

Investigation of the effects of hydrogenotrophic denitrification and anammox on the improvement of the quality of the drinking water supply system

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A drinking water supply system operates at Chyasal (in the Kathmandu Valley, Nepal) for purifying the groundwater that has high levels of ammonium nitrogen ($\text{NH}_4\text{-N}$). However, high $\text{NO}_3\text{-N}$ concentrations were seen in the water after treatment. To further improve the quality of the drinking water, two types of attached growth reactors were developed for the purification system: (i) a hydrogenotrophic denitrification (HD reactor) and (ii) a concurrent reactor with anammox and hydrogenotrophic denitrification (AnHD reactor). For the HD reactor fed by water containing $\text{NO}_3\text{-N}$, the denitrification efficiency was high (95–98%) for all $\text{NO}_3\text{-N}$ feed rates (20–40 mg/L). The nitrite-nitrogen ($\text{NO}_2\text{-N}$) and nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations in the effluent were ~ 0.5 mg/L. On the other hand, the AnHD reactor fed with water containing $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ was operated under varying flow rates of H_2 (30–70 mL/min) and intermittent supply periods (1–2 h). The efficiency of the anammox process was found to increase with decreasing H_2 flow rates or with increasing intermittency of the H_2 supply, while the efficiency of denitrification decreased under these conditions. For the optimal condition of 1.5 h intermittent H_2 supply, the anammox and denitrification efficiencies of the AnHD reactor reached 80% and 42%, respectively, while the concentrations of both $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ in the effluent were < 1.0 mg/L, and no $\text{NO}_3\text{-N}$ was detected. From the experimental results, it is clear that both HD and AnHD reactors can function as efficient and critical units of the water purification system.

Keywords: Hydrogenotrophic denitrification, anammox, $\text{NH}_4\text{-N}$ contamination, drinking water, intermittent H_2 supply.

Introduction

Groundwater is commonly used as potable water in many areas, such as Chyasal in Nepal^[1,2] and Hanoi in Vietnam.^[3,4] However, these groundwater sources contain contaminants such as ammonium-nitrogen ($\text{NH}_4\text{-N}$) in concentrations higher than that limits prescribed by the World Health Organization (WHO). Even though the consumption of low levels of $\text{NH}_4\text{-N}$ does not have any negative effects on human health, high concentrations can make the water unsuitable for consumption due to bad taste and smell. Moreover, its derivatives such as nitrite-nitrogen ($\text{NO}_2\text{-N}$) and nitrate-nitrogen ($\text{NO}_3\text{-N}$) are believed to be

responsible for the occurrence of ‘blue baby syndrome’ and stomach cancer in infants and adults, respectively.^[5,6]

Recent research works^[7–9] propose the use of biological processes such as anaerobic ammonium oxidation (anammox) and nitrification for removing $\text{NH}_4\text{-N}$ from water and groundwater. In the anammox process, $\text{NH}_4\text{-N}$ is removed as N_2 in the presence of $\text{NO}_2\text{-N}$, as shown in Eq. 1.^[10] However, some amount of $\text{NO}_3\text{-N}$ is present in the treated water, as a by-product of this process. Because the anammox system was developed for treating $\text{NH}_4\text{-N}$ rich wastewater, the remnant amounts of $\text{NO}_3\text{-N}$ in the treated water are within the acceptable limit prescribed by the United States Environmental Protection Agency (US EPA) for effluent wastewater. The anammox process is an ideal candidate for purification of the drinking water due to advantages like no external carbon addition, low sludge waste production and low energy consumption. However, to develop the anammox system for purification of drinking water, the $\text{NO}_3\text{-N}$ produced should be removed so as to maintain the limit lower than the prescribed limits.

