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Surveying Denitrification Efficacy in Up-Flow Packed Bed Bioreactor Operated under Heterotrophic Condition Using Autotrophic Bacteria

Ali Asghar Neshat [1], *Abdomajid Gholizadeh* [1]*, *Babak Jahed* [2], *Pouria Nikvand* [2]

[1] *Department of Environmental Health Engineering, Esfarayen Faculty of Medical Sciences, Esfarayen, Iran.*
[2] *Department of Public Health, School of Nursing and Midwifery, Iranshahr University of Medical Sciences, Iranshahr, Iran.*

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***Corresponding Author:**
Abdomajid Gholizadeh

Email:
gholizadeh_eng@yahoo.com

Tel:
+985837223504

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ABSTRACT

Introduction: The biological denitrification process is an interesting cost-effective technique to remove nitrate from water supplies. Acetic acid can be used as a carbon source in this process, but its consumption rate is a critical issue and, in some cases, it is quite different from stoichiometric constants. The current study aimed to investigate the nitrate removal in an up-flow packed bed bioreactor. Furthermore, various parameters affecting this process were investigated and optimized. In this study, the autotrophic bacteria were used for the heterotrophic process.

Materials and Methods: Initially, the autotrophic bacteria were cultured and used for the following heterotrophic conditions in distinct reactors. A pilot-scale anoxic up flow bioreactor packed was constructed using the polyethylene media and applied to remove nitrate from the aqueous environment. Consequently, the effects of hydraulic retention times (HRT) and different acetic acid concentrations as carbon source were evaluated. During the study, the amounts of alkalinity, pH, temperature, and nitrate were checked.

Results: The designed bioreactor removed an average of over 88% of nitrate, while the acetic acid consumption was 2 mg/mg NO_3-N, which was lower than the stoichiometric constant for heterotrophic process. Moreover, in the three studied HRTs (1.5, 3, and 5 h), the Alkalinity increased from 14.2 to 19.8 %.

Conclusion: The results of this study showed high efficiency in nitrate removal via heterotrophic denitrification using acetic acid as carbon source for autotrophic bacteria.

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Introduction

Nitrate is commonly found in drinking water considering the human activities such as excessive use of chemical fertilizers, incomplete septic system, and inappropriate disposal of industrial, human, and animal wastewaters. Since nitrate is soluble in water, it can enter into the groundwater aquifer and drinking water resources [1, 2]. The concentration of nitrate in surface waters is usually low (0-18 mg/L), but can increase as a result of the agricultural water and contamination with human or animal wastes. Water analyses in the United States and Canada showed that nitrate concentration in water supplies was about 10 mg/L [3]. Moreover, the concentrations of nitrate in ground waters of the rural areas in New York [4], India [5], North China Plain [6], Korea [7], Spain [8], etc. were higher than the standard level (45 mg/L), showing

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that this is a global problem.

Nitrate is not often hazardous to health in low and intermittent consumptions, but high levels of nitrate anion in aquatic environments is a serious environmental problem in which its continuous and excessive usage can have adverse the health effects, especially for infants and pregnant women [9, 10]. Lord et al. reported that nitrate in drinking water can cause disturbance in the digestive tract cancer [11], which is most likely due to the formation of nitrosamines in water [12, 13].

Since nitrate ion is very water soluble, it cannot be removed efficiently by conventional purification methods such as coagulation, adsorption, etc. [14]. Ion exchange, reverse osmosis (RO), nanofiltration, and electrodialysis are the common processes applied for nitrate removal from aquatic environments [2, 15]. The utility of the above-mentioned processes has been limited due to expensive operation, low efficiency in some cases, and subsequent disposal problem of the nitrate concentrate and generated sludge [16, 17]. On the other hand, the biological techniques are among the best strategies for nitrate removal, mainly due to causing high efficiency, producing reusable sludge, and converting nitrate to harmless nitrogen gas as the major end product [18]. This method was promoted by the European Strategy as a reference opposed to the physiochemical treatment alternatives due to its good performance, low cost, and large available quantities [19, 20]. A study specified that granular sludge sequencing batch reactors could denitrify the nitrate bearing acidic effluents directly without a prior treatment [21].

