

## Effect of saline drainage water on performance of denitrification bioreactors

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### ABSTRACT

Excessive use of nitrate fertilizers in agriculture causes harm to humans and the environment. The most suitable nitrate removal process is heterotrophic biological denitrification. The purpose of the present study was to evaluate the performance of three shapes of denitrification bioreactors: triangular, rectangular and semicircular. The main element that was used to remove nitrate was beech woodchips. The concentration of inlet nitrate was 75 mg/l and the salinity of the solution was 1 ds/m and 5 ds/m, for a period of six months. The results showed that the efficiency of the triangular bioreactor with a salinity level of 1 ds/m was 90%, which is more efficient than the rectangular and semicircular bioreactors with performances of 55.8% and 53.8%, respectively. The results also indicated that at a salinity level of 5 ds/m, the semicircular bioreactor with a performance of 50.8% inlet nitrate removal was the best of the three shapes of bioreactors tested, the efficiencies of the triangular and rectangular bioreactors were 49.9% and 48.6% respectively. Also, it was observed that at the salinity level of 1 ds/m, a high hydraulic retention time had a high positive effect on denitrification, on the other hand at the salinity level of 5 ds/m, there was better performance of denitrification if the hydraulic retention time was lower.

**Key words** | beech woodchips, dimensions of bioreactors, hydraulic retention time

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### HIGHLIGHTS

- The shape of denitrification beds can affect the performance of the bioreactors.
- The performance of three shapes of denitrification bioreactors was evaluated.
- The performance of denitrification beds using different concentrations of saline drainage water was evaluated.
- Saline drainage water decreased the efficiency of denitrification beds.
- The highest nitrate removal of saline drainage water occurred in the triangular shaped bed.

### INTRODUCTION

It is estimated that the world population will increase to more than 9.8 billion by 2050 (United Nations 2017). Therefore, to meet the world's growing food demands, providing a timely and sufficient supply of irrigation water is essential for permanent production of agricultural and food products (Das *et al.* 2015; Masunga *et al.* 2016). One of the challenges in the long term is the issue of water quality decrease due to improper use

of fertilizers and pesticides available in the runoff from agricultural land and plantations. In agricultural production, most of the nitrogen needs of plants are provided through fertilizers (Frink *et al.* 1999). However, the impact on water quality caused by water draining from agricultural land is of great concern (Blann *et al.* 2009; Christianson *et al.* 2012a; Addy *et al.* 2016). Many physicochemical and biological approaches have

been used to remove nitrate from water (Hakeem *et al.* 2017). As a low cost and useful technology, denitrification woodchip bioreactors are increasingly being used to remove nitrate in groundwater and surface water (Jaynes *et al.* 2008; Christianson *et al.* 2012a). Denitrification in bioreactors is influenced by temperature, which promotes the activity of anaerobic bacteria (Bock *et al.* 2015). However, most studies on woodchip bioreactors have been performed at relatively high temperatures and are not relevant in the regions where the temperature may be low (below 10 °C) for extended periods. Earlier studies indicate that the efficiency of bioreactors in colder climates is not completely understood. Research conducted by Hassanpour *et al.* (2017) showed 100% reduction in concentration of NO<sub>3</sub>-N when the temperature was above 16 °C. In contrast the reduction ratio for the same solution was below 30% when the temperature was below 5 °C. Feyereisen *et al.* (2016) also found that NO<sub>3</sub>-N removal rates (RRs) are generally 4–5 fold lower under cool (1.5 °C) conditions compared with those under warm (15.5 °C) conditions.

