrease because in the same operating conditions, the specific denitrification rate increases by increasing the C/N ratio. The drop also demonstrated the reduction of organic carbon available to the heterotrophic denitrifying bacteria. The results show that when acetic acid was present in excess, higher nitrate conversions could be achieved.

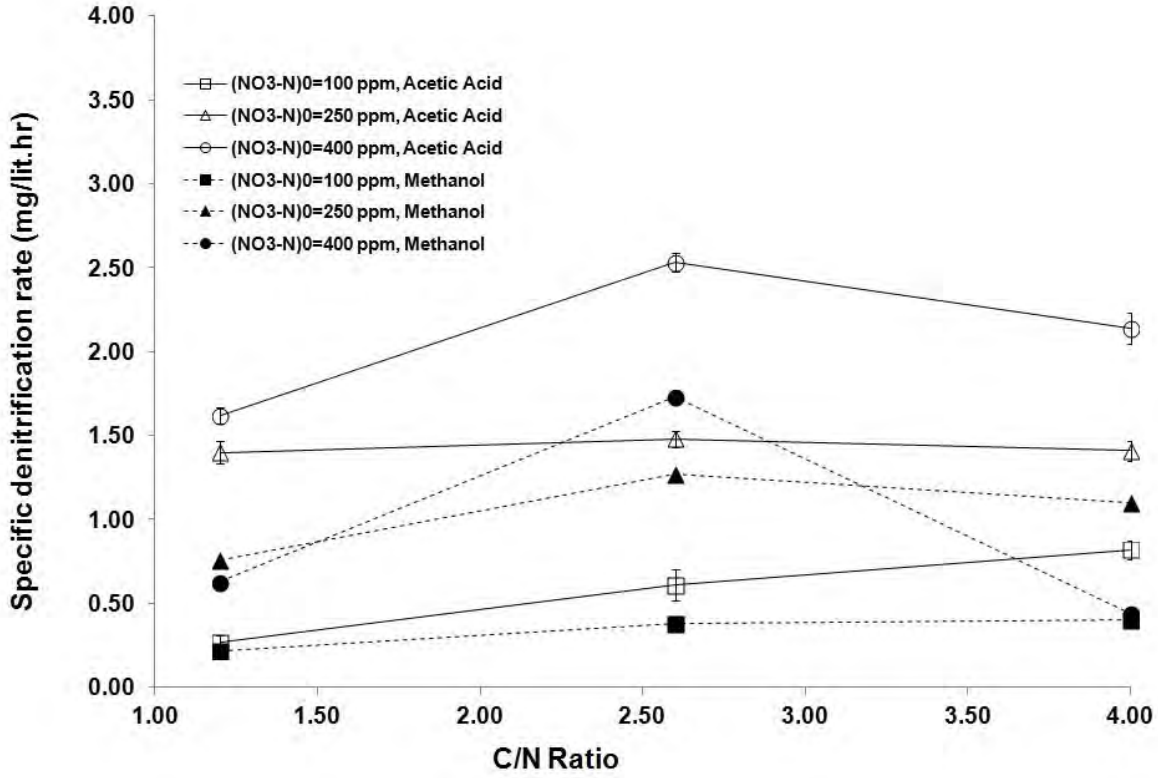

Figure 1. Variation of specific denitrification rate with C/N ratio

It is seen from Figure 2 that nitrate removal is done simultaneously by COD decrease and nitrate has not been trapped in the cells [26]. The highest COD reduction (63.4%) appeared in C/N ratio of 1.2, NO_3-N concentration 250 mg/l and acetic acid as carbon source. C/N ratio is very critical because if there is lack of carbon source, the bacterial activity decreases and if carbon source is in excess, a pollutant will be added to the denitrified water.

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۸۸۶۷۱۶۷۶ (۰۲۱) - ۰۹۱۹۷۵۵۶۴۲۴
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

