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Denitrification of nitrate-contaminated groundwater in an anoxic rotating biological contactor: a case study

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ABSTRACT

In this study, denitrification of Tehran (Iran) oil refinery groundwater in an anoxic rotating biological contactor (ARBC) was investigated. Influence of different parameters such as hydraulic retention time (HRT), Carbon/Nitrogen ratio, and nitrate loading in the influent, on the nitrate, nitrite, ammonium, alkalinity, and chemical oxygen demand concentration of effluent were evaluated. Acetate was used as a carbon source in a heterotrophic process. Nitrate concentration of treated water in all experiments was far below the standard limitation and nitrate removal efficiency reached over 97% in optimum value of Carbon/Nitrogen = 1.25 and HRT = 8 h. Nitrite accumulation wasn't observed up to nitrate loading of 105 mg/L. Based on the results of this experiment, ARBC reactor could be an adequate and convenient method in denitrification of groundwater.

Keywords: Anoxic rotating biological contactor; Denitrification; Carbon/Nitrogen ratio; Hydraulic retention time; Nitrate loading

1. Introduction

In most countries, especially those located in droughty areas, groundwater is widely used in agricultural, industrial, and domestic usages as main water source. However, overuse of chemical fertilizers and pesticides in agriculture as well as domestic, animal, and industrial discharge overshadows the usage of this water sources. Nitrate is one of the most common contaminants that has been increased worldwide over the last decades. Natural fixation of nitrogen gas and soil erosion are the main natural sources of nitrate existence in groundwater, but human agricultural and industrial activities, and direct discharge of wastewater are the actual reason of nitrate contamination in groundwater. Consumption of nitrate-contaminated water poses a serious threat to human health. The best known effect of nitrate is methemoglobinemia that causes blue-baby syndrome in infants. In addition, presence of nitrate in the digestive tract forms N-nitroso compounds, proven as carcinogenic compounds. According to these problems, specific regulations have been established by different organizations. For example, the US Environmental Protection Agency (USEPA) and World Health Organization (WHO) limited nitrate concentration in drinking water to 44.43 mg/L (measured as Nitrate—10 mg/L as Nitrogen) and 50 mg/L (measured as Nitrate), respectively [1,2].

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Ion exchange [3], catalytic denitrification [4,5], adsorption [6], electrodialysis [7], Bioelectrochemical and electrochemical technology [8,9], Nanoalumina and Nanozero-valent iron [10–12], and reverse osmosis [13,14] are conventional methods in nitrate removal from water. But these methods are too expensive to be used in large scale. In addition, in most cases, high level of nitrate-contaminated brine remains that need further treatment or disposal. Biological denitrification is an alternative process that is less costly and instead of accumulating nitrate, converts it to harmless nitrogen gas (Eq. (1)).

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

Biological denitrification includes autotrophic and heterotrophic process under anaerobic conditions. Autotrophic bacteria needs inorganic compound (hydrogen [15] and sulfur [16]) as electron donor in order to reduce nitrate. Moreover, they use inorganic carbon source (carbon dioxide and carbonate) as energy source. Heterotrophic bacteria use nitrate as electron acceptor and organic carbon as electron donor and energy source. In this process, nitrate reduces to nitrogen gas, and carbon dioxide is released. Different carbon sources like methanol, ethanol, acetate and acetic acid, citrate [17], toluene [18], glucose [19,20], and wheat straw [21] have been utilized in water treatment. According to the recent investigation, acetate shows the best performance [20,22,23].

Different biological reactors have been used in water and wastewater denitrification. Aslan and Cakici [24] investigated a heterotrophic process in a slow sand filter and reported that slow sand filter can reduce nitrate under the drinking water limitation in different filtration rate. Shen et al. [20] used a modified anoxic/oxic-membrane bioreactor for the treatment of a high strength nitrate waste. They found that under running condition of Carbon/Nitrogen (C/N) molar ratio = 1.56, using sodium acetate as carbon source, pH = 7.5-8.5 and hydraulic retention time (HRT) = 30 h, nitrate removal was almost complete without nitrite accumulation. Wang et al. [25] developed a fiber-based biofilm reactor in order to effectively treat nitrate polluted groundwater. The experimental results demonstrated that the optimum reaction parameters were pH 7-7.5, C/N=1.25, and HRT = 8 h, under which over 99% of NO₃–N was removed, almost no NO2-N accumulated, and chemical oxygen demand (COD) was nearly zero in treated

water when the concentration of NO₃–N was around 100 mg/L in influent.