Woodchips are the most widely used materials because of their low cost, availability, hydrological properties, and ability to provide a long lasting carbon source. Biochar is a good sorbent of organic pollutants that occur in subsurface drainage tile lines (Ashoori *et al.* 2019; Hassanpour *et al.* 2019). When NO<sub>3</sub>-N is ingested by humans, it can be converted to nitrite (NO<sub>2</sub>-N), which can combine with amines to form carcinogenic nitrosamines. Ingestion at concentrations significantly above the drinking water standard at 10 mg l<sup>-1</sup>, set by the United States Environmental Protection Agency, can lead to several types of cancer (DeSimone *et al.* 2009; McCasland *et al.* 2012; U.S. EPA NPDWR 2017). Woodchip bioreactors have a wide range of reported NO<sub>3</sub>-N removal rates, but a typical range is 13% to 100%, depending on conditions and location (Greenan *et al.* 2009; Christianson *et al.* 2012b, 2018; Hassanpour *et al.* 2017). To maximize NO<sub>3</sub>-N removal, bioreactors must be engineered to optimize denitrification by considering landscape placement, shape, biomass source, carbon source, drainage treated, peak flow conditions, and hydraulic residence time (HRT). Due to a significant possibility of soil, surface and ground water pollution, and risks of harm to human health; efforts to develop sustainable and cost-effective strategies for produced water treatment and reuse have been intensifying (Ahmadun *et al.* 2009; Riley *et al.* 2018; Sardari *et al.* 2018). Understanding the temporal and spatial variability

of produced water composition plays an important role in the selection of the optimum treatment process and its design (Ozgun *et al.* 2013; Qetjen *et al.* 2018). A major hindrance to refining wastewater and using the refined water is the high salinity of refined water. The total dissolved solids (TDS) can include sodium, potassium, bromide, calcium, fluoride, nitrate, phosphate, chloride, sulfate and magnesium (Cakmakci *et al.* 2008; Chittick & Srebotnjak 2017; Freedman *et al.* 2017). High salinity is likely to affect physicochemical parameters and biological activities, as well as system performance, which causes complex challenges due to their dynamic interactions (Lay *et al.* 2010; Qui & Ting 2013). Elevated salinity has been reported to have inhibitory or toxic effects on the microbial activity and population in bioreactors, and it reduces organic carbon removal efficiency (Wang *et al.* 2014; Frank *et al.* 2017). Anaerobic treatment in bioreactors has increased in many applications in municipal wastewater treatment (MWT) in the last decade, while presenting an advanced technology for environmental protection and resource preservation (Pretel *et al.* 2015; Stazi & Tomei 2018). The combination of a membrane and an anaerobic bioreactor (Anaerobic membrane bioreactor (AnMBR)) paved the way for a sustainable wastewater treatment process with complete biomass retention, and with additional advantages such as less sludge production, high quality effluent and net energy production as the organic matter is converted into high-value products (volatile fatty acids (VFAs)) and energy in the form of gas (Ozgun *et al.* 2013; Gouveia *et al.* 2015). There are different construction designs of bioreactors, but generally these are structures filled with solid organic carbon material (e.g. woodchips) through which nutrient rich water passes (Addy *et al.* 2016).

Therefore, we investigated the effect of the shape of bioreactors and temperature on the nitrate removal rate, at two salinity levels. Our findings help in understanding the importance of bioreactor shape in the design of bioreactors based on water quality and the weather conditions in the area.

## MATERIAL AND METHODS

### Study site

In order to evaluate the performance of different denitrification bioreactors at different salinity levels, an experiment

was conducted in 2018–2019 on Aburaihan Agricultural Campus, University of Tehran, Tehran, Iran. Aburaihan Agricultural Campus Research Farm is located 25 km south-east of Tehran, 33 degrees north latitude, 51 degrees east longitude, with an altitude of 1,180 metres above sea level. The test site is considered to be a warm and semi-arid region in terms of climate. The average annual temperature is approximately 16.8 °C. A schematic of the experiment is provided (Figure 1).

### Design and construction of bioreactors

In this study, three denitrification bioreactors of triangular, rectangular and semicircular shape were investigated. In order to investigate the effect of the shape of the bioreactors on nitrate removal, the volume of all three bioreactors was equal to 0.7 m<sup>3</sup> and the depth of all of them was equal to 0.6 m, so the lengths of these bioreactors were different. The specifications of the bioreactors are presented in Table 1. First, the bioreactors were designed by Tri-Di max software. In the next stage, 0.6 m was excavated in the field and these bioreactors were created in the ground using brick and cement. In order to pass the output current, a one-inch polyethylene pipe was installed at the end of each bioreactor at a height of 50 cm from the floor, then the bioreactors were completely insulated using a waterproof membrane.