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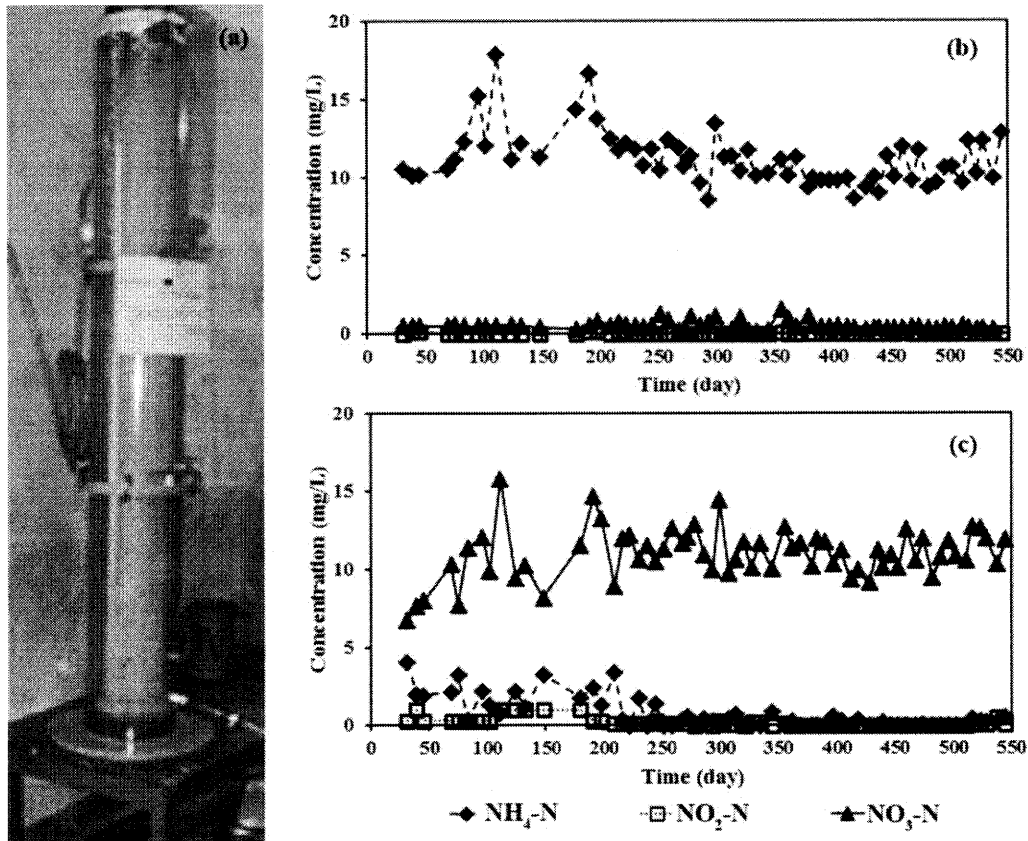
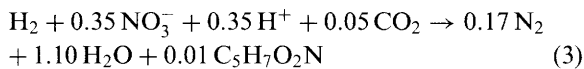
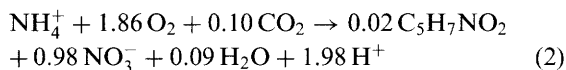
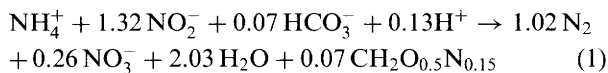


Fig. 1. (a) Pilot-scale nitrification reactor ($\phi 25 \times 160$ height cm) at Chyasal (Kathmandu Valley, Nepal) and nitrogen concentrations in (b) raw groundwater and (c) treated water (color figure available online).

On the other hand, the nitrification process (in the presence of oxygen) removes NH₄-N by converting it to NO₃-N, as shown in Eq. 2.^[11] The nitrification system has been implemented for cleaning water supplies in several locations;^[3,12] however, high levels of NO₃-N (above the limits prescribed by the WHO) were reported.^[13] Thus, an additional unit is required to remove the NO₃-N present in the effluent from both the nitrification and anammox processes.



Recently, hydrogenotrophic denitrification has become a popular process for the removal of NO₃-N from drinking water and groundwater.^[14-16] The significant advantage

of this process is the absence of any residual organic carbon, which tends to degrade the quality of treated water by increasing the biochemical oxygen demand (BOD) value. In the hydrogenotrophic denitrification process, NO₃-N is converted to N₂ in the presence of H₂ (Eq. 3^[17]).