Considering the advantages of anoxic rotating biological contactor (ARBC) such as small land area requirement, easy construction, compact design, simplicity of operation, low operating and maintenance costs, short HRT, and high biomass concentration per reactor volume [22], this reactor grows interest in water denitrification. However, there are few studies conducted with this type of reactor. Cortez et al. [22,26] and Teixeira and Oliveira [27] studied the denitrification of wastewater and landfilled leachate in an ARBC reactor. They used poly-methyl methacrylate discs with 3 mm thickness and spacing of 20 mm. Their experiment showed that although completely submerged discs need more time to set up, in terms of denitrification, it's more efficient. At C/N ratio of 1.5, using acetate as carbon source, the nitrate removal efficiency was above 90% and nitrite build-up was at its lowest. Increasing Nitrate load causes sudden decrease in nitrate removal efficiency till biofilm acclimate and removal efficiency recover. They reported that HRT less than 10 h is adequate, but decreasing HRT results in slight nitrite accumulation. They, also, studied the effect of phosphorous concentration in denitrification process, since it is one of the most important factors in microbial growth. Shortage of phosphorous concentration could prevent proper growth of denitrifying micro-organism leading to nitrite accumulation; while on the other hand, optimization of phosphorous concentration is necessary in order to prevent an excessive increase in biomass and for environmental protection and economic reasons. Teixeira and Oliveira [17] concluded that it is possible to optimize the phosphorous concentration in the range of 2-20 mg/L and based on this study, Cortez et al. [22,26] considered 10 mg/L of phosphorous concentration as phosphate in their investigations. Mohseni-Bandpi et al. [28-30] investigated different effective parameter of groundwater denitrification in an ARBC reactor. According to nitrate removal rate and removal efficiency, acetate was the most effective carbon source with optimum C/N ratio of 1.7. No significant changes have been seen at HRT further than 8.8 h. They mentioned that increasing the nitrate concentration in influent leads to drop of nitrate removal efficiency and increasing nitrite concentration in effluent. These changes were more significant in limited carbon source condition.

The purpose of this study is to investigate the pilot-scale ARBC for treatment of synthetic water in different HRT, C/N, and nitrate concentration.

2. Materials and methods

2.1. Synthetic water

Groundwater was simulated according to previous study of Mirbagheri and Amirsoleymani [31] on denitrifving groundwater of Tehran oil refinery located in industrial zone in south of Tehran that was compatible with evaluation on groundwater quality of this zone conducted by Nassery and Falsafi [32]. Different nitrate concentrations and C/N ratio were considered in this experiment. In this regard, synthetic water was prepared by potassium nitrate and acetate, chosen as carbon source, according to different C/N and nitrate concentrations. The phosphorous concentration of 10 mg/L P-K₃PO₄ was considered in order to guarantee proper microbial growth. The pH of the initial solution was kept in the range of 7-7.5. All the salt and minerals used in this experiment were provided from Merck & Co pharmaceutical company.

2.2. Experimental setup

Fig. 1 represents the schematic diagram of pilot used in this study. The ARBC reactor is a rectangular tank with total volume of 46.55 L containing four discs placed on a horizontal shaft. Each disc consists of a circular poly-methyl methacrylate plate with 1 mm thickness. Additionally, polyurethane foam with thickness of 1 cm was attached to each side of discs. ARBC parameters are listed in Table 1. Rotating speed of the discs was fixed at 4 rpm to gain the suitable mix in reactor. In order to create anoxic condition, ARBC reactor was completely sealed. Two submerge pumps were used for influent and effluent and flow rate was set by manual valves.



Fig. 1. Schematic representation of pilot, MV: manual valve, SP: submerge pump.

Table 1 Summary of ARBC unit parameters

Parameter	Value
Reactor volume (L)	46.55
Numbers of discs	4
Disc spacing, center to center (cm)	7
Disc diameter (cm)	25
Disc thickness (cm)	2.1
Disc's type material	polyurethane foam
Disc submergence (%)	100
Shaft diameter (cm)	1.8
Unit length (cm)	38
Rotational speed (rpm)	4

As it can be seen in Fig. 1, the settling tank possesses the following cross-section: height of 17.5 cm, and bottom and top width of 8 and 35 cm, respectively, followed after the ARBC reactor. The filter column was filled with quartz sand in the range of 1–2 mm to the height of 35 cm.