In this study, sawdust from beech wood was used as a natural carbon material. Therefore, after insulation of the floor and

the walls of each bioreactor, they were completely filled with beech woodchips and then covered with plastic and soil was poured on them to keep the plastic in place. The inlet drain was supplied from a 1,000 litre tank. A 16 mm tube was placed under the plastic cover at the front of each bioreactor and the drain water entered the bioreactors by means of gravity and exited from the opposite end of the bioreactors.

### Initial water supply

The study was performed over two time periods of 90 days with two salinity levels of 1 ds/m and 5 ds/m, but the amount of nitrate input to the bioreactors in both time periods was the same and equal to 75 mg/l. The water used in this study was supplied from well water on the Aburaihan campus, the salinity of this water was 1 ds/m and its nitrate content was 35 mg/l. To increase the nitrate concentration of the water to 75 mg/l, we added 65.8 mg of potassium nitrate per litre to the 1,000 litre tank. Also, to increase the salinity concentration to 5 ds/m, we added 2.08 mg of NaCl per litre to the 1,000 litre tank.

### Sampling

Sampling was carried out over 180 days from October 25th, 2018 until April 23rd, 2019, between 12 noon and 2 pm, every three days, once from the water entering each bioreactor and once from the water leaving each bioreactor. In the first period the concentration of nitrate entering the



**Figure 1** | Flowchart: In the first stage, bioreactors were designed by Tri-Di max software and the necessary excavation was performed. In the second stage, the bioreactors were made of brick and cement, insulated with a waterproof membrane, and filled with beech woodchips. In the last step, the bioreactors were covered with plastic and by adding the necessary materials to the tank, the experiment was started, sampling was performed and the data were analyzed.

**Table 1** | Details of denitrification bioreactors (triangle, rectangle and semicircle)

	Length (m)	Width (m)	Depth (m)	Angle (Degree)	Radius (m)
Triangle	3.5	0.69	0.6	60	–
Rectangle	1.5	0.78	0.6	–	–
Semicircle	1.2	–	0.6	–	0.6

bioreactors was 75 mg/l and the salinity of the water was 1 ds/m. In the second period the concentration of nitrate entering the bioreactors was 75 mg/l and the salinity of the water was 5 ds/m. During these 180 days, water flowed continuously through the bioreactors and the experiment was performed under completely saturated conditions. During these 180 days, the 1,000 litre tank was refilled twice every 24 hours and the above mentioned ingredients were added.

Sampling was performed using 80 ml plastic bottles. Samples were stored at minus 4 °C until analysis.

### Data analysis

Samples were used in the laboratory to measure nitrate, ammonium, potassium, salinity, and pH. The concentration of outlet nitrate was measured using a spectrophotometer (DR3900 manufactured by Hach Company USA) at 410 wavelengths, using the Nessler method (Golterman & De Oude 1991), and potassium was measured by a flame photometer. The pH of the tested samples was read using a pH-meter (model 712 made by Metrohm, Switzerland). The ammonium content of the samples was measured using a micro-Kjeldahl apparatus. The EC measurement of the specimens was carried out using the Lovibond SD 320 Con EC-meter. Nitrate removal efficiency was calculated using the following equation:  $R\% = (C_i - C_{ef}) / C_i \times 100$ ; the inlet ( $C_i$ ) and outlet ( $C_{ef}$ ) nitrate concentrations are both in mg per litre (Ghane et al. 2015).

