ORIGINAL ARTICLE

Biological Nitrate Removal from Groundwater by Filamentous Media at Pilot Scale, 2015

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Abstract

Background: The compounds which contain nitrogen entering the environment can cause some problems, such as eutrophication for water resources and potential risk for human health because of methemoglobinemia and cancer. Biological techniques are effective in removing nitrate. The aim of this study was to remove nitrate from groundwater using denitrification. The main objectives of this research were determining the reduction of water nitrate based on different retention time and also the effect of using grape extract as organic matter and electron acceptor in biological nitrate removal from water.

Methods: In this experimental study, the effect of heterotrophic Pseudomonas separated from Shiraz wastewater treatment plant on removing nitrate from groundwater was investigated at pilot scale using grape extract as carbon source and filamentous media at constant pH (7±0.1) and temperature (20±1 °C). During this study, 2 pilots were made. Pilot number 1 was used for separation and growth of the above-mentioned bacteria (Pseudomonas) that are able to remove nitrate. Pilot number 2 was also used for surveying the removal of nitrate by these bacteria. At least, 13 samples were examined in every retention time and each test was repeated for 2 or 3 times. Statistical analysis was performed in SPSS (ver.19) software using one-way repeated measures ANOVA, and Bonferroni tests.

Results: According to the results, nitrate removal rates were 49%, 55%, 67% and, 67% at retention times of 1, 1.5, 2, and 2.5 hours, respectively. The best retention time was 2 hours with 67% removal rate ($P<0.05$).

Conclusion: The results showed that using grape extract as the carbon source and proper growth of bacteria in filamentous media led to a significant increase in the removal rate.

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Introduction

One of the valuable water resources is groundwater, especially among arid and semi- arid area like the central and southern parts of Iran.[1] Groundwater provides necessary water for 2 billion people and also 40% of food production in the world.[2,3] One of the most important components of the earth's atmosphere is nitrogen and it appears in different forms including elemental nitrogen, nitrate and ammonia. Nitrate is one of the main elements which is obtained through natural nitrogen cycle. Moreover, nitrate concentrations have been increasing by anthropogenic sources, especially in groundwater area.[4,5]

Nitrogen, as well as Carbon, Hydrogen and Oxygen, is one of the major components which is

important in forming the living matter. Persistent changes are happening in Nitrogen and compounds related to it, especially synthesis process in which organic nitrogen compounds are created from nitrates and gaseous nitrogen, and organic dead material catalysis due to which the released ammonia changes into nitrates and free nitrogen.[6]

Nitrogen is required in growing plant, and nitrogenous fertilizers are utilized in global agricultural lands for increasing crop production.[7] Nitrogen mostly appears in the shape of nitrates in underground waters, since they solve in water easily, they do not engage in soil sorption complex and to some extent they are drained away from the soil fast. In addition, ammonium ions are usually nitrified to nitrates. Apart from nitrates, there are also nitrites in trace amounts.[6]

Since the past decades, there was a Nitrate pollution problem in most parts of the world.[8,9] Because of excessive usage of inorganic nitrate and organic fertilizers in agriculture and supplying lands by wastewater, Nitrate can be drained away from the soil to groundwater. As in rural regions of China 100 mg/L of Nitrate have been found in groundwater.[10] As a result excessive usage of fertilizers, disposal of untreated municipal and industrial wastes create a serious environmental problem in the world.[7] So, the nitrogen load discharged to receiving waterways increases by this extravagant usage of fertilizers, comprehensive exploitation of farms and significant presence of industries.[4,11-17] Moreover, extensive Nitrate contamination of groundwater happens by uncontrolled use of chemical fertilizers and using untreated (or poorly treated) industrial wastewater.[2]

Nowadays, we can see the importance of Nitrate as a main environmental matter because of its role in affecting human and animal health.[4] Eutrophication of water bodies created by extensive usage of nitrate includes inland seas, lakes and ponds. Humans and animals health also can be endangered by drinking these kinds of waters. Eutrophication arouse algae and aquatic plants growth which can endangere the aquatic life and water quality.[7,8,18] This excessive concentration of nitrate can be dangerous for humans and animals, too. In addition, microorganisms in human body can change the form of nitrate to nitrate with more toxicity (Methemoglobinemia or blue baby syndrome in infants and gastrointestinal cancer in adults are caused by reduction of Nitrate to nitrite in the intestines).[2,7,8,10] This can harm the livestock, as well.