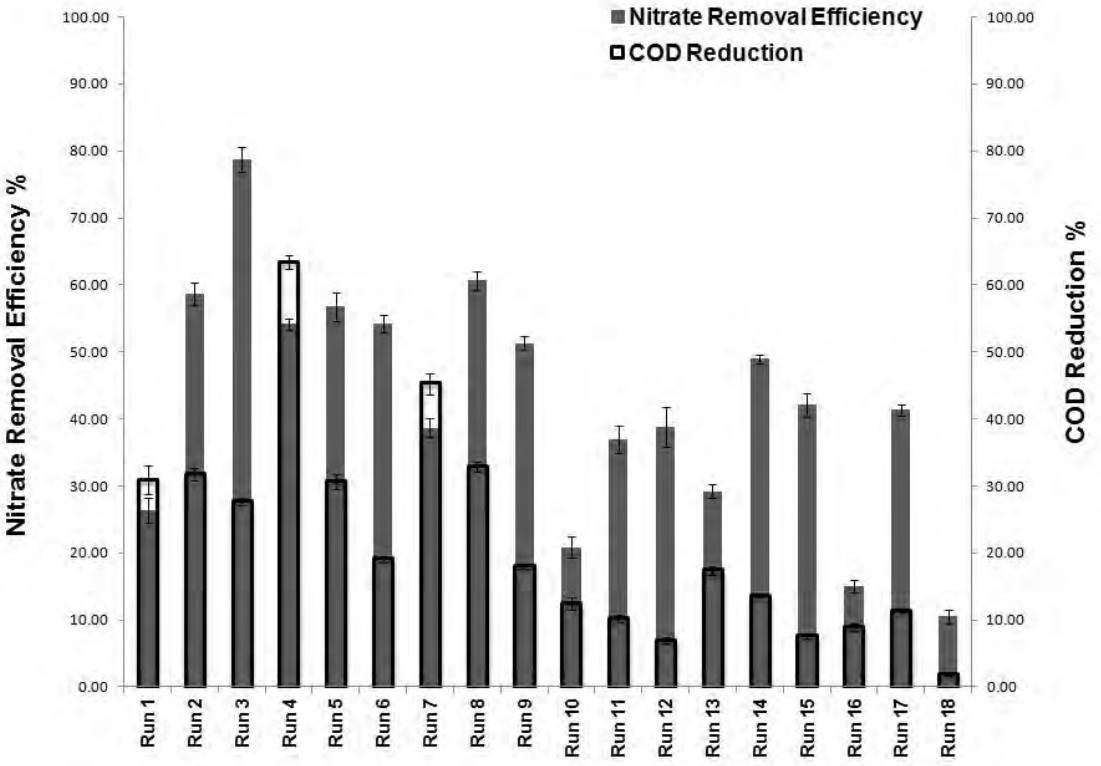

Figure 2. Comparing nitrate removal efficiency and COD reduction

3.2. Effect of NO_3-N concentration on nitrite accumulation

Usually, biological denitrification consists of a sequence of enzymatic reactions leading to the evolution of nitrogen. In this process, microorganisms first reduce nitrates to nitrites and then produce nitric oxide, nitrous oxide, and finally nitrogen gas. The pathway for nitrate reduction is given in equation 1. To reduce the risk to public health and since the nitrite is more toxic than nitrate; the formed nitrite must be minimized. When carbon is insufficient, it will be consumed quickly for the first step and no more carbon will be left for the other steps. Consequently, the limited carbon conditions would result in the increase of NO_2-N concentration in the 4-step process of denitrification [5, 18, 27]. Figure 3 shows the nitrite accumulation at different C/N ratios and carbon sources. The experimental results show that the use of methanol as a carbon source gives much more accumulation of nitrite than the use of acetic acid, except for $(NO_3\text{-}N)_0$ concentration of 250 mg/l and C/N ratio of 2.6. Probably, acetic acid is a much more easily used carbon source for *Hyphomicrobium denitrifican*, in comparison with methanol. With increasing $(NO_3\text{-}N)_0$ concentration, the nitrite accumulation increases for both carbon sources. Showing that for the same amount of C/N ratios, increasing the $(NO_3\text{-}N)_0$ concentration results in the carbon shortage

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۰۹۱۹۷۵۵۶۴۲۴ - (۰۲۱) ۸۸۶۷۱۶۷۶
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

and all steps of denitrification process cannot be completed and finally, there will be a large amount of nitrite accumulated in the solution. However, the nitrite accumulation decreases by increasing the C/N ratio due to the enough available carbon sources for *Hyphomicrobium denitrifican*.

