Hydrogenotrophic denitrification in an attached growth reactor under various operating conditions
Wilawan Khanitchaidecha and Futaba Kazama

ABSTRACT
To study the performance of an attached growth reactor on nitrate-nitrogen (NO₃-N) removal by a hydrogenotrophic denitrification process, the reactor was operated under various H₂ flow rates (30, 50 and 70 mL/min), hydraulic retention times (HRTs) (2.6, 3.1, 4.7 and 6.7 h) and inorganic carbon (IC) concentrations (excess and no IC addition). Synthetic NO₃-N water was prepared in accordance with the groundwater quality of the study area: Chyasal in Kathmandu Valley, Nepal. The results show an increase in denitrification efficiency with H₂ flow rate and HRT. Further, for effective denitrification, the dissolved hydrogen (DH) during operation should be maintained at greater than 0.1 mg/L. A sharp decrease in the denitrification efficiency from ∼96% (under excess IC) to 10–35% (under no IC) suggested occurrence of denitrification inside the attached growth reactor by a hydrogenotrophic process (using H₂) as well as heterotrophic process (using internal organic carbon). The good performance of the attached growth reactor in terms of high efficiency, low H₂ supply and short HRT requirement indicates the reactor’s appropriateness for groundwater remediation through NO₃-N removal.

Key words | attached growth reactor, groundwater remediation, hydrogenotrophic denitrification, NO₃-N removal

INTRODUCTION
Nitrate-nitrogen (NO₃-N) is one of the common contaminants in groundwater. A high NO₃-N ranging from 12 to 60 mg/L are reported in the groundwater of several developing countries such as the Philippines, India, Nepal, and China (Bouman et al. 2002; Ju et al. 2006; Suthar et al. 2009; Chapagain et al. 2010). Groundwater is a major source of drinking water in those countries. Consumption of the NO₃-N contaminated water (exceeding the limiting value of 11.5 mg/L for drinking water (WHO 2004)) can cause methemoglobinemia (blue baby syndrome) in infants and stomach cancer in adults (Bouman et al. 2002). The sources of NO₃-N contamination in groundwater can be various, such as fertilizer use, pesticide residue, industrial discharge and sewage effluent (Umezawa et al. 2008; Suthar et al. 2009). The NO₃-N concentration could also be enhanced in supplied water if a nitrification process (NH₄-N + O₂ → NO₃-N) has been used to treat NH₄-N-contaminated groundwater to comply with NH₄-N guidelines for drinking water (Ha et al. 2006). Therefore, both nitrification and denitrification processes are necessary to remove NH₄-N as well as NO₃-N if water quality guidelines related to all the forms of nitrogen are to be complied with.

The denitrification process is an attractive option for remediation of NO₃-N contaminated groundwater for the following reasons: (1) NO₃-N is efficiently and selectively removed by conversion to nitrogen gas (N₂); (2) no toxic waste is generated; and (3) the system is cost-effective to use in developing countries (Mansell & Schroeder 2002). The most common denitrification processes are heterotrophic and hydrogenotrophic. The heterotrophic process requires the addition of organic carbon such as acetate and methanol (as shown in Equation (1) from Barber & Stuckey 2000) whereas the hydrogenotrophic process requires a hydrogen gas (H₂) supply (as shown in Equation (2) from...
Ghafari et al. 2009). Hydrogenotrophic denitrification is preferred over heterotrophic to remove NO$_3$-N from groundwater/drinking water for the following reasons (Kurt et al. 1987; Chang et al. 1999; Rezania et al. 2005; Vasiliadou et al. 2009): (1) it is less harmful to human health; (2) there is no biological instability as H$_2$ do not persist in the treated water; (3) there is no need for post-treatment to remove either excess H$_2$ or its derivatives; (4) the low sludge treatment cost due to relatively low biomass yield; and (5) the low cost of the system because of the lower cost of hydrogen compared to methanol or acetate.