Here are the samples of nitrate poisoning symptoms in livestock: cyanosis in non-pigmented areas (mouth and eyes), shortness of breath, rapid heartbeat, staggered gout, frequent urination, and collapse.[19] In severe cases, we will see coma and death of the patients within a few hours. Since there is a connection between health problems and immoderate existence of nitrate in drinking water, World Health Organization (WHO) and regulatory agencies in different parts of the world have provided an agreement about nitrate concentration limits.[7] As reported by WHO (WHO, 2011), Iranian and European Union, nitrate concentration in drinking water should not exceed 50 mg/L.[2] On the other hand, the US Environmental Protection Agency has determined 44 mg NO_3^-/L (EPA, 2009). In Australia, the recommended limit is 50 mg NO_3^-/L for infants up to 3 months old and 100 mg NO_3^-/L for adults and children over the age of 3 months (National Health and Medical Research Council, 2011). South Africa, however, has considered a much lower permissible level, i.e. 20 mg NO_3^-/L.[7,20] Additionally, both USEPA and China have determined the Maximum Contaminant Levels (MCL) to be 10 mg/L nitrate nitrogen (NO_3^-- N) and 1 mg/L nitrite nitrogen (NO_2^- -N) by.[8,10]

Nitrate tendency for deposition and surface assimilation is low because of its high stability and solubility. Therefore, removing it from water using conventional water treatment technologies is difficult.[21] Up to now, many advanced physiochemical and biological treatment techniques have been recommended for extracting excessive nitrate from water so far. These include Reverse Osmosis (RO), ion exchange, electro-dialysis, adsorption, denitrification, algae growth, disposal of the harvest, and a combination of ozonation and sand/activated carbon filtration.[7,8,11,22,23]

Some of the above-mentioned methods, including ion-exchange, RO, and electro-dialysis are known to be effective in removing nitrates. Nevertheless, these methods are relatively expensive and produce waste concentrates (brines) containing other ions and high concentrations of nitrate, which require additional treatment or disposal. In many inland places, local regulations regarding discharge of brines to the wastewater system limit the application of physicochemical technologies.[2,11,24]

As an alternative treatment method, biological denitrification, in both heterotrophic and autotrophic modes,[7,9] does not produce waste brine, but it requires an intensive post-treatment step to remove the potential water contamination by organic matter and bacteria. Moreover, application of biological treatment of drinking water is restricted by health concerns and public acceptance limitations.[8,11] Reduction of nitrate to dinitrogen gas using denitrifying bacteria occurs through biological removal of nitrition from drinking water.[10,25] Conventionally, in two separate reactors Nitrogen is removed by nitrification and denitrification. The first reactor is oxidization of ammonia to nitrite (NO_2^-) and then to nitrate (NO_3^-)

in aerobic reactor by autotrophic nitrifiers with oxygen as the electron acceptor.

Subsequently, by heterotrophic microorganisms using organic matter as the carbon source, conversion of nitrate to nitrogen gas happens in anoxic reactor.[2]

Although biological nitrate removal has been done by different kinds of media, it seems that filamentous media have not been used in this regard so far. Therefore, the present study aimed at biological nitrate removal from groundwater by filamentous media at pilot scale. The main objectives of this research are determining the reduction of water nitrate based on different retention times and also the effect of using the grape extract as the organic matter and electron acceptor in biological nitrate removal from water.

Materials and Methods

This research was conducted in the first half of 2015 at one of the cities of Fars province.