However, the design and operation of such systems as proposed in previous research works^[14-19] are not suitable for implementation in remote areas (i.e., Chyasal). The major hindrance to the implementation is the high energy demands for maintaining a saturated dissolved hydrogen level of 1.6 mg/L during operation,^[18,19] as well as the limited electricity supply in these areas.

The aim of this research is to investigate the effectiveness of two efficient, economic and low-energy reactors in the removal of nitrogen contaminants from drinking water. One reactor was operated to study the hydrogenotrophic denitrification process; this reactor was fed with water containing varying NO₃-N contents. The second reactor was operated to study the concurrent effects of anammox and hydrogenotrophic denitrification processes; this reactor was fed by water containing NH₄-N and NO₂-N contaminants, and was operated under varying H₂ flow rates and for

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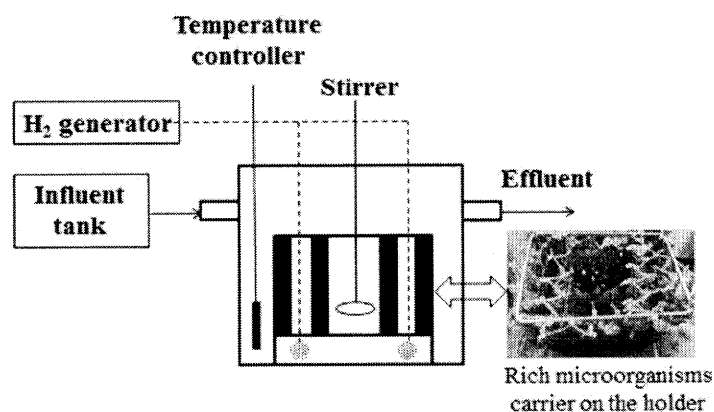


Fig. 2. Schematic diagram of the attached growth reactors; HD reactor fed by NO₃-N containing water and AnHD reactor fed by water containing NH₄-N and NO₂-N (color figure available online).

intermittent supply periods. The outcomes of this research work will provide suggestions for modification of the purification system to improve the quality of drinking water in this locality.

Materials and methods

Study area

Chyasal is a local area in the Lalitpur sub-metropolitan city of Kathmandu Valley, Nepal. This area is currently facing

serious problems arising from the high levels of NH₄-N contamination present in the groundwater. The concentration of NH₄-N ranges from 10–20 mg/L, which is higher than the acceptable limit of 1.5 mg/L.^[6] A purification system including a NH₄-N removal unit has been set up to provide safe drinking water for the residents.^[12] The NH₄-N removal unit (labeled as the pilot-scale nitrification reactor in Fig. 1a) and its recent performance parameters are presented in Figures 1b–1c. Due to the nitrification process, the NH₄-N and NO₂-N concentrations in the treated water were relatively low (~1 mg/L), and the NO₃-N levels were high (~13 mg/L) in comparison with the standard limits.^[6]

Table 1. Operating conditions for the experiments.

Reactor	Experiment	Influent conc. (mg/L)			H ₂ flow rate (mL/min)	H ₂ supply	Experimental days
		NH ₄ -N	NO ₂ -N	NO ₃ -N			
Hydrogenotrophic denitrification (HD) reactor	i	—	—	20	70	Continuous	30
		—	—	30	70	Continuous	30
		—	—	40	70	Continuous	30
Anammox and hydrogenotrophic denitrification (AnHD) reactor	i	10	10	—	70	Continuous	30
		10	10	—	50	Continuous	30
		10	10	—	30	Continuous	30
	ii	10	10	—	70	1.0 h	30
		10	10	—	70	intermittent*	30
		10	10	—	70	1.5 h intermittent* 2.0 h intermittent*	30

*Intermittent H₂ supply during all 30 days of experimental days.