2.3. Experimental procedure

The ARBC was inoculated with denitrifying bacteria from activated sludge of South Tehran wastewater treatment containing anoxic basin. About 10 L of sludge was exerted into the reactor, and then the reactor was filled with synthetic water to the volume of 40 L. The reactor was operated in batch mode for 6 d in order to form and accumulate the biofilm on the discs. Afterward, the reactor was operated in continuous mode with synthetic water, C/N of 1.50, and nitrate concentration of 75 mg/L, for 1 week. The C/N ratio was a little higher than theoretical value according to McCarty et al. [33] equation, considering carbon consumption due to presence of oxygen in influent water.

Three different groups of experiments on HRT, C/N ratio, and nitrate concentration were carried out in order to evaluate capability and optimum operational parameters of the reactor. Under each experiment, reactor was kept running for a period equivalent to 5 HRT, in order to ensure reaching the steady state condition. All the experiments were conducted under room temperature (≈ 26 °C).

2.4. Analytical method

Influent solution was prepared daily. The concentration of NO_3^- , NO_2^- , NH_4^+ , COD, and alkalinity were measured with single-beam spectral photometer SpectroDirect (Loviband Co.). Concentration of each

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compound was determined according to the following procedure: nitrate was tested according to 2,6-Dimethylphenole method at the wavelength of 340 nm, nitrite was tested according to N-(1-Naphthyl)-ethylenediamine method at the wavelength of 545 nm, NH_4^+ was tested according to Indophenole-blue method at the wavelength of 676 nm, COD was tested according to Dichromate/H₂SO₄ method at the wavelength of 620 nm, and alkalinity was tested according to Acid/Indicator method at the wavelengths of 551 and 615 nm.

3. Results and discussion

3.1. Hydraulic retention time

As it was expected, increasing the HRT resulted in decreasing the nitrate concentration in effluent. As it is been indicated in Table 2, the NO_3^- declined from 3.10 mg/L in HRT equal to 4 h to not detected value (<2.2 mg/L) in greater HRTs. According to this, denitrification efficiency was above 97% for HRT \geq 8 h. This result was completely similar with Mohseni-Bandpi and Elliott [29]. In both works, nitrate removal efficiency decreases significantly when HRT was less than 8 h, however, it didn't change significantly in HRT farther than 8 h.

On the other hand, nitrite concentration in effluent is approximately constant and nitrite production in denitrification process seems to be independent of HRT. Accumulation of nitrite is an undesirable phenomenon in biological denitrification process. Due to nitrite toxicity, its accumulation could inhibit the bacterial growth, inhibit the denitrification process, and cause the presence of nitrite in effluent. The nitrite accumulation in this study could be related to the different micro-organisms present in the medium, since they show different activity patterns. Cortez et al. [22] mentioned the micro-organisms, which just reduced nitrate to nitrite and were unable to take the denitrification process further to reduce nitrite to nitrogen gas. Fabbricino and Petta [34] also named parasite process as a compatible process that inhibits the denitrification process. Since the activated sludge provided from wastewater treatment plant is a mixed culture, it is probably enriched with different microbial species that some of them are the reason of nitrite presence in the effluent. Therefore, the nitrite concentration in effluent was not effected by HTR and acted independently.

Ammonium is the result of dissimilatory nitrate reduction to ammonium (DNRA) or aggregation and mortality of bacteria. Fig. 2 shows descending trend of NH_4^+ -N concentration in effluent. Increasing HRT provides enough time for bacteria to nitrify the released ammonium. On the other hand, ammonium could be involved directly in denitrification process as electron donor [35]. As a result of these reactions, decreasing in ammonium concentration with increasing HRT has been observed. Alkalinity is a product of heterotrophic denitrification process. As it is illustrated in Fig. 3, the