1. Making pilot number 1. First, in order to identify and separate the bacteria that are able to remove nitrate, 90 liter of wastewater of Shiraz municipal wastewater plant was conveyed to laboratory before chlorination plant. For surveying the existence of Pseudomonas bacteria, a microbial test was done on Acetamide broth culture medium and the results revealed the existence of this microorganism. Then, filamentous media that were plastic threads were prepared for growth and increasing the number of bacteria and were put into a 90-liter container. Wastewater was also added to this container and was kept there for 60 days at a constant and suitable condition in order to grow and increase the number of Pseudomonas bacteria.

As shown in Figure 1, the effluent of the wastewater container was connected to a holed pipe on the end of the rectangular shaped container, which was full of the filamentous media. The wastewater entered the space among these media via holes and let the bacteria connect to and grow in the filamentous media. The pump, which was located there, let the wastewater return to the 90-liter container for continuation of this process. After 60 days, microbial test was done to assess the existence of Pseudomonas bacteria in the special culture medium, and results proved the existence of this microorganism.

2. Pilot number 2. This pilot consisted of three tanks (Figure 2):

A: Glucose tank (electron donor), which included grape extract in this study.

B: Filamentous media given from pilot number 1.

C: Underground water with nitrate concentration of 63±0.5 mg/L.

According to this figure, there were two pipes with holes at the end of the tank containing filamentous media. One of these pipes was connected to the drinking water tank containing nitrate that was in the lower pipe, and water containing nitrate entered the space in the tank. The other pipe (upper) was used for entering the grape extract into the tank. It should be mentioned that these two pipes were located separately for preventing the holes from clogging.

Since the effluent from this pilot must be disinfected and disinfection in our country is mostly done with