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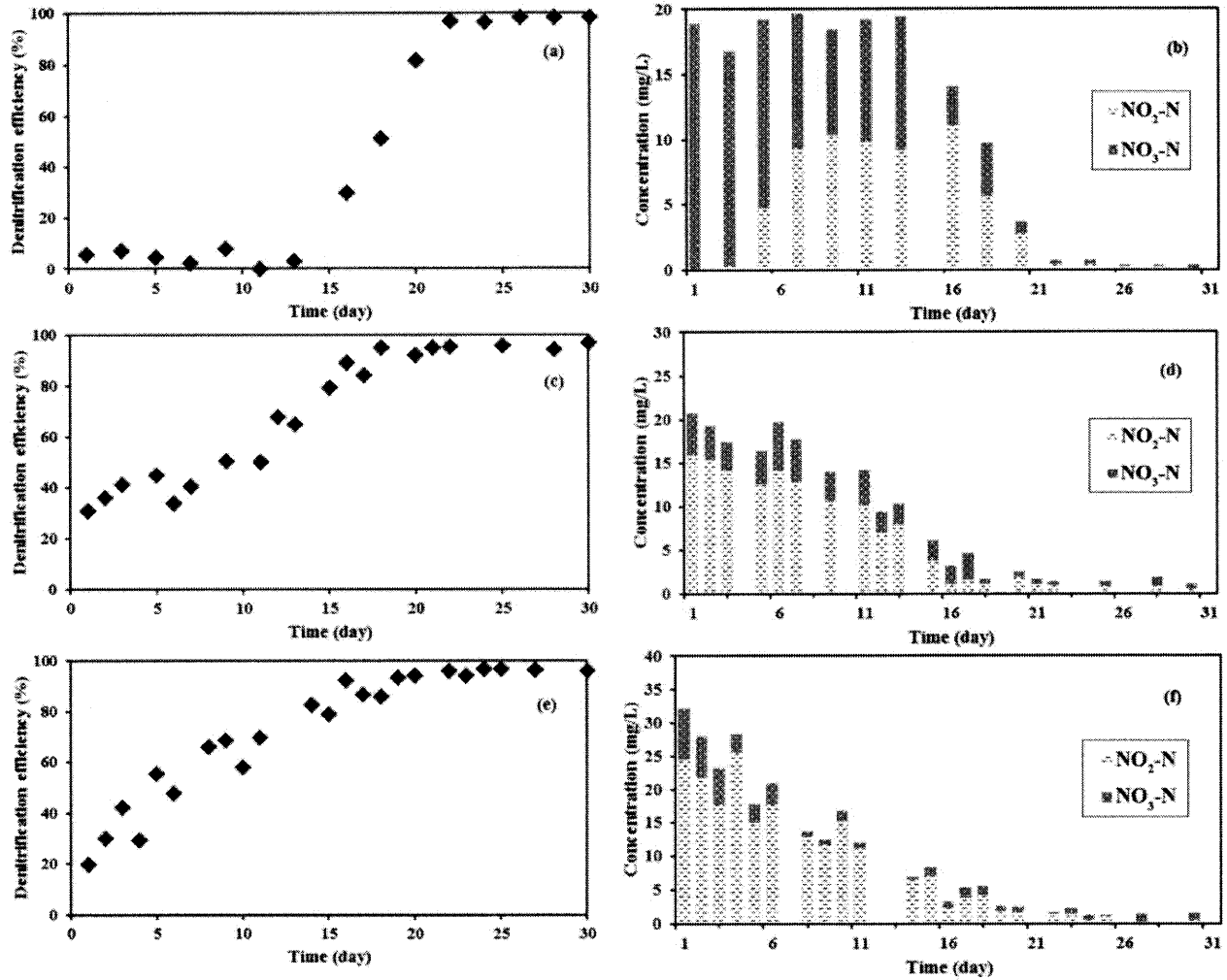


Fig. 3. Denitrification efficiency and nitrogen concentrations of the HD reactor at various $\text{NO}_3\text{-N}$ feed rates: (a)–(b) 20 mg/L, (c)–(d) 30 mg/L, and (e)–(f) 40 mg/L.

Reactor setup and operation

Reactor setup

The lab-scale attached growth reactors were comprised of a rectangular acrylic container with 3 L working volume

($11.5 \times 16 \times 16 \text{ cm}^3$) and contained 660 cm^2 (area) of fiber carrier (NET Co. Ltd, Japan). The fiber carrier consisted of a polyester mono-filament as the frame and absorbent acrylic fiber as the holder of the microorganisms. The fiber carrier formed a cover over a stainless steel holder (Fig. 2), and the assembly was kept compacted in the reactors.