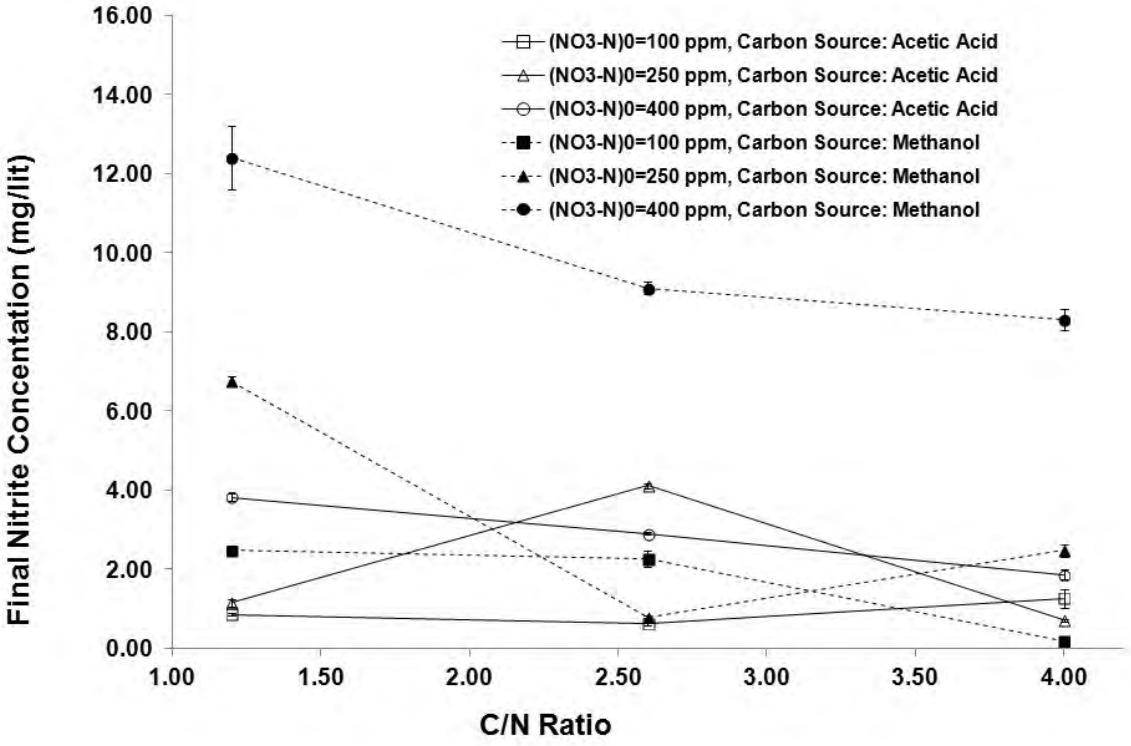

Figure 3. Nitrite accumulation at different C/N ratios and carbon sources

No matter what the pathways are, the amount of NO_2-N formed was much less than NO_3-N reduced at all times (Figure 4), indicating that the most of the influent NO_3-N was biologically transformed into gaseous nitrogen, as expressed by equation 2.

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۸۸۶۷۱۶۷۶ (۰۲۱) - ۰۹۱۹۷۵۵۶۴۲۴
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