## RESULTS

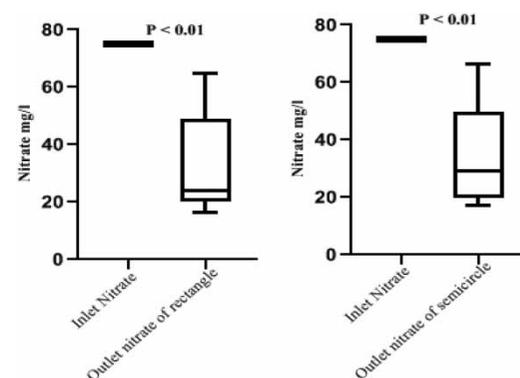
### Performance of rectangular and semicircular bioreactors

The performance of the rectangular and semicircular bioreactors is very close because their dimensions are almost

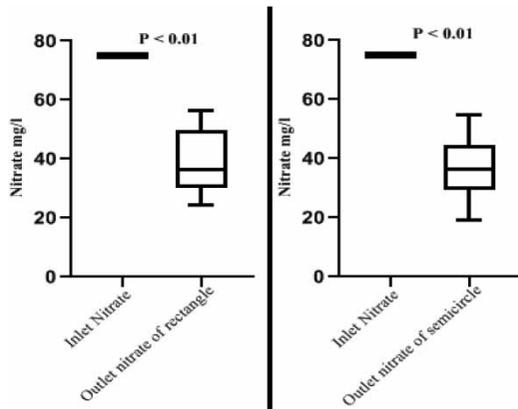
the same, so we analyzed the performance of these two bioreactors together. The results showed that in the first period of the experiment with a salinity of 1 ds/m and a nitrate concentration of 75 mg/l, the concentration of nitrate output from the rectangular and semicircular bioreactors had a significant difference ( $p \leq 1\%$ ) compared to the amount of nitrate entering these bioreactors (Figure 2). The average nitrate output in this period for the rectangular and semicircular bioreactors was 33.57 mg/l and 34.62 mg/l, respectively. In addition, it was observed that in these bioreactors denitrification has a direct correlation with temperature, with the rise and fall of temperature, the performance of both bioreactors is affected. The passage of time increases the denitrification rate if the temperature is high.

In the second period, which started with a salinity of 5 ds/m immediately after the first period, the results showed that the average nitrate removal for the rectangular and semicircular bioreactors was 38.49 mg/l and 36.86 mg/l, respectively, and there was a significant difference ( $p \leq 1\%$ ) compared to the nitrate input to these bioreactors (Figure 3). The effect of salinity on the performance of the bioreactors is clear. But after heterotrophic anaerobic microorganisms adapt to salinity, their function returns to normal. A temperature higher than 20 °C disrupted the function of the microorganisms for several days, but they adapted and returned to their original state after a few days.

The amount of ammonium input to these bioreactors was almost zero, but the ammonium output from the rectangular and semicircular bioreactors in the first period ranged from



**Figure 2** | Box plot diagrams for the first period for the semicircular and rectangular bioreactors.



**Figure 3** | Box plot diagrams for the second period for the semicircular and rectangular bioreactors.

1.84–2.34 mg/l and 1.35–1.63 mg/l, respectively, and in the second period ranged from 1.98–2.41 mg/l and 1.91–2.34 mg/l respectively. There was a significant difference ( $p \leq 1\%$ ) compared to the input ammonium. Changes in ammonium did not follow a specific order and increased over time.

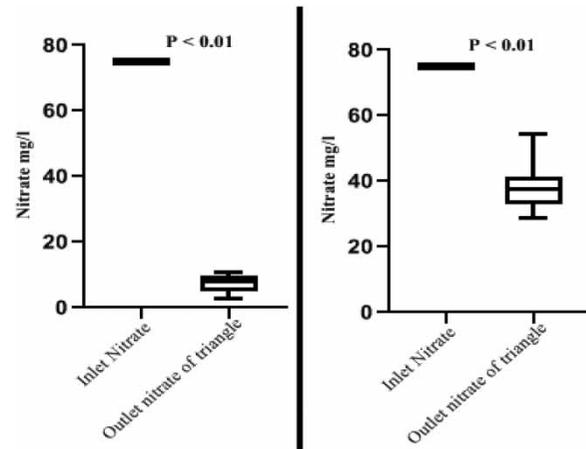
The pH of the input to these bioreactors was in the range of 7.55–7.66 in both periods, while the pH of the outputs of the rectangular and semicircular bioreactors in the first period were in the range of 7.75–7.99 and 7.79–7.98, respectively, and in the second period were in the range of 7.64–7.99 and 7.61–7.7, respectively. There was a significant difference ( $p \leq 1\%$ ) compared to the input pH.