Table 2. Summary of the denitrification efficiencies and rates at increasing concentrations of $\text{NO}_3\text{-N}$ in the HD reactor.

$\text{NO}_3\text{-N}$ feeds (mg/L)	Final denitrification efficiencies (%)	Denitrification rates (mg/L-h)	Effluent Conc. (mg/L)	
			$\text{NO}_2\text{-N}$	$\text{NO}_3\text{-N}$
20	98	0.35	0.5	0
30	95	0.28	0.5	0.5
40	95	0.15	0.5	0.5

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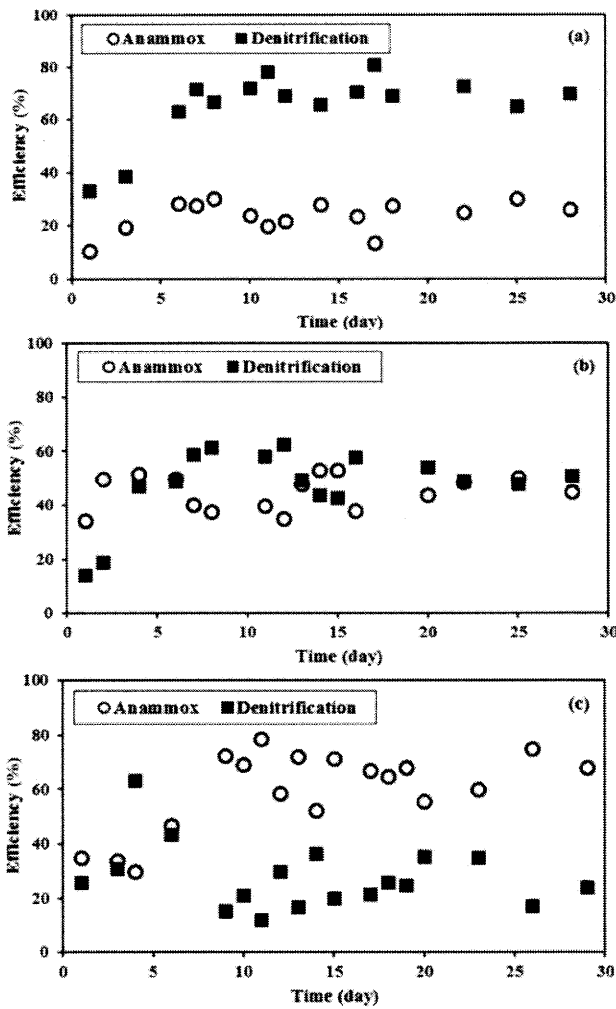


Fig. 4. Anammox and denitrification efficiencies of AnHD reactor at decreasing H₂ flow rates; (a) 70 mL/min, (b) 50 mL/min, and (c) 30 mL/min.

Because the intention was to develop lab-scale reactors using on-site microorganisms, the fiber carrier of the pilot-scale nitrification reactor at Chyasal that was fed with groundwater for 2 years was used for this purpose. The fiber carrier was found to have microorganisms responsible for nitrification as the dominant, while other complex microorganism communities could have been present as well. This would have resulted in the co-existence of microorganisms responsible for anammox and hydrogenotrophic denitrification in the reactors.

Operation of hydrogenotrophic denitrification (HD) reactor

The HD reactor was operated in a continuous feed mode with a feed rate of 9.6 L/day of water containing NO₃-N. The NO₃-N concentration was varied between 20 and 40 mg/L (Table 1) to determine the reactor performance at varying NO₃-N feeds. H₂ gas produced from a H₂-generator (HG260, GL Science, Japan) was supplied continuously to the reactor at a high flow rate of 70 mL/min. The H₂ gas was converted to dissolved hydrogen (DH), which saturated the water; however, residual H₂ gas was released to the atmosphere via the influent and effluent pipes. A slow and continuous stirring at 150 rpm was provided to the reactor to enhance H₂ solubility and microorganism activity. The *in situ* temperature was maintained at 30°C.