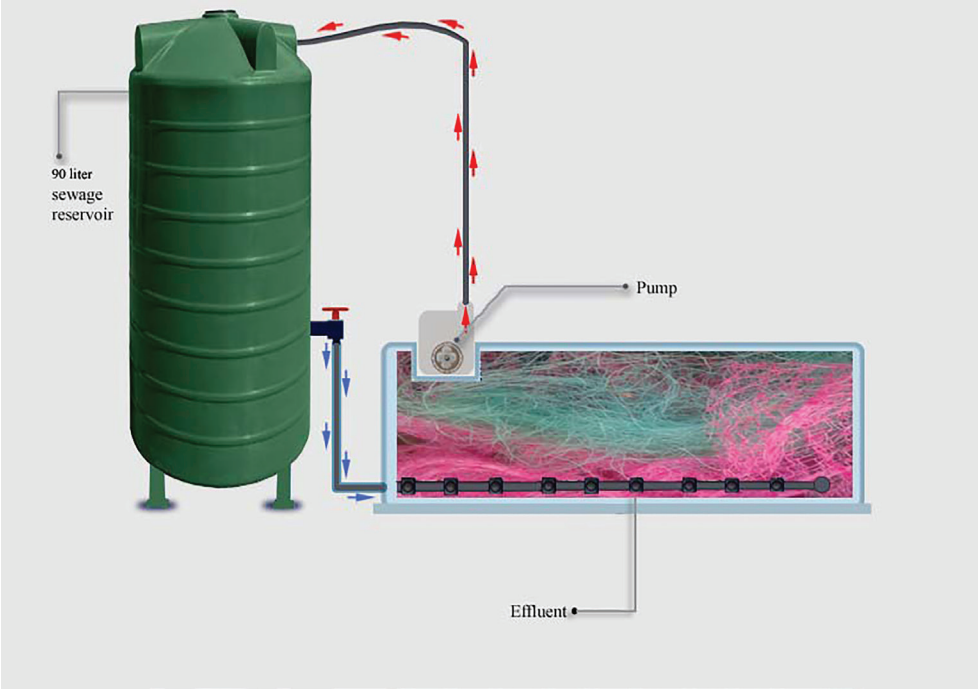

Figure 1: This figure is the schematic picture of pilot number 1

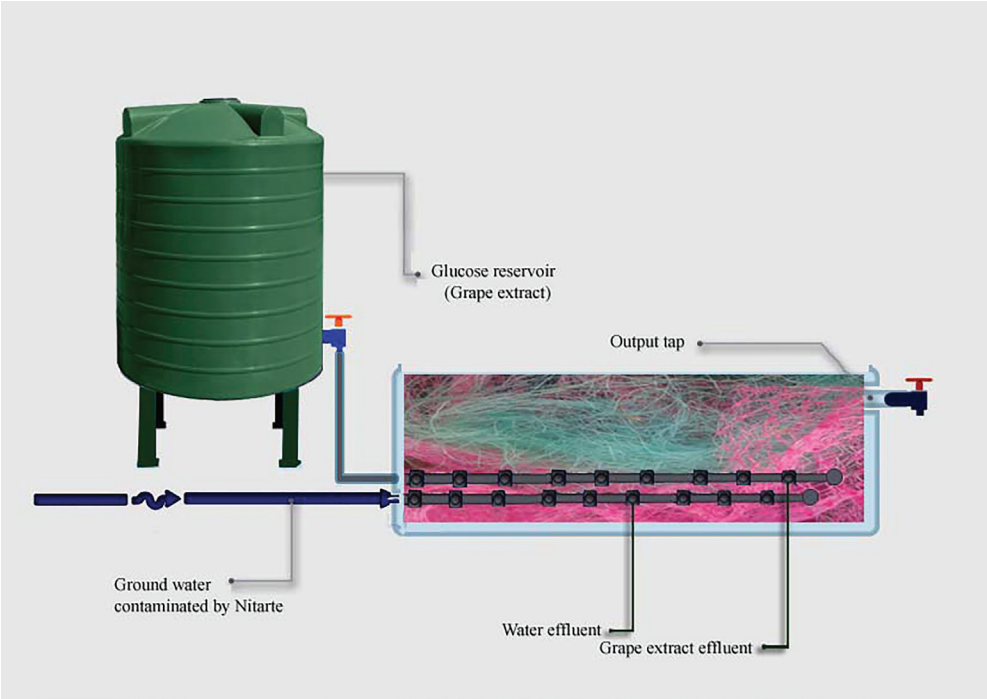

Figure 2: This figure is the schematic picture of pilot number 2

chlorine compounds, existence of organic matters in the effluent increases the probability of carcinogenic compounds by the combination of this material with chlorine. Therefore, determining the exact amount of the organic matter used is necessary. The results of the tests indicated that glucose comprised of 87% of the grape extract. Thus, the following equation (Eq1) was used for obtaining the suitable amount of grape extract:

Eq1. $C_6H_{12}O_6 + 4NO_3 \rightarrow 6CO_2 + 6H_2 + 2N_2$

180 60

X 65

X=195

195 mg/Lit ≈ 0.2 gr / Lit → 0.2×90=18 gr

18 gr 20 min → 1 gr 1 min

1.5 Kg 24 hour 1.5 (Kg in 24 hour)

The used substrate should be suitable with the amount of nitrate in the water so that there is no excessive BOD in the effluent water. In this study, the evaluation index was the amount of nitrate before and after the experiment. Hence, changes in the amounts of the effluent nitrate and nitrite with change in the retention time were examined in every sample. Moreover, the weight of the used media in this pilot was 500 grams. In other words, 5.5 grams of the plastic media were used for every liter of the tank volume.

The media used and the pilot made for removing nitrate in this study are shown in Figures 3 and 4.

Sampling was done from pilot number 2 reactor output daily and the intended parameters (amounts of nitrate and nitrite) were calculated using laboratory methods. At least, 13 samples were examined in every retention time and each test was repeated for 2 or 3 times (Table 1).