Table 2Parameters on influent and effluent of different experimental procedure

Experiment parameter			Influent	Effluent				
HRT (h)	C/N	Flow rate (L/h)	NO_3^- (mg/L)	NO ₃ ⁻ (mg/L)	NO ₂ ⁻ (mg/L)	NH ₄ ⁺ -N (mg/L)	COD (mg/L)	Alkalinity (mg/L as CaCO ₃)
4	1.5	10.00	75	3.10	1.12	0.34	_	180
8	1.5	5.00	75	ND*	1.35	0.32	_	210
12	1.5	3.33	75	ND	1.28	0.30	_	220
16	1.5	2.50	75	ND	1.25	0.28	_	240
20	1.5	2.00	75	ND	1.15	0.26	_	270
8	1.00	5.00	75	2.22	1.05	_	110	_
8	1.25	5.00	75	ND	1.02	-	120	-
8	1.50	5.00	75	ND	1.25	-	125	_
8	1.75	5.00	75	ND	1.38	-	160	_
8	2.00	5.00	75	ND	1.12	-	270	_
8	1.25	5.00	60	ND	0.79	-	_	-
8	1.25	5.00	75	ND	1.02	-	_	_
8	1.25	5.00	90	ND	1.18	-	_	_
8	1.25	5.00	105	ND	8.23	-	_	-
8	1.25	5.00	120	ND	13.16	-	_	-

*Note: ND: not detected.



Fig. 2. NH_4^+ -N concentration in effluent over different HRTs at C/N = 1.5 and nitrate concentration of 75 mg/L.

alkalinity concentration increases with HRT enhancement. This indicates that although the nitrate concentration in not detected in effluent with HRT ≥ 8 h, the denitrification process still goes on.

According to the experimental results at HRT = 8 h, all the parameters reach their optimum value, although at HRT = 4 h the concerning term concentration is far below the standard limitation. Therefore, HRT = 8 h was used in subsequent experiments.

3.2. C/N ratio

Table 2 shows that nitrate concentration in effluent was undetectable in all C/N ratio, except C/N = 1.00. The NO_3^- concentration was 2.22 mg/L at C/N = 1.00. The concentration of nitrite was in the range of previous experiment in larger values of C/N, but slightly decreased in C/N = 1.00 and 1.25. Decreasing the C/N ratio limits the carbon source and it could affect the activity of nondenitrifying and parasite micro-organism, reducing nitrite accumulation. COD of treated water increased with increasing of C/N



Fig. 3. Alkalinity concentration in effluent over different HRTs at C/N = 1.5 and nitrate concentration of 75 mg/L.

ratio, shown in Fig. 4, Below C/N ratio of 1.5, COD variation is too small which could be a sign of complete use of acetate. Experiment with C/N = 1.00 was even insufficient due to carbon limitation, which resulted in residual nitrate in effluent. At C/N ratio of 1.75 and 2.00, extreme increase in effluent COD was observed, indicating presence of excess acetate in effluent. Moreover, at C/N = 2.00 with overgrowth of bacteria, sludge rising in reactor occurred. Thus, it can be concluded that at C/N ratio further than 1.5 carbon source provided was more than required for denitrification process and it just passed the reactor to the effluent without any use.

Theoretical oxidation–reduction reaction of acetate and nitrate is as follows:

$$NO_{3}^{-} + 0.625CH_{3}COO^{-} + 0.374H^{+} \rightarrow 1.25HCO_{3}^{-} + 0.5N_{2} + 0.5H_{2}O$$
(2)

According to (Eq. (2)), theoretical C/N ratio for denitrification using acetate as carbon source is 1.07 (mg C–CH₃COO/mg N–NO₃). C/N ratio is always higher than theoretical value. Carbon source is consumed for biomass growth in the reactor. On the other hand, dissolved oxygen which is presence in water is inevitable in a continuous reactor, increases the C/N ratio. According to McCarty et al. suggestion, using acetate as carbon source, the denitrification reaction would be as follows [33]:

$$\begin{array}{l} \mathrm{NO}_{3}^{-} + 0.826\mathrm{CH}_{3}\mathrm{COO}^{-} + 1.816\mathrm{H}^{+} \\ \rightarrow 1.302\mathrm{CO}_{2} + 0.07\mathrm{C}_{5}\mathrm{H}_{7}\mathrm{O}_{2}\mathrm{N} + 1.9\mathrm{H}_{2}\mathrm{O} + 0.467\mathrm{N}_{2} \end{array} \tag{3}$$

According to (Eq. (3)), C/N ratio considering biomass growth is 1.416. It is notable that the nature of



Fig. 4. COD concentration in effluent corresponding to different C/N ratio at HRT = 8 h and nitrate concentration of 75 mg/L.

bacterial species plays an important role in sufficient C/N ratio required for complete denitrification.