Heterotrophic and autotrophic are two types of biological denitrification. Hydrogen, iron, or sulfur compounds are used as energy source and inorganic carbon compounds, such as carbon dioxide and bicarbonate are applied as carbon source by autotrophic denitrifiers. The most common denitrifiers in the nature are heterotrophic denitrifiers that use organic carbon compounds as carbon source.

However, heterotrophic bacteria can utilize different carbon compounds as electron donor. The type of carbon source used in biological processes has an important role in the heterotrophic denitrification rate. Commonly, sugar, glucose, acetone, acetic acid, ethanol, and methanol are available as carbon sources [20, 22-24]. In comparison, acetic acid is more readily metabolized than methanol and ethanol. Higher denitrification rate, high buffering capacity, and absence of toxic effects are from the advantages of applying acetic acid in the denitrification process [25, 26]

Equation 1 shows the utilization of acetic acid as a carbon source for denitrification process [27]:

Equation 1:

$$0.84CH_3COOH + NO_3^- \rightarrow 0.08C_5H_7O_2N + HCO_3^- + 0.3CO_2 + 0.92H_2O + 0.46N_2$$

As shown, approximately 4.1 g acetic acid is required to remove 1 g of $NO_3 - N$. Usually, a defined rate of acetic acid is utilized in the stoichiometric calculation, but this rate can be different in practice depending on the used system and its operation [27]. Many aspects of the biological denitrification have not been revealed yet. Therefore, studying the effective factors and optimizing them play a significant role in improving the performance of this process. The autotrophic process does not require a carbon source, but is slow and efficient. Moreover, in the heterotrophic process, the nitrate removal rate and yield are greater, but need adding a large amount of the organic carbon [28]. In this study, autotrophic bacteria were used in the reactor to play the role of heterotrophic bacteria by adding acetic acid as a carbon source. Moreover, we aimed to compare the performance of up-flow packed bed bioreactor fed by autotrophic bacteria in heterotrophic condition to remove nitrate from water supply and optimize different factors affecting this system.

Materials and Methods

Pilot design and configuration

All experiments were conducted in the laboratory scale. A schematic of up-flow packed bed denitrification bioreactor constructed from polycarbonate was used in the current study (Figure 1). The denitrification process was operated under anoxic condition (The DO concentration was near zero, but bound oxygen

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existed in NO_3 and SO_4).

Polyethylene media manufactured by Aria PetroPaak Company (Arak, Iran) with a 45 µm pore size, active surface of 500 m^2/m^3 and a density of 95 kg/m^3 filled 70% of the reactor volume and increased the retention time of the microbial community in the reactor. Useful volume of the reactor was 7 L. To prevent flow return, two check valves were contrived in the bioreactor. Furthermore, two peristaltic dosing pumps were injected by a desirable flow rate of raw water into the reactor. Furthermore, a raw water tank was equipped with nitrogen gas diffusers at the bottom to remove the dissolved oxygen from the water that entered the tank.

Reactor operation

Initially, the autotrophic bacteria were cultured and used for the followed heterotrophic conditions. For this purpose, the anoxic sludge was collected from selector unit of wastewater treatment plant in South of Tehran as the denitrifier microorganism source and added to distinct 1L reactors, which had autotrophic conditions. Elemental sulfur and sodium bicarbonate provided the energy and carbon sources and no organic matter was found in the reactors.

After 10 days, when the bacteria were established and the nitrate was removed over 95%, the autotrophic bacteria were ready to be injected into the main reactor. Before conducting denitrification experiments, the reactor was operated continuously for two weeks to form a satisfactory biofilm on the media packed. Then, the reactor was operated during 60 days in three 20-day periods (1-20, 21-40 and 41-60 days). The pH and temperature parameters were kept constant using a thermostat in 7.5 ± 0.5 and 30 ± 2 °C, respectively.

The effect of Hydrulic Retention Time (HRT) was elucidated on reactor performance by selecting three HRT (1.5, 3, and 5 h). Moreover, to survey the effect of carbon source concentration, different concentrations of acetic acid including 90, 102, 135, 205, 225, and 270 mg/L were considered. The tap water, which its dissolved oxygen reached to lower than 0.2 mg/L by injecting N_2 gas for a half hour, was entered the reactor. Then, the concentration of nitrate was adjusted as 40.6 ± 8.7 mg/L and subsequently injected into the anoxic reactor, as shown in Table 1. After two weeks of continuous operation, the necessary hydraulic retention times were adjusted after modifing the inlet flow rate when the nitrate concentration reached around zero. The flow injected into reactor using two peristaltic pumps so that to provide distinct hydraulic retention times (1.5, 3 and 5h). Considering the useful volume of reactor was equal to 7 L, the inlet flows were changed from 1.4 L/h to 4.6 L/h.