In this study, nitrate value was determined using spectrophotometer Dr-5000 (U.S. made HACH model) with 1-centimeter-diameter Quartz cell at 220 nm wave length. Besides, water samples were examined using the method mentioned in the Standard Method section.[1]

Figure 3: This figure shows the used plastic media

Figure 4: This figure shows the used pilot

Table 1: The number of samples in each retention time

Number	pH	Temperature (°C)	Retention time (hour)	Number of samples
1	6.9	20	0.5	15
2	7	19	1	13
3	7.05	19	1.5	16
4	7	20.5	2	20
5	7.05	20.5	2.5	20

To prepare the calibration curve, nitrate solutions with concentrations 5, 10, 15, 20 and 25 mg/lit were made using dilution of stock solution with 100 mg/lit concentration.

1 mL HCl (1 N) was added to 50 mL of the above solutions and the adsorption rate of the solutions was read using UV/vis spectrophotometer DR-5000at 220 nm wavelength. Then, using excel software, we shaped the standard curve. Standard curve of nitrate solution is shown in Figure 5.

The obtained results at different retention times are presented in Table 2.

After all, the data were entered into the SPSS statistical software, version 19 and analyzed using descriptive statistics, one-way repeated measures ANOVA, and Bonferroni tests.9

Results and Discussion

The maximum and minimum amounts of remained nitrate were related to 30 min and 2 hours, respectively (Table 2). The results of one-way repeated measures ANOVA showed a significant difference between the mean amounts of nitrate at different retention times ($P=0.001$). The result of Bonferroni test (for comparison of means between two groups) also revealed a significant difference between the mean amounts of nitrate in different groups at different retention times ($P<0.05$).

The changes in output nitrate concentration at different times with the initial concentration of 62–63 mg/L are presented in Figure 6.

The efficiency of nitrate removal at different retention times is shown in Figure 7.

Changes in output nitrite concentration at different retention times with the initial concentration of 0.001 mg/L NO_3^- are illustrated in Figure 8.

Biological treatment of drinking water has been recently considered by engineers as a new and growing bioenvironmental biotechnology to remove impurities of water, such as nitrate, nitrite, iron, manganese, and biodegradable organic matters.[26] The present study aimed to assess the possibility of nitrate removal from water with Pseudomonas bacteria using biological methods, filamentous media, and grape extract.

The results of the study by Hamedani et al. showed

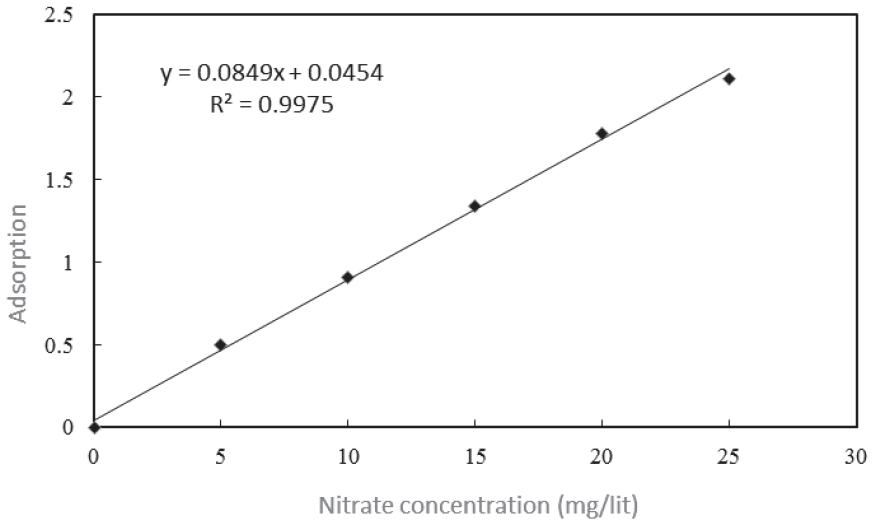

Figure 5: Nitrate standard curve.