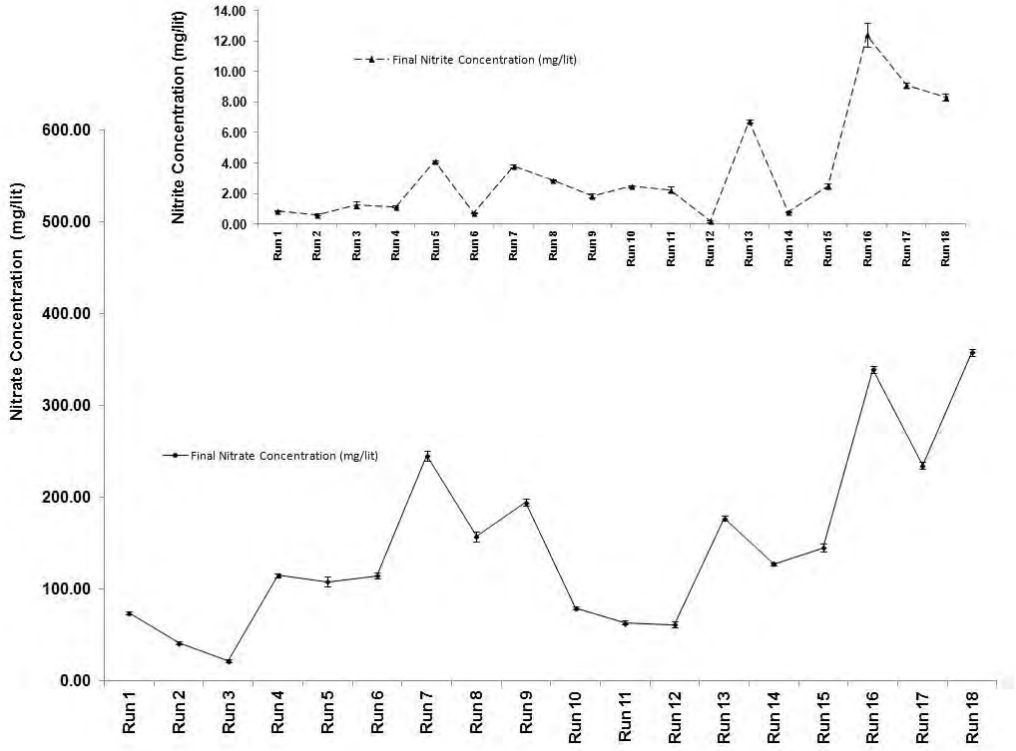

Figure 4. Final nitrate and nitrite concentrations (mg/lit)

3.3. Effect of $(NO_3\text{-}N)_0$ concentration on removal efficiency

In order to optimize the amount of carbon source to be added to achieve efficient NO_3-N removal, the efficiency of the system was studied at varying carbon to nitrogen ratios for each carbon source. As shown in Figure 5, nitrate removal efficiency was markedly affected by C/N ratio. The decrease in nitrate removal efficiency at higher $(NO_3\text{-}N)_0$ concentrations may result from the lack of soluble carbon compared to high NO_3-N loading. In other words, the C/N ratio was too low to supply sufficient carbon source for completing the 4-step process of biological denitrification. Therefore, microorganism metabolism was inhibited and low nitrate removal efficiency was observed at higher nitrate concentrations [15, 24].

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۰۹۱۹۷۵۵۶۴۲۴ - (۰۲۱) ۸۸۶۷۱۶۷۶
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

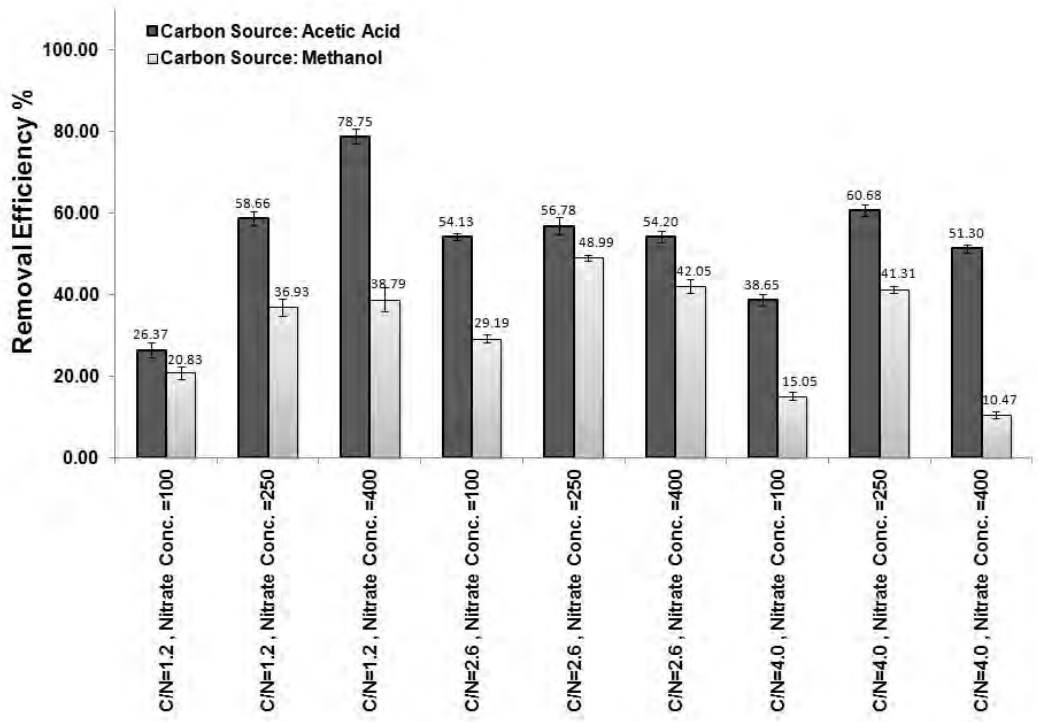

Figure 5. Nitrate removal efficiency for different C/N ratios, $(NO_3\text{-}N)_0$ concentrations and carbon sources