Heterotrophic denitrification:

$$5(\text{organic-C}) + 2\text{H}_2\text{O} + 4\text{NO}_3^- \rightarrow 2\text{N}_2 + 4\text{OH}^- + 5\text{CO}_2$$

(1)

Hydrogenotrophic denitrification:

$$2\text{NO}_3^- + 5\text{H}_2 \rightarrow \text{N}_2 + 2\text{OH}^- + 4\text{H}_2\text{O}$$

(2)

Recently, several configurations of a hydrogenotrophic denitrification system (i.e., membrane reactor, packed bed reactor, sequencing batch reactor) have been proposed at laboratory scale (Mansell & Schroeder 2002; Mo et al. 2005; Vasiliadou et al. 2009; Ghafari et al. 2010). However, their complicated systems and high investment cost limit their application for many developing countries. An attached growth reactor which is simple in design and cost-effective in operation (Khanh et al. 2010) could be an attractive option to use in many developing countries. Besides, it has a potential configuration to be developed for hydrogenotrophic denitrification.

The objective of this research is to develop an attached growth reactor for hydrogenotrophic denitrification. To determine an optimal operating condition for the reactor, the effects of H$_2$ flow rate, hydraulic retention time (HRT) and inorganic carbon (IC) concentration on denitrification efficiency were studied. The mechanisms for denitrification in the reactor are also discussed in this paper.

**MATERIALS AND METHODS**

**Reactor set-up and operation**

The attached growth reactor, comprising of a rectangular acrylic container with 3 L working volume ($11.5 \times 16 \times 16$ cm$^3$) and containing 660 cm$^2$ area of fibre carrier was set up in the laboratory. The carrier (from NET Co. Ltd, Japan) was covered over a stainless steel holder, and made of a polyester monofilament with absorbent acrylic fibre as a bacteria holder (Figure 1). The reactor was operated in a continuous feed mode using a peristaltic pump with influent (synthetic NO$_3$-N water) flow rate of 0.4–1.1 L/h. H$_2$ gas was produced by an H$_2$ generator (HG260, GL Science, Japan) and continuously fed to the reactor with a controlled

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**Figure 1** | Schematic diagram and experimental set-up of attached growth reactor.
flow rate of 30–70 mL/min. The liquid inside the reactor was completely mixed by a stirrer at the rate of 150 rpm. During operation, temperature was maintained at 30 °C and samples from the influent and effluent were taken frequently for further analysis.

To start the experiment, seed sludge was required. It was prepared by cultivating the sludge taken from the activated sludge system of a drinking water plant in Kofu city (Yamanashi, Japan) for three weeks. During the cultivation, the reactor (without fibre carrier) was operated with influent and \( \text{H}_2 \) flow rates of 0.4 L/h and 50 mL/min, respectively. Other conditions (i.e., synthetic NO\(_3\)-N water and its temperature) during the cultivation were the same as that to be used in the experiment. After cultivation, 300 mL of concentrated sludge was added into the attached growth reactor (with fibre carrier) to start the experiment.

**Synthetic NO\(_3\)-N water preparation**

In this research, synthetic NO\(_3\)-N water was prepared (or standardized) in accordance with the effluent quality of the nitrification unit installed in the drinking water supply system at Chyasal (Kathmandu Valley, Nepal). The nitrification reactor and effluent water quality is detailed in Khanitchaidecha et al. (2011). The drinking water supply system is designed for remediation of NH\(_4\)-N contaminated groundwater, however high NO\(_3\)-N of approximately 10–15 mg/L was found in the treated water. The synthetic NO\(_3\)-N water for studying the denitrification process was prepared by mixing the following chemicals (g/L): 0.12 of NaNO\(_3\), 0.48 of NaHCO\(_3\), 0.05 of KCl, 0.11 of CaCl\(_2\)-2H\(_2\)O, 0.10 of MgSO\(_4\)-7H\(_2\)O and 0.02 of Na\(_2\)HPO\(_4\)-12H\(_2\)O. Concentration of quality parameters (i.e., \( \text{K}^+ \), \( \text{Ca}^{2+} \), \( \text{Mg}^{2+} \)) in the synthetic NO\(_3\)-N water was based on the groundwater quality in Chyasal (Khanitchaidecha et al. 2010), while the NO\(_3\)-N, PO\(_4\)-P and inorganic carbon (IC) were controlled at 20, 2 and 70 mg/L, respectively.