The amount of potassium entering the bioreactors in both periods was 133.5 mg/l, while in the first period, the amount of potassium leaving the rectangular and semicircular bioreactors ranged from 17.84–82.37 mg/l and 28.31–84.11 mg/l, respectively, and in the second period, it ranged from 54.46–150.38 mg/l and 42.26–139.92 mg/l respectively. There was a significant difference ( $p \leq 5\%$ ) compared to the input potassium in both periods.

No significant changes were observed in the EC output of the bioreactors.

### Triangular bioreactor function

The results showed that the performance of the triangular bioreactor in the first 90 days with salinity of 1 ds/m and nitrate concentration of 75 mg/l had a significant difference ( $p \leq 1\%$ ) compared to the amount of nitrate output from this



**Figure 4** | Box plot diagrams for the first and the second periods for the triangular bioreactor, respectively.

bioreactor (Figure 4). The average nitrate output in this bioreactor was 7.44 mg/l, in addition, the effects of temperature on this bioreactor in the first period were very low. The passage of time increased the rate of nitrate removal.

With the start of the second period and increasing the salinity to 5 ds/m, nitrate removal in the triangular bioreactor was strongly affected negatively. The average nitrate output in this period was 37.86 mg/l and there was a significant difference ( $p \leq 1\%$ ) compared to the input nitrate (Figure 4). At the beginning of this period, the nitrate removal process decreased, but after a few days, the heterotrophic anaerobic microorganisms adapted to the new conditions and the amount of nitrate removal increased. An increase in temperature from 20°C also affected the function of the microorganisms for a few days, but after a short time the microorganisms returned to their original state. The effect of salinity on this bioreactor was greater than in the rectangular and semicircular bioreactors.

The amount of ammonium input to this bioreactor was almost zero, but the ammonium output from it, in the first period in the range of 1.77–2.42 mg/l, and in the second period in the range of 2.05–2.34 mg/l, had a significant difference ( $p \leq 1\%$ ) compared to the input ammonium.

The input pH of this bioreactor was in the range of 7.55–7.66 in both periods, while the output pH was 7.79–8.1 in the first period and 7.66–7.99 in the second period, and had a significant difference ( $p \leq 1\%$ ) compared to the input pH.

The amount of potassium input to this bioreactor in both periods was 133.5 mg/l, while in the first period the amount

of potassium leaving the bioreactor in the range of 33.54–91.09 mg/l, and in the second period in the range of 120.74–150.83 mg/l, was significantly different ( $p \leq 5\%$ ) compared to the input potassium.

No significant changes were observed in the EC output of the bioreactor.

## DISCUSSION

According to the results obtained in the first stage, it is inferred that denitrification is directly related to temperature. In the first stage, the performance of the triangular bioreactor is much higher than the other two bioreactors because the hydraulic retention time in this bioreactor is much longer than the other two bioreactors due to its dimensions. It is known that the untreated water remains longer in the woodchip bioreactor and more denitrification occurs because microorganisms have more time to interact with the untreated water. Therefore, the dimensions of bioreactors are very important in nitrate removal (Figure 5).

The effect of temperature on the triangular bioreactor is less than for the rectangular and semicircular bioreactors, which is also due to the long length and consequently high hydraulic retention time in this bioreactor. The length of bioreactors can be increased to prevent the negative effect of low temperature on the nitrate removal process.