Table 2: The mean amounts of remained nitrate at different retention times

Retention Time (hour)	Number of samples	Mean±SD (mg/lit NO3-)	Range (mg/lit NO3-)	P value*
Input	-	63±0.08	62–64	0.001
0.5	15	62±0.78	61–63	
1	13	32±0.78	28–37	
1.5	16	28±2.95	24–34	
2	20	20±2.17	17–24	
2.5	20	20±1.66	18–23	

*One-way repeated measures ANOVA; Temperature=20±1 °C, pH=7±0.1

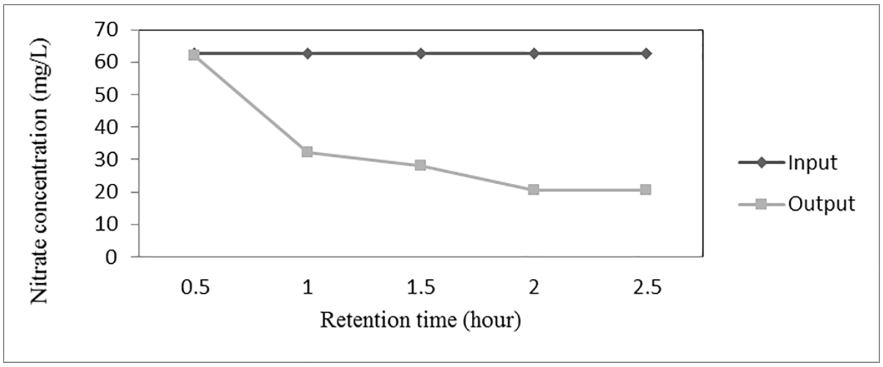

Figure 6: Comparison of output nitrate concentration at different retention times with the initial concentration of 62–63 mg/lit NO_3^-

that Pseudomonas bacteria could not use methanol as the carbon source.[27] On the other hand, Kirstein et al.[28] and Burcherding et al.[29] conducted a study on Pseudomonas bacteria and reported that these bacteria had high enzymatic activity for reduction of nitrate. This kind of bacteria can also be used in micro titer or biological sensors technology. These results were consistent with those of the present study.

Seid-Mohammadi and his collogues demonstrated that hydraulic retention time is very effective on nitrate removal and the optimum retention time on their study was 2.4 hours.[30] In Ha and Ong's study, 75% of total nitrogen was removed at an HRT of 3 hours and the result of this study was similar to ours.[31] Also, the study of Wang his collogues showed that optimum HRT was 8 hours and at this time about 99% of NO_3^- - N was removed.[32] In the same line, Kessru and colleagues showed that ethanol could be a proper carbon source for biological nitrate removal using Pseudomonas bacteria. Additionally, they recommended that carbon/nitrogen ratio had to be above 3.[33] Overall, they indicated that biological nitrate removal using Pseudomonas bacteria with succinate as the carbon source could reduce very high concentrations of nitrate (about 800 mg/L nitrate-nitrogen). However, it was not able to convey these values to effluent standards. Accordingly, the