The optimum C/N ratio was reported differently in previous studies using acetate and acetic acid as carbon source. Shen et al. [20] considered C/N = 1.34using A/O-MBR reactor, while Cortez et al. [22] and Mohseni-Bandpi and Elliott [29] both using RBC reactors, reported optimum C/N ratio of 1.5 and 1.7, respectively. According to the results of this study, C/N = 1.25 is the optimum value that is lower that the theoretical value obtained by (Eq. (3)). As Rittmann and McCarty [36] suggested, heterotrophic denitrification reaction is a combination of energy production and cellular synthesis, while nitrate is the electron acceptor in first reaction and nitrogen source in second one. Comparing the optimum C/N ratio (1.25) obtained in this study with theoretical C/N obtained from (Eq. (2)) illustrates that biomass growth is happening in ARBC reactor, but the cellular synthesis reaction contribution in this study was less than what McCarty assumed in (Eq. (3)). This phenomenon could be related to the bacterial species that undoubtedly play an important role in denitrification process.

3.3. Nitrate concentration

As it is illustrated in Table 2, increasing $NO_3^$ loading didn't have any influence on NO₃⁻ concentration in effluent and NO_3^- remains undetectable. On the other hand, increasing NO_3^- loading from 60 to 120 mg/L, increased NO_2^- from 0.79 to 13.16 mg/L. According to Fig. 5 up to the nitrate loading of 90 mg/L, NO_2^- concentration remains within standard limitation, but at NO_3^- loading of 105 and 120 mg, NO_2^- concentration of effluent is far beyond this limit. Mohseni-Bandpi et al. [30] observed decrease of nitrate removal efficiency from 99 to 83% when they changed the influent nitrate concentration from 40 to

2.0 0.0 75 80 85 90 95 100 105 110 115 120 125 55 60 65 70 Nitrate concentration of influent (mg/L)

Fig. 5. Nitrite concentration in effluent corresponding to nitrate loading at HRT = 8 h and C/N = 1.25.

250 mg/L in a continuous ARBC reactor with constant C/N and acetic acid as carbon source. In addition, nitrite concentration of effluent varied from 0.05 to 8 mg/L, however, this variation in 130–250 mg/L range of influent nitrite concentration was 1.5–8 mg/L. Wang et al. [25], during their study on fiber-based biofilm reactor operated with methanol as carbon source, mentioned that although the influent nitrate loading did not have influence on NO_3^- -Nremoval, the NO_2^- -N in effluent increased from 0.01 to 0.75 when the NO_3^- -N loading increased from 49.28 to 148.66 mg/L.

4. Conclusions

Denitrification of Tehran oil refinery groundwater in an ARBC, operated in heterotrophic process using acetate as carbon source, was considered in this study. NO_3^- concentration of effluent in all experiments was far below the standard value, according to WHO and EPA and the nitrate removal efficiency in optimum condition, HRT = 8 h and C/N = 1.25, was always above 95%. Ammonium and alkalinity concentration of effluent shows the progress of denitrification process by HRT increasing. According to COD, nitrate concentration, and nitrite concentration of effluent, C/N ratio above 1.25 is economically and environmentally undesirable. The COD concentration in effluent rises from 120 to 270 mg/L with increasing the C/N ratio from 1.25 to 2.00, indicating the presence of excess acetate in effluent. The NO₂⁻ accumulation was the result of nondenitrifying micro-organisms; however, its concentration in all experiments effluent was below the standard value, except in influent nitrate loading of 105 and 120 mg/L, NO_2^- concentration increased to 8.23 and 13.16 mg/L, respectively. Accumulation of NO_2^- in high nitrate loading of influent is due to inadequate retention time leading to incomplete denitrification. Prolonging HRT or adding an aerating basin after ARBC at high nitrate loading would help to solve this problem.

Based on the result of this experiment, ARBC reactor is an adequate and convenient method in denitrification of groundwater.

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