**Experimental procedure**

The attached growth reactor was set-up and operated for two experiments: the effect of \( \text{H}_2 \) flow rate and HRT, and the effect of \( \text{H}_2 \) flow rate and IC concentration. The first experiment was run under various \( \text{H}_2 \) flow rates of 30, 50 and 70 mL/min, and various HRTs of 2.6, 3.1, 4.7 and 6.7 h. An IC concentration of 70 mg/L (more than the amount required for a complete denitrification (Mansell & Schroeder 2002)) was maintained to avoid IC limitation for denitrification. Another experiment was run under various \( \text{H}_2 \) flow rates of 30 and 70 mL/min, and with no IC addition. However, an IC concentration of approximately 5 mg/L was found in the synthetic NO\(_3\)-N water, due to the IC existing in tap water used to prepare the synthetic water. The operating conditions of these two experiments are summarized in Table 1.

**Analytical methods**

Concentrations of NO\(_2\)-N and NO\(_3\)-N in influent and effluent were measured using colorimetric and ultraviolet spectrophotometric screening methods, respectively, in accordance with the standard method for the examination of water and wastewater (APHA 1998). The denitrification efficiency was calculated using Equation (3). Dissolved hydrogen (DH) in the reactor was measured by a hydrogen meter (KM2100DH, Japan).

\[
\text{Denitrification efficiency} = \left( 1 - \frac{\text{Effluent (NO}_2\text{-N + NO}_3\text{-N)}}{\text{Influent NO}_3\text{-N}} \right) \times 100
\]

### Table 1: Operating conditions of the attached growth reactor

<table>
<thead>
<tr>
<th>Experiment</th>
<th>( \text{H}_2 ) flow rate (mg/L)</th>
<th>NO(_3)-N (mg/L)</th>
<th>IC (mg/L)</th>
<th>Flow rate (L/h)</th>
<th>HRT (h)</th>
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<td>1</td>
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<td>70</td>
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<td>0.6</td>
<td>4.7</td>
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<td></td>
<td></td>
<td>0.9</td>
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RESULTS AND DISCUSSION

The results of operating the attached growth reactor are discussed in the following sequence: effect of H₂ flow rate, effect of HRT, carbon consumption during NO₃-N removal, effect of IC concentration, and comparison of the performance with other reactors.

Effect of H₂ flow rate

The denitrification efficiency increased with H₂ flow rate, particularly at a short HRT of 2.6 h; the efficiencies were approximately 3, 27 and 90% for the H₂ flow rates of 30, 50 and 70 mL/min, respectively (Figure 2(a)). By contrast, the effect of H₂ flow rate on the efficiency was not obvious at long HRTs. For example, at an HRT of 6.7 h, high efficiency (~96%) was achieved for all H₂ flow rates (Figure 2(a)). However, dissolved hydrogen (DH) concentration for the HRT of 6.7 h varied in accordance with H₂ flow rate; high DH of 1.6 mg/L, which is saturated H₂ in water (Kurt et al. 1987), was found at the highest H₂ flow rate of 70 mL/min, and decreased to 0.3 and 0.1 mg/L for the flow rates of 50 and 30 mL/min, respectively (Figure 2(b)).

This is because of the increase in the amount of H₂ with flow rate. Therefore, sufficient H₂ fed results in effective denitrification, and the excess H₂ appears as a DH value. Based on this experiment, 0.2–0.5 L H₂ (at ambient temperature (25 ± 2°C) and ambient pressure) needs to be fed to remove 1 mg NO₃-N, and the DH should be maintained at >0.1 mg/L to achieve denitrification. According to the above conditions, the denitrification was not inhibited by H₂ limitation.

Furthermore the H₂ flow rate significantly affected the denitrification rate, as shown in Figure 3(a)–(c). The denitrification rate was calculated by the reduction of total

![Figure 2](a) Denitrification efficiency and (b) dissolved hydrogen (DH) concentration at various H₂ flow rates and HRTs.