Operation of anammox and hydrogenotrophic denitrification (AnHD) reactor

The AnHD reactor was continuously fed with water containing NH₄-N and NO₂-N at a flow rate of 9.6 L/day. The NH₄-N and NO₂-N concentrations were kept constant at 10 mg/L. To study the optimisation of anammox and hydrogenotrophic denitrification processes in the single reactor, two experiments were setup (Table 1):

- (i) In the first one, H₂ was continuously fed at varying flow rates of 30–70 mL/min, and
- (ii) H₂ was intermittently fed for periods varying from 1–2 h at a constant flow rate of 70 mL/min.

Table 3. Summary of the anammox and denitrification efficiencies and effluent concentrations at various H₂ flow rates and intermittent H₂ supply periods of AnHD reactor.

Experimental condition	Efficiency (%)		Effluent conc. (mg/L)		
	Anammox	Hydrogenotrophic denitrification	NH ₄ -N	NO ₂ -N	NO ₃ -N
<i>Various H₂ flow rates</i>					
70 mg/L	25	70	6.5	0.5	0
50 mg/L	45	53	4.5	0.5	0
30 mg/L	65	25	2.5	0.5	0
<i>Various intermittent H₂ supply periods</i>					
1.0 h	65	42	4.0	0.5	0
1.5 h	80	42	1.0	0.5	0
2.0 h	80	15	1.0	1.5	0

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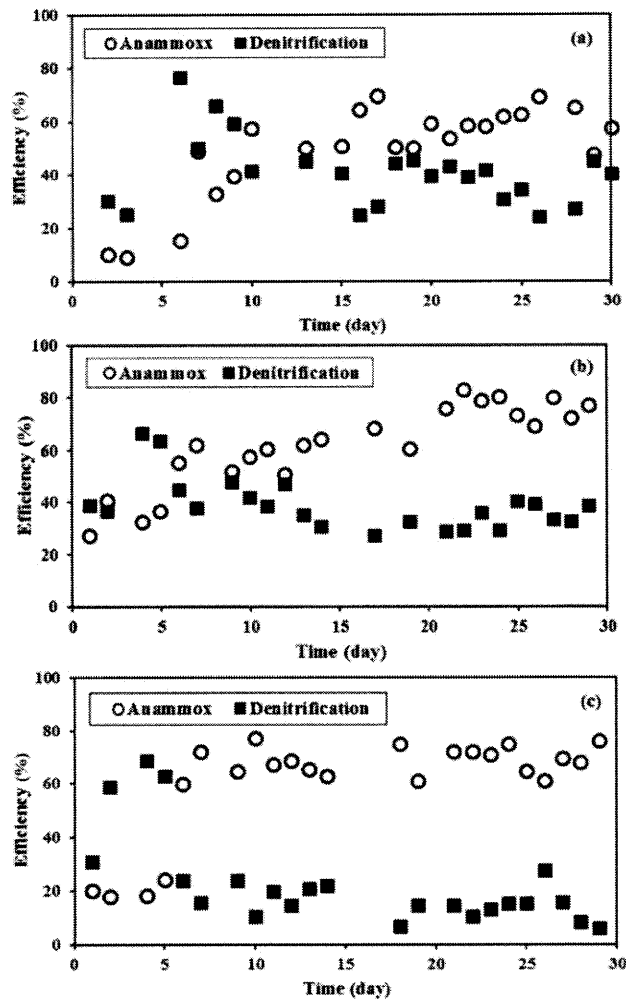


Fig. 5. Anammox and denitrification efficiencies of AnHD reactors at increasing intermittency of the H₂ supply periods; (a) 1.0 h, (b) 1.5 h, and (c) 2.0 h.

The liquid inside the reactor was also mixed using a stirrer (at 150 rpm) in both cases and the temperature was maintained at 30°C.