4. Conclusion

The results show that biodenitrification of high nitrate concentrations is possible with pure strain of *Hyphomicrobium denitrifican* DSM 1869 and addition of an external carbon source. *Hyphomicrobium denitrifican* was demonstrated to use nitrate and nitrite as electron acceptors and produce N_2 as reduction product. The present study clearly shows that maximum reduction of nitrate (2.53 mg/lit.hr) was observed at C/N ratio 2.6 and nitrate level of 400 mg/lit. Acetic acid was an effective and safe source of carbon and energy for *Hyphomicrobium denitrifican*, especially for high nitrate concentrations. The results of this research showed that using *Hyphomicrobium denitrifican* is a promising technology that may compete successfully with systems that use activated sludge of wastewater treatment plants, which may have many pathogens. Nitrite production may sometimes be observed, especially when using methanol as carbon source.

References

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۸۸۶۷۱۶۷۶ (۰۲۱) - ۰۹۱۹۷۵۵۶۴۲۴
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

1. Nuhoglu, A., Pekdemir, T., Yildiz, E., Keskinler, B. and Akay, G., *Drinking water denitrification by a membrane bio-reactor.* Water Research, 2002. 36: p. 1155–1166.

2. Ghafari, S., Hasan,M. and Kheireddine Aroua, M., *Bio-electrochemical removal of nitrate from water and wastewater-A review.* Bioresource Technology, 2008. 99: p. 3965–3974.

3. Sperl, G.T.a.H., D. S., *Denitrification with methanol: a selective enrichment for Hyphomicrobium Species.* Journal of Bacteriology, 1971. 108(2): p. 733-736.

4. Murray, R.E.a.K., R., *Production of NO and N_2O in the presence and absence of C_2H_2 by soil slurries and batch cultures of denitrifying bacteria.* Soil Biology & Biochemistry, 2003. 35: p. 1115–1122.

5. Nair, R.R., Dhamole, P. B., Lele, S.S. and Souza, S. F. D, *Biological denitrification of high strength nitrate waste using preadapted denitrifying sludge.* Chemosphere, 2007. 67: p. 1612–1617.

6. Chu, L.a.W., J., *Denitrification performance and biofilmcharacteristics using biodegradable polymers PCL ascarriers and carbon source.* Chemosphere, 2013. 91: p. 1310–1316.

7. Warneke, S., Schipper, L. A., Matiasek, M. G., Scow, K. M., Cameron, S., Bruesewitz, D. A. and McDonald, I. R., *Nitrate removal, communities of denitrifiers and adverse effects in different carbon substrates for use in denitrification beds.* Water Research, 2011. 45: p. 5463-5475.

8. Huang, G., Huang, Y., Hu, H., Liu, F., Zhang, Y. and Deng, R., *Remediation of nitrate–nitrogen contaminated groundwater using a pilot-scale two-layer heterotrophic–autotrophic denitrification permeable reactive barrier with spongy iron/pine bark.* Chemosphere, 2015. 130: p. 8–16.

9. Wasik, E., Bohdziewicz, J. and Błaszczyk, M., *Removal of nitrates from ground water by a hybrid process of biological denitrification and microfiltration membrane.* Process Biochemistry, 2001. 37: p. 57–64.

10. Zhang, Y.a.A., I., *A new method for in situ nitrate removal from groundwater using submerged microbial desalination-denitrification cell (SMDDC).* Water Research, 2013. 47 p. 1827-1836.

11. Shen, Z., Zhou, Y., Liu, J., Xiao, Y., Cao, R. and Wu, F. , *Enhanced removal of nitrate using starch/PCL blends as solid carbon source in a constructed wetland* Bioresource Technology, 2015. 175: p. 239–244.

12. Bautista-Toledo, M.I., Espinosa-Iglesias, D., Carrasco-Marín, F., Perez-Cadenas, A. F. and Maldonado-Hodar, F. J., *Influence of the physicochemical properties of inorganic supports on the activity of immobilized bacteria for water denitrification.* Journal of Environmental Management, 2015. 156: p. 81-88.