![Figure 3](a) Change of NO₂-N, NO₃-N and total N (NO₂-N + NO₃-N) at various H₂ flow rates of (a) 30 mL/min, (b) 50 mL/min, and (c) 70 mL/min.
N (NO$_2^-$N + NO$_3^-$N) in a linear function from the batch test. The rate was approximately 5 mg/L/h at an H$_2$ flow rate of 30 mL/min, which was later increased to 6 and 12 mg/L/h at H$_2$ flow rates of 50 and 70 mL/min, respectively. The results suggest that it takes 3 h to completely remove the NO$_3^-$N at a high H$_2$ flow rate (i.e., 70 mL/min); while the operation at a lower H$_2$ flow rate (i.e., 30 and 50 mL/min) takes as long as 5 h. In addition, the NO$_2^-$N concentration increased sharply from 0–2 to 10–15 mg/L in the first two hours of all three batch tests, and decreased gradually to zero in the subsequent hours. This phenomenon illustrated that the denitrification process took place in the attached growth reactor firstly by NO$_2^-$N consumption and conversion to NO$_2^-$N, and then by conversion of NO$_2^-$N to N$_2$. Interestingly, the bacteria preferred to consume NO$_3^-$N rather than NO$_2^-$N, and they had no denitrification inhibition from NO$_2^-$N accumulation. In this study, no inhibition from NO$_2^-$N was found even at a higher NO$_2^-$N of 10–15 mg/L in the reactor which is very good compared with a previous study (Zhou et al. 2007), in which inhibition from NO$_2^-$N was found even at a low NO$_2^-$N of 3 mg/L.

**Effect of HRT**

As shown in Figure 2(a), the denitrification efficiency for all the H$_2$ flow rates increased with HRT; reaching a maximum of 96% at the longest HRT of 6.7 h. However, at a low H$_2$ flow rate (of 30 mL/min), the impact of HRT on the denitrification efficiency was significant. The efficiency was 3–15% for the short HRTs of 2.6–3.1 h, and increased sharply to approximately 96% for the long HRTs of 4.7–6.7 h. The amounts of H$_2$ (L H$_2$/mg NO$_3^-$N) fed to the reactor for the HRTs of 2.6, 3.1, 4.7 and 6.7 h were 0.08, 0.09, 0.14 and 0.20, respectively (at H$_2$ flow rate of 50 mL/min). As discussed above in the effect of H$_2$ flow rate section, a complete denitrification requires 0.2 L/mg N of H$_2$. Therefore, the low denitrification efficiency at the short HRT was due to H$_2$ limitation.

Another analysis was carried out to estimate the capacity of the attached growth reactor (g N removed/day) at the high H$_2$ flow rate (of 70 mL/min) to ensure no H$_2$ limitation for denitrification. The results show a continuous increase in reactor capacity with decreasing HRT (Figure 4). The maximum reactor capacity in this experiment was approximately 0.5 g N/day at the shortest HRT of 2.6 h, however the reactor capacity is likely to increase at HRT of <2.6 h. The results suggest that the attached growth reactor can be operated with efficient denitrification at relatively short HRTs (of <2.6 h), compared to previous studies (Mo et al. 2005; Park et al. 2005; Terada et al. 2006). For operation with lower H$_2$ flow rates of 30 and 50 mL/min, maximum reactor capacity of 0.3 and 0.4 g N/day, respectively, was reached at HRT of 4.7 and 5.1 h (data not shown). With these results, it can be concluded that NO$_3^-$N removal capacity of the attached growth reactor can be enhanced by operating it under high H$_2$ flow rate.

Based on the experiments to analyse the effects of H$_2$ flow rates and HRTs, the optimal operating condition of the attached growth reactor for NO$_3^-$N removal was obtained. Sufficient H$_2$ in the reactor is the key for effective denitrification. The sufficient H$_2$ is related to H$_2$ flow rate and HRT; an operation with a high H$_2$ flow rate can achieve high denitrification efficiency at a short HRT, whereas an operation with a low H$_2$ flow rate needs a longer HRT for a complete denitrification.