Preparation of synthetic water

Water containing NO₃-N. The water containing NO₃-N (influent for the HD reactor) was prepared in accordance with the quality of the groundwater at Chyasal; the concentrations of K⁺, Ca²⁺, Mg²⁺, P and inorganic carbon were kept at 25, 30, 10, 1 and 70 mg/L, respectively.^[12] However, the NO₃-N concentration was increased step-wise (by 10 mg/L) from 20 to 40 mg/L. During the preparation of the water containing NO₃-N, the dissolved oxygen (DO) in tap water (used as the water source for synthetic water

preparation) was reduced to <0.3 mg/L by feeding H₂ gas for 1 h.

Water containing both NH₄-N and NO₂-N. For the water containing both NH₄-N and NO₂-N (influent for the AnHD reactor), the concentrations of K⁺, Ca²⁺, Mg²⁺, P and inorganic carbon were also maintained at 25, 30, 10, 1 and 70 mg/L, respectively. Moreover, the NH₄-N and NO₂-N concentrations were kept at ~10 mg/L each. The water containing NH₄-N and NO₂-N was prepared everyday and the DO level was maintained at <0.3 mg/L.

Batch tests

The batch testing was done in the HD reactor to evaluate the denitrification rate. 3 L of water containing NO₃-N (with 20 mg/L of NO₃-N) was prepared for filling the reactor. The reactor was operated at a temperature of 30°C and stirred at a rate of 150 rpm. Water samples were taken every hour (for 8 h) and the NO₂-N and NO₃-N levels were analysed. The batch tests were done after the completion of each experiment with NO₃-N feed rates of 20, 30 and 40 mg/L.

Analytical methods

Influent and effluent water from the HD and AnHD reactors were sampled to determine the NH₄-N, NO₂-N and NO₃-N levels. The concentrations of NH₄-N, NO₂-N and NO₃-N were measured using phenate, colorimetric and ultraviolet spectrophotometric screening methods, respectively, as prescribed by the standard methods for examination of water and wastewater.^[20] The denitrification and anammox efficiencies and the denitrification rate were calculated using Eqs. 4–7. The *in situ* DH level was measured using a hydrogen meter (KM2100DH, Japan).

Equations used for calculations

For the HD reactor.

$$\begin{aligned} \text{Denitrification efficiency} \\ &= \left(\frac{\text{Influent NO}_3\text{-N} - (\text{Effluent NO}_2\text{-N} + \text{Effluent NO}_3\text{-N})}{\text{Influent NO}_3\text{-N}} \right) \\ &\quad \times 100 \end{aligned} \quad (4)$$

$$\begin{aligned} \text{Denitrification rate} \\ &= \frac{\text{NO}_3\text{-N}_{t=0} - (\text{NO}_2\text{-N} + \text{NO}_3\text{-N})_{t=t}}{\text{time}} \end{aligned} \quad (5)$$

For the AnHD reactor.

$$\begin{aligned} \text{Anammox efficiency} \\ &= \left(\frac{\text{Influent NH}_4\text{-N} - \text{Effluent NH}_4\text{-N}}{\text{Influent NH}_4\text{-N}} \right) \\ &\quad \times 100 \end{aligned} \quad (6)$$

$$\text{Denitrification efficiency} = \frac{((\text{Influent NO}_2\text{-N} - \text{Effluent NO}_2\text{-N}) - (\text{Influent NH}_4\text{-N} - \text{Effluent NH}_4\text{-N}))}{\text{Influent NO}_2\text{-N}} \times 100 \quad (7)$$

Results and discussion

The results are discussed in terms of (i) the cultivation of the microorganisms and (ii) the performance of the HD and AnHD reactors under different conditions. Suggestions have been provided for improving the quality of the drinking water supply system at Chyasal.

Hydrogenotrophic denitrification (HD) reactor

Cultivation of the microorganisms. In the start-up, the HD reactor containing fiber carrier from the pilot-scale nitrification reactor (a treatment unit of the drinking water supply system at Chyasal) was operated under a NO₃-N feed of 20 mg/L. The start-up results including the denitrification efficiency and the NO₂-N and NO₃-N concentrations in the effluent are shown in Figs. 3a–b. In the beginning, the denitrification efficiency was relatively low (<5%); however, the conversion of NO₃-N to NO₂-N continued to increase from zero to 10 mg/L, which shows that the NO₃-N-reducing microorganisms co-existed in the pilot-scale nitrification reactor and they grew at an earlier time compared to the NO₂-N-reducing microorganisms.