Understanding the effect of hydraulic retention time (HRT) of a laboratory-scale controlled system is essential for optimizing the design of a woodchip bioreactor in a variety of conditions (Martin et al. 2019). The most important factors in nitrate removal in bioreactors are high hydraulic

retention time and carbon source (Rivas et al. 2020). Also Ghane et al. (2015) stated that if water remains in a bioreactor for a longer time, the denitrification process will be more efficient. In general, temperature has a significant effect on the speed of biochemical reactions, including the speed of harvesting and consumption of food and the growth rate of microorganisms, therefore one of the parameters limiting the performance and efficiency of the denitrification process is the reduction of temperature, especially in cold seasons (Nichols et al. 2007). The results of the studies of Carrera et al. (2003) showed that to compensate for the effect of low temperature in reducing the rate of denitrification reactions, the hydraulic retention time of the effluent in the bioreactor should be increased. Similar to these results Christianson et al. (2012a) also reported that temperature increase is one of the factors influencing the rate of nitrate removal and in general the rate of biological reactions increases with increasing temperature (Schipper et al. 2010).

With the start of the second period and increasing the salinity to 5 ds/m, the performance of the bioreactors changed compared to the previous stage. In this stage, it was observed that the performance of the triangular bioreactor, which was very high in the first stage, drastically decreased (Figure 6). One of the important results is that with increasing salinity, the effect of high hydraulic retention time is reversed because salinity has a negative effect on the function of microorganisms. As a result, if the water salinity increases, the long hydraulic retention time has a negative effect on nitrate removal. Of course, after a few days of increasing the salinity to 5 ds/m, the microorganisms' performance improves and they adapt to the new conditions,

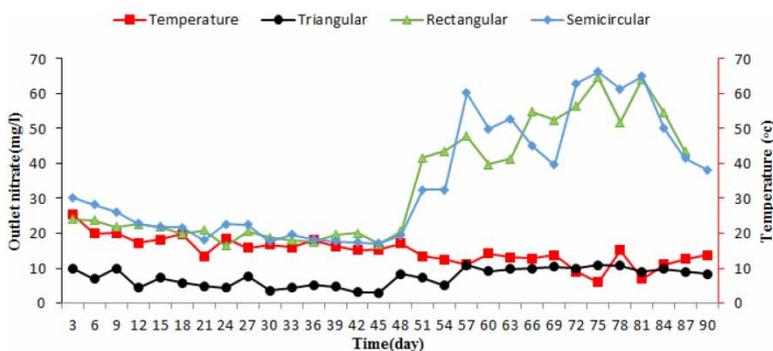
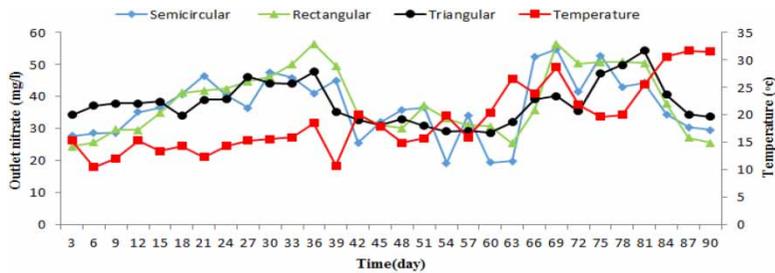


Figure 5 | Comparison of nitrate removal in the three types of bioreactor at a salinity of 1 ds/m.



**Figure 6** | Comparison of nitrate removal in the three types of bioreactor at a salinity of 5 ds/m.

but it takes a long time for them to perform as well as before. The performance of the semicircular bioreactor in this period is better than the triangular and rectangular bioreactors because its hydraulic retention time is low. These findings indicate that the design of bioreactors should be in accordance with the quality of input water as well as the climatic conditions of the target area.