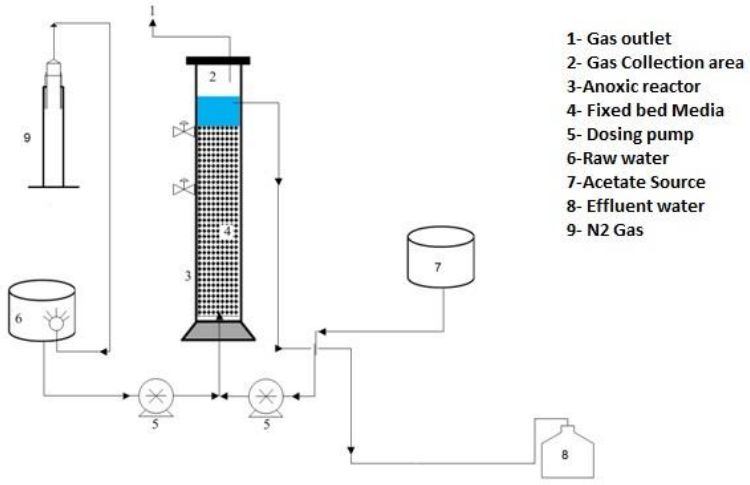

Figure 1: Schematic of up-flow packed bed denitrification bioreactor

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Analysing and measurments

The supernatant of samples was separeted by a centrifuge device, and their nitrate concentrations were measured using a DR5000 spectrophotometer (Hach Co., Loveland, USA). Alkalinity was determined using titration method. All parameters were performed in accordance with the Standard Methods for the Examination of Water and Wastewater [29]. Data were statistically analyzed through T-Test in SPSS V. 19. s.

Results

The results considering effects of different HRTs on NO_3-N, alkalinity, and acetic acid consumption are summarized in Table 1. As shown, in HRT of 1.5 h the rate of acetic acid consumption to remove NO_3-N is lower than the stoichiometric rate (Table 1).

Table 1: Results of the heterotrophic denitrification process in different HRT

HRT (h)	Influent		Effluent		Acetic acid consumption (mg/mg NO_3-N)
	NO_3-N (mg/L)	Alkalinity (mg/L $CaCO_3$)	NO_3-N (mg/L)	Alkalinity (mg/L $CaCO_3$)	
1.5	40.6 ± 8.7	210 ± 46.4	12.4 ± 3.1	245 ± 55.5	2.27 ± 0.9
3	40.6 ± 8.7	210 ± 46.4	5.8 ± 5.7	252.8 ± 53	1.91 ± 0.55
5	40.6 ± 8.7	210 ± 46.4	0.58 ± 0.95	262 ± 48	2 ± 0.7

According to Table 1, the influent concentration of parameters was constant in three HRTs and the last column of Table 1 shows the acetic acid consumption in every HRT.

Figure 2 shows the effect of HRT on NO_3-N removal troughout the study. According to Figure 2, the nitrate removal efficiency improved with increase of the hydraulic retention time. It was observed that, at HRT of 1.5 h, 68.7% nitrate removal was obtained; in which, the output nitrate concentration exceeds the maximum allowable limit of the regulated standards [30]. The average nitrate removal rates of 97% and 98.5% were obtained at HRTs of 3 and 5 h, respectively; in which, concentrations of the output nitrate fulfilled the standard limits.

According to the statistical parameteric analysis, a significant difference was observed between the average removal percentage of the studied hydraulic retention times ($p < 0.05$). This indicates that the nitrate removal efficiency increased as the hydraulic retention time increased from 1.5 to 3 and 5 h.