13. Cheikh, A., Grib, H. , Drouiche, N., Abdi, N. , Lounici, H. and Mameri, N., *Water denitrification by a hybrid process combining a new bioreactor and conventional electrodialysis.* Chemical Engineering and Processing, 2013. 63: p. 1– 6.

14. Ravnjak, M., Vrtovšek, J. and Pintar, A., *Denitrification of drinking water in a two-stage membrane bioreactor by using immobilized biomass.* Bioresource Technology, 2013. 128: p. 804–808.

15. Ovez, B., Ozgen, S. and Yuksel, M., *Biological denitrification in drinking water using Glycyrrhiza glabra and Arunda donax as the carbon source.* Process Biochemistry, 2006. 41: p. 1539–1544.

مجموعه مقالات ششمین کنفرانس آب، پساب و پسماند
شماره شابک مقالات: ۲ – ۴۷ – ۸۰۴۵ – ۶۰۰ - ۹۷۸
سوم دی ۱۳۹۴، ایران، تهران، مرکز همایش‌های صدا و سیما
۰۹۱۹۷۵۵۶۴۲۴ - (۰۲۱) ۸۸۶۷۱۶۷۶
مجریان: هم اندیشان انرژی کیمیا و
انجمن علمی مهندسی حرارتی و برودتی ایران
www.PASAB.ir

16. Wang, Q., Feng, C. , Zhao, Y. and Hao C., *Denitrification of nitrate-contaminated groundwater with a fiber-based biofilm reactor* Bioresource Technology, 2009. 100 p. 2223–2227.

17. Vasiliadou, I.A., Karanasios, K.A., Pavlou, S. and Vayenas, D.V., *Experimental and modelling study of drinking water hydrogenotrophic denitrification in packed-bed reactors.* Journal of Hazardous Material, 2009. 165: p. 812-824.

18. Yang, X., Sh. Wang and Zhou, L., *Effect of carbon source, C/N ratio, nitrate and dissolved oxygen concentration on nitrite and ammonium production from denitrification process by Pseudomonas stutzeri D6.* Bioresource Technology, 2012. 104: p. 65-72.

19. Zou, G., Papirio, S., van Hullebusch, E.D. and Puhakka, J.A., *Fluidized-bed denitrification of mining water tolerates high nickel concentrations.* Bioresource Technology, 2015. 179: p. 284–290.

20. Moon, H.S., Shin, D. Y., Nam, K. and Kim, J. Y., *A long-term performance test on an autotrophic denitrification column for application as a permeable reactive barrier.* Chemosphere, 2008. 73: p. 723–728.

21. Sahinkaya, E., Yurtsever, A., Aktas, O., Ucar, D. and Wang, Z., *Sulfur-based autotrophic denitrification of drinking water using a membrane bioreactor.* Chemical Engineering Journal, 2015. 68: p. 180–186.

22. Xu, Y., Qiu, T. L., Han, M. L., Li, J. and Wang, X. M., *Heterotrophic Denitrification of Nitrate-Contaminated Water Using Different Solid Carbon Sources.* Procedia Environmental Sciences, 2011. 10: p. 72–77.

23. Sarmiento, C.V., Iglesias, D. E., Toledo, I. B., Merino, M. A. A., Hodar, F. J. M., Marin, F. C. and Cadenas, A. F. P., *Bacteria supported on carbon films for water denitrification.* Chemical Engineering Journal, 2015. 259 p. 424–429.

24. Labelle, M.A., Juteau, P., Jolicoeur, M., Villemur, R., Parent, S. and Comeau, Y., *Seawater denitrification in a closed mesocosm by a submerged moving bed biofilm reactor.* Water Research, 2005. 39: p. 3409–3417.

25. Clesceri, L.S., Greenberg, A. E. and Eaton, A. D., in *Standard Methods for the Examination of Water and Wastewater*. 1998, American Public Health Association, American Water Works Association, Water Environment Federation: Maryland.

26. Buttiglieri, G., Malpe, F., Daverio, E., Melchiori, M., Nieman, H. and Ligthart, J., *Denitrification of drinking water sources by advanced biological treatment using a membrane bioreactor.* Desalination, 2005. 178: p. 211-218.

27. Isaka, K., Kimura, Y., Osaka, T. and Tsuneda, S., *High-rate denitrification using polyethylene glycol gel carriers entrapping heterotrophic denitrifying bacteria.* Water Research, 2012. 46: p. 4941-4948.