These results are similar to the results of studies of [Chen \*et al.\* \(2016\)](#) which stated that high salinity is a key problem that negatively affects the biological treatment process of wastewater. The results of studies of [Gao \*et al.\* \(2020\)](#) showed that the microbial population decreases with increasing salinity concentration in nitrate removal bioreactors. [Wu \*et al.\* \(2008\)](#) evaluated the denitrification potential at salinity levels between zero ppt and 30 ppt in wastewater saturated wetlands. The results of their research show a decrease in denitrification potential at the highest salinity level. [Jafari \*et al.\* \(2015\)](#) investigated the performance of a rotary bioreactor to remove nitrate at several salinity levels (1 to 20 g/l sodium chloride salt). The researchers observed that at the beginning of the experiment, denitrification was a function of salinity, so that with increasing salinity, the nitrate removal efficiency decreased and after a few days of testing, it returned to stable conditions (100% nitrate reduction). The results of this study show that the denitrifiers in the bioreactor are resistant to salinity stress over an extended period of time and are able to repeat the metabolic conditions for the biological denitrification process. [Di Capua \*et al.\* \(2015\)](#) investigated the effect of sodium chloride salt concentration on heterotrophic and autotrophic denitrification simultaneously, based on sulfur and methanol as a source of organic carbon. The researchers observed complete nitrate removal of up to 3.5% sodium chloride salt and reported that increasing salinity to 5‰ and 4‰ reduced nitrate removal efficiency to 78% and 48%, respectively. [Mutlu \*et al.\*](#)

[\(2019\)](#) also reported that salinity negatively affects the physiology of anaerobic bacteria and reduces denitrification performance. The table below shows the nitrate removal efficiency in all three bioreactors ([Table 2](#)).

In both periods, in all three bioreactors, the process of ammonium changes did not follow a specific order but was almost constant. During the dissimilatory nitrate reduction to ammonium (DNRA) process, nitrate is converted to ammonium by anaerobic bacteria ([Van Rijn \*et al.\* 2006](#)). Similar to these results, in a field study by [Robertson \*et al.\* \(2000\)](#) a layer of wood as a bioreactor was placed under septic tanks, increasing ammonium nitrogen by 12%. Studies have shown that in an anaerobic environment with a high carbon to nitrate ratio, ammonium production may increase relative to denitrification ([Van Rijn \*et al.\* 2006](#)).

The increase in pH in both periods in the three bioreactors did not follow a specific order. This increase in pH in the bioreactors can be due to the effect of denitrification, because carbon dioxide and hydroxide are produced simultaneously with the denitrification process and can cause the pH to rise. Similar to these results, [Rivett \*et al.\* \(2008\)](#) reported that the denitrification process can lead to an increase in pH by producing hydroxide and carbon dioxide. However, the researchers added that if the production of hydroxide is greater than carbon dioxide, then an increase in pH occurs in the environment. [Hernandez & Guerrero \(2008\)](#), during their research on the use of pine bark and

**Table 2** | Nitrate removal efficiency in all three bioreactors

	semicircular	rectangular	triangular
Salinity of 1 ds/m	53.8%	55.8%	90%
Salinity of 5 ds/m	50.8%	48.6%	49.9%

coconut fiber as an organic substance to intensify the removal of iron pollution, especially nitrate from drainage water, stated that the rate of change in the acidity of the drainage water did not follow regular conditions.

The amount of potassium input to the bioreactors is high, but in the first period the output potassium in all three bioreactors is less than the input potassium. Some of the potassium is probably absorbed by the woodchips, and in the second period, because a long time has passed since the start of the experiment and the temperature was high and the hot water has softened the beech woodchips, more potassium is released.

## CONCLUSIONS

The results of this study show that if the temperature remains high during access to a carbon source and also with the passage of time, the activity of heterotrophic microorganisms increases and the denitrification rate rises. Denitrification has a direct correlation with temperature, but if the temperature rises, it has a negative effect on denitrification over a short period (20 days). Increasing hydraulic retention time is a good counter measure against the negative impact of temperature. In water without salinity, the longer the hydraulic retention time, the greater the denitrification, and in saline water, if the hydraulic retention time is shorter, the effect of salinity on denitrification is less. In water without salinity, the best yield was obtained for the triangular bioreactor with 90% nitrate removal efficiency, and in saline water, the best performance was for the semi-circular bioreactor with 50.8% nitrate removal efficiency.

## DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories ([https://drive.google.com/file/d/1fda1W-Q544aleIkRrFJQWpzjXj\\_3EgBFv/view?usp=sharing](https://drive.google.com/file/d/1fda1W-Q544aleIkRrFJQWpzjXj_3EgBFv/view?usp=sharing)).

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