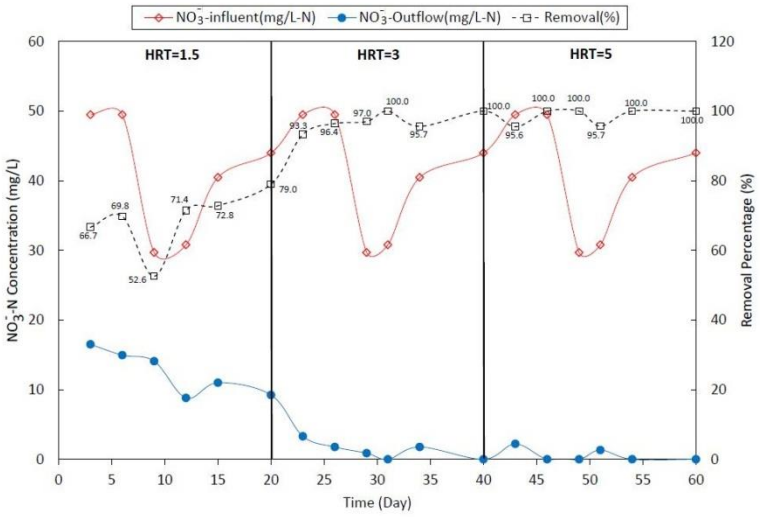

Figure 2: Effects of different HRTs on NO_3^--N removal in the up-flow packed bed bioreactor

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Further, Figure. 3 a, b, and c show the concentrations of acetate, nitrate, and alkalinity in the influent and effluent at variables for HRTs of 1.5, 3, and 5 h. Alkalinity is increased in heterotrophic process, unlike the autotrophic denitrification.

It is clear that by increase of the nitrate removal, the acetic acid consumption also augmented and the average acetic acid consumption was 1.91 ± 0.55 mg per each mg of the removed NO_3-N in HRT of 3 h.

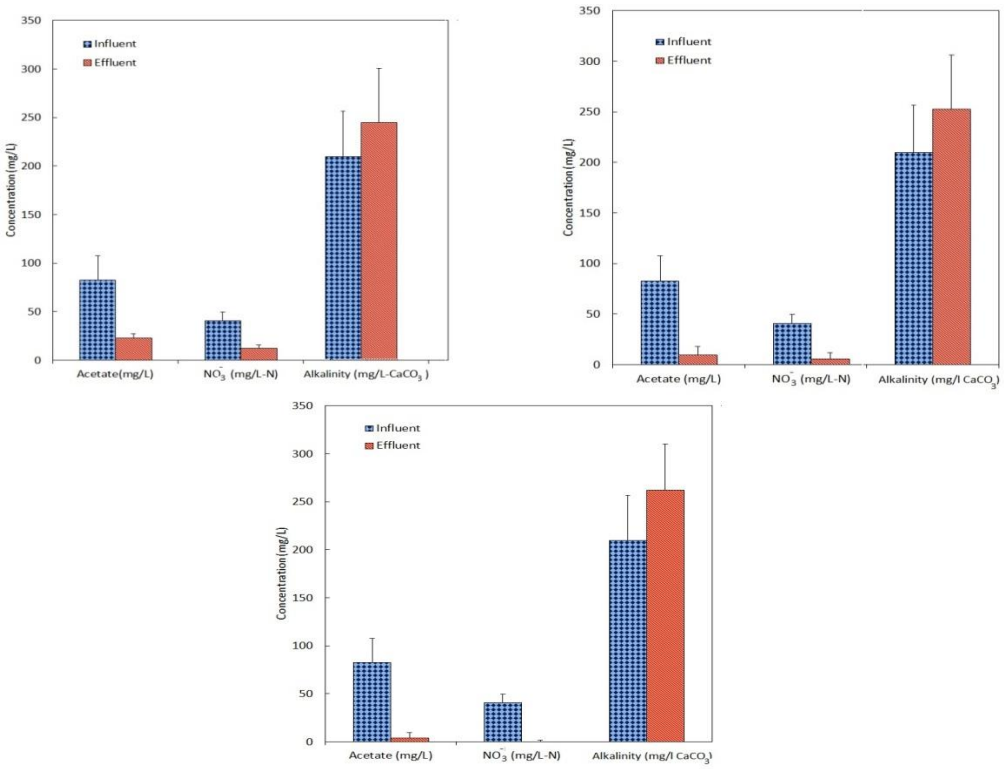

Figure. 3: Concentrations of acetate, nitrate, and alkalinity in influent and effluent at different HRTs. A: 1.5 h, B: 3 h, and C: 5h.