Due to the absence of the NO₂-N-reducing microorganisms, the effectiveness of denitrification could not be determined. After 2 weeks, the denitrification efficiency started to gradually increase, reaching ~98%; this indicates a drastic increase in both the NO₃-N- and NO₂-N-reducing microorganisms in the HD reactor. These results reveal the complexity of the microorganism community including microorganisms responsible for hydrogenotrophic denitrification in the pilot-scale nitrification reactor. However, the denitrification process (indicated by a decrease in the NO₃-N levels) was not found to occur during the operation of this pilot-scale nitrification reactor at the site (Fig. 1c). The absence of the denitrification process is because of the high oxygen levels in the water (~6 mg/L).^[12] Therefore, under low oxygen conditions (< 0.3 mg/L), the number of hydrogenotrophic denitrification microorganisms increased to rich amounts in the HD reactor.

Performance under increasing NO₃-N concentrations. As seen from the results in the previous experiment, a high denitrification efficiency of 98% was achieved when the HD reactor was operated with a NO₃-N feed of 20 mg/L. To determine the reactor performance at high NO₃-N feeds, the NO₃-N concentrations were increased from 20 to 40 mg/L (in increments of 10 mg/L), and the

results are shown in Figures 3c–f. The denitrification efficiency dropped to 20–30% immediately after the NO₃-N concentrations were increased, leading to high NO₂-N levels (15–25 mg/L) remaining in the effluent. These results suggest that the rapid and instantaneous increase in the NO₃-N concentrations had a negative effect on the activity of the NO₂-N-reducing microorganisms. However, these microorganisms were capable of adapting to the high NO₃-N conditions (i.e., 30 and 40 mg/L) in 3 weeks, as indicated by the high denitrification efficiencies (95%) achieved at that point in time.

Batch tests were done to clarify the effects of high NO₂-N accumulation. The results showed that the denitrification rates decreased from 0.35 mg/L-h for the lowest NO₃-N feed of 20 mg/L, to 0.28 and 0.15 mg/L-h when the NO₃-N feeds were increased to 30 mg/L and 40 mg/L, respectively (Table 2). Although NO₂-N accumulation occurred only for 2 weeks, high denitrification efficiencies (95%) being achieved in the subsequent weeks (days 15–20 in Figs. 3c–3f), the high levels of accumulated NO₂-N hindered the activity of hydrogenotrophic denitrification microorganisms. However, a long retention time of operation of the HD reactor of 7.5 h was sufficient to achieve hydrogenotrophic denitrification process at decreasing activities of the microorganisms. For the three NO₃-N feeds used, low concentrations of both NO₂-N and NO₃-N (< 1.0 mg/L) were present in the effluent.

Anammox and hydrogenotrophic denitrification (AnHD) reactor

Cultivation of Microorganisms. The AnHD reactor containing fiber carrier from the pilot-scale nitrification reactor (similar to the case of the HD reactor) was operated under H₂ flow rate of 70 mL/min. In this reactor, NH₄-N was removed by the anammox process, whereas NO₂-N was removed by both anammox and hydrogenotrophic denitrification. The efficiencies of anammox and denitrification during startup are presented in Figure 4a.

The increasing anammox and denitrification efficiencies reflect an increase in the amounts of both microorganisms in the AnHD reactor. After a week of operation, a high denitrification efficiency of 70% was achieved, while the anammox efficiency was still low (~25%). The considerable difference between the anammox and denitrification efficiencies was due to the high H₂ supply rate of 70 mL/min. Under the high H₂ flow rate, the hydrogenotrophic denitrification became the dominant process for NO₂-N removal, resulting in a deficiency of NO₂-N for the anammox process.

These results reveal the complexity of the microorganism communities involved in anammox and hydrogenotrophic denitrification in the pilot-scale nitrification reactor, and the fact that these microorganisms could be cultivated in a short period. During the startup period, ~0.5 mg/L of