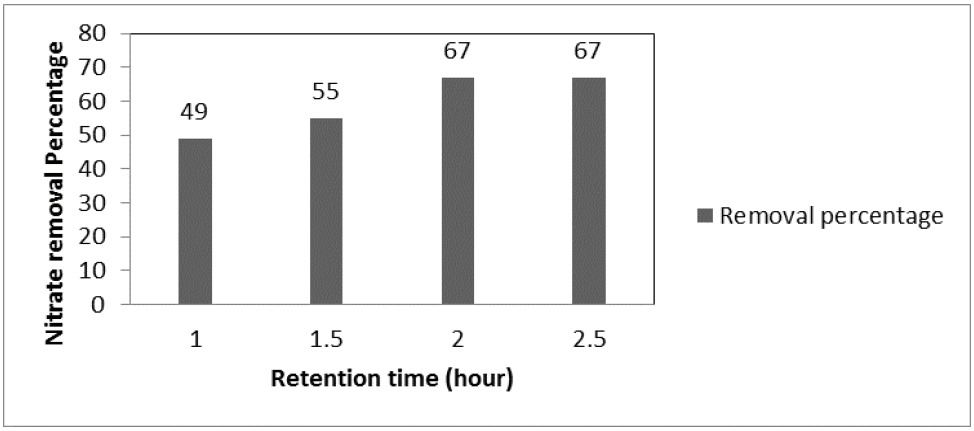

Figure 7: The effect of retention time on nitrate removal efficiency

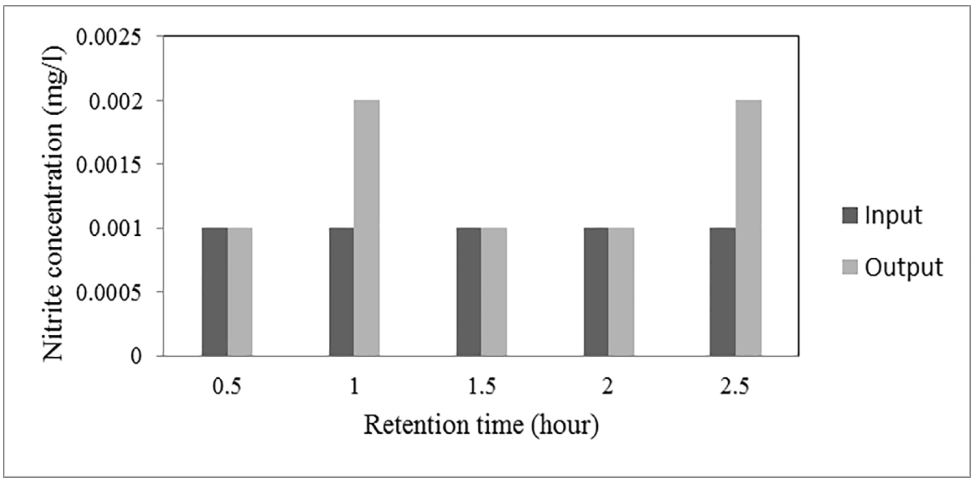

Figure 8: Output nitrite concentration at different retention times with the initial concentration of 62–63 mg/lit NO_3^-

concentration of the produced nitrite was more than the recommended standards.[34] Similar results were also obtained by Foglar and colleagues in a study on high concentrations of nitrate using bacterial consortium.[35] In that study, nitrate prime growth affected the maximum growth of the responsible microorganisms for nitrate removal. Besides, the effect of the initial concentration of nitrate could be modeled by Monod equation.[35] Moreover, the proper pH for nitrate removal by Pseudomonas bacteria was 7.2 where the process showed maximum efficiency. In the same line, Ovez and colleagues concluded that pH between 6.5 and 8 could not have negative effects on nitrate removal process. In other words, nitrate removal was well done in this pH range.[34]

For a proper nitrate removal process with the initial nitrate-nitrogen concentration of 200 mg/L and succinate as the carbon source, at least 3×10^8 CFU/ml Pseudomonas bacteria is required. Although nitrate removal also occurs in lower concentrations of bacteria, the reactor's setting time becomes longer. In field conditions, low concentrations of bacteria lead to an increase in the volume of the reactor and cost of nitrate removal.[36]

Conclusion

This experimental study evaluated the impact of heterotrophic bacteria separated from Shiraz municipal wastewater treatment plant on removal of nitrate and nitrite from the underground water at pilot scale using grape extract as the carbon source and filamentous media at the constant pH (7±1) and temperature (20±1 °C). The following results were obtained:

Nitrate removal rates were 49%, 55%, 67%, and 67% at 1, 1.5, 2, and 2.5-hour retention times, respectively. Thus, the best retention time was 2 hours with 67% removal rate. The Nitrite concentration was approximately 0.001 mg/lit NO_3^- at all retention times, which was lower than the standard limit. The use of grape extract as the carbon source and proper growth of Pseudomonas bacteria in filamentous media significantly increased the removal rate of nitrate ($P<0.05$).

Overall, the results demonstrated that biological methods could be a suitable alternative for physicochemical methods for removing nitrate from drinking water resources. Moreover, by choosing proper media for growing nitrate and nitrite reducer

bacteria and using available, cheap, and natural carbon sources, such methods can be utilized widely.

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