As seen in Figure 2 and Figure. 3, by increase of the retention time from 1.5 to 3 h, the nitrate reduction is obvious.

In the HRT of 5 h, acetic acid and NO_3 were reduced considerably, so that their concentrations were near zero in the effluent. It should be noted that like the pervious retention time, the average rate of acetic acid consumption was lower for the nitrate removal than stoichiometric constant; the obtained value for the mentioned constant was 2.07.

Discussion

The results considering effects of different HRTs on the nitrate removal showed the efficiency improved with increase of the hydraulic retention time. Increasing the hydraulic retention time give suficient time to microbes for reducing nitrogen and thereby removing it from wastewater. Addy et al, concluded that in beds with less hydraulic retention times, the nitrate removal (mass per volume) was significantly lower. This argues for the fact that bed designs incorporate sufficient time for nitrate removal [31]. Similar results were found in the study of Christianson et al, who suggested that the minimum design retention times (7.5–79 h) should be increased to achive sufficient mass nitrogen reduction. However, they mentioned that caution should be considred in this regard, because by increase of the design retention times and enlargement of the corresponding bioreactors, the detrimental by-products may exacerbate under low flow conditions. According to Wang and Chu as well as Ovez et al., decrease of HRT to certain values increased the effluent nitrate concentrations

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and nitrite accumulation [32, 33].

Bed designers should optimize the system to address the expected flow rates and ensure the sufficient time for nitrate removal. Nowadays, many denitrifying bed designes incorporate with the hydraulic control components and adjust to the bypass flow in high flow events. Such design features provide the extended HRT and permit the flexibility, so that nitrate removal can be examined under different HRT strategies [34, 35].

The statistical parameteric analysis showed a significant difference between the average removal percentage of the studied hydraulic retention times ($p < 0.05$). This shows that by increase the hydraulic retention time from 1.5 to 3 and 5 h, the nitrate removal efficiency increased. Furthermore, Wang et al. studied the efficiency of a laboratory-scale denitrification reactor packed with biodegradable snack ware in a low-temperature condition. They found that at a concentration of 50 mg/L for NO_3-N, 5 h of HRT was needed to complete the nitrate removal [36].

Meanwhile by increasing of the nitrate removal, the acetic acid consumption also augmented and the average acetic acid consumption was 1.91 ± 0.55 mg per each mg of the removed NO_3-N in HRT of 3 h. Some studies obtained the same results; for example, Sukias et al. found that the acetic acid requirement was 3.5 mg acetic acid per mg NO_3-N removal [37]. Mohseni and Elliott reported that the acetic acid to nitrate nitrogen (A/N) ratio was in the range of 4.2 to 4.3 [38].

So, we can conclude from these findings that another source of organic materials is probably available for heterotrophic bacteria in reactors such as the organic materials in influent (raw water) and the residue of died microorganisms. This source causes a decrease of acetic acid consumption and consequently lowers the constant of acetic acid consumption to the removed NO_3-N in comparison to the stoichiometry calculation.

Alkalinity is increased in heterotrophic process, unlike the autotrophic denitrification. In autotrophic process, a part of the alkalinity is consumed as an inorganic carbon source [39], but in heterotrophic denitrification, the existing organic carbon is consumed and changed to inorganic carbon; so, the alkalinity is increased in the effluent [40]. Zhao J. et al. found that the dominant denitrifiers, in a woodchip-based solid-phase denitrification (W-SPD) bioreactor, were carbonaceous compound degrading bacteria and fermentative bacteria. Furthermore, this system was able to remove 92.5% – 96.4% of the nitrate [41].

Conclusion

The results show that, at HRTs of 1.5 h, 3 h, and 5 h 68.7%, 97%, and 98.5% nitrate removal were obtained, respectively. It is clear that by increase of the nitrate removal, the acetic acid consumption augmented. In the current study, we showed that constant rate of the acetic acid consumption to remove NO_3-N was lower than the stoichiometric with a proportion of 2:1 (acetic acid to NO_3-N). According to the results of this study, the autotrophic bacteria can remove nitrate from water under heterotrophic condition and high efficiency removal of nitrate decreased the consumption of organic matter (acetic acid).

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Conflict of Interest

All authors declare they have no conflict of interest regarding the publication of this article.

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