**Carbon consumption during NO$_3^-$N removal**

As suggested by Mansell & Schroeder (2002), 4.2 mg/L of IC is required to remove 20 mg NO$_3^-$N/L by hydrogenotrophic denitrification. To avoid the IC limitation for the denitrification process and to use it as a buffer, IC of 70 mg/L (excess than the amount required for a complete denitrification) was added in the synthetic NO$_3^-$N water while studying the effect of H$_2$ flow rate and HRT. An analysis of effluent IC concentration shows no correlation with the denitrification
efficiency (Figure 5(a)) and linear relation with H₂ flow rate (Figure 5(b)). The average IC concentrations were approximately 54, 71 and 92 mg/L for the H₂ flow rates of 30, 50 and 70 mL/min, respectively. The decrease in IC concentration (compared to the initial concentration of 70 mg/L) at a low H₂ flow rate (of 30 mL/min) reflects that some IC was consumed for the hydrogenotrophic denitrification process. By contrast, at a high H₂ flow rate of 70 mL/min, the effluent IC was higher than the initial IC. This reflects that some IC was produced during the denitrification process and the production was higher than consumption. Consequently, the IC produced during denitrification remained in the reactor and effluent. The IC production increased with H₂ flow rate (Figure 5(b)). The possible process for increasing IC in the reactor could be the occurrence of the heterotrophic denitrification process (see Equation (1)). The following discussion supports this possibility.

**Increasing IC from heterotrophic denitrification process**

The heterotrophic denitrification process requires organic carbon (OC) to remove NO₃-N, as shown in Equation (1). Although OC was not added while preparing the synthetic NO₃-N water, internal OC can be produced from hydrolysis and degradation of bacteria inside the reactor (Janning et al. 1997). Some other possible internal sources of OC, as discussed in the literature, could be soluble microbial products (SMP) derived during biomass growth and decay (Jarusutthirak & Amy 2007), volatile fatty acids (VFA) produced by acetogenic bacteria, and extracellular polymeric substances (EPS) which are produced by biomass to promote attachment (Ergas & Reuss 2001; Lee & Rittmann 2002). Those OC produced internally are the carbon source for the heterotrophic denitrification process. Moreover, the hydrogenotrophic denitrifying bacteria are able to grow autotrophically on hydrogen and heterotrophically on organic carbon (Smith et al. 1994; Szekeres et al. 2002), therefore both hydrogenotrophic and heterotrophic denitrification occur in the attached growth reactor. The heterotrophic denitrification process produces IC as a by-product (see Equation (1)), resulting in an increase in IC in the reactor and a higher IC concentration (than the initial IC) in the effluent for the reactor operated at an H₂ flow rate of 70 mL/min.

The hypothesis of IC and OC produced during NO₃-N removal was determined in two batch tests: NO₃-N contained water (Figure 6(a) and (b)) and no NO₃-N contained water (Figure 6(c) and (d)). The IC concentration increased over time with H₂ flow rate (Figure 6(a)). At the end of the batch test (6 h operation), the concentration of IC increased from 77 mg/L to 79, 84 and 88 mg/L for H₂ flow rates of 30, 50 and 70 mL/min, respectively. The concentrations of OC at various H₂ flow rates were notably different, being approximately 1–2 mg/L (Figure 6(b)). In contrast, for no NO₃-N contained water, the IC was in a range of 72–75 mg/L for all the H₂ flow rates (Figure 6(c)), whereas the OC was more likely constant at a low H₂ flow rate of 30 mL/min, and increased over time at a high H₂ flow rate of 70 mL/min (Figure 6(d)). These results reveal enhancement of internal OC production with the high H₂ flow rate. The OC was consumed for heterotrophic denitrification and the IC, a by-product of heterotrophic denitrification, was also increased during the process. The IC concentration in Figure 6(c) was stable because no denitrification occurred with no NO₃-N contained water. It can be concluded that at a low H₂ flow rate, only the

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**Figure 5** Relationship between (a) effluent IC concentration and efficiency, and (b) effluent IC concentration and H₂ flow rate.
hydrogenotrophic denitrification process was dominant, resulting in a decrease in effluent IC concentration (see Figure 5(a)). Whereas at a high H₂ flow rate, both hydrogenotrophic and heterotrophic denitrification occurred, resulting in an increase in effluent IC concentration (see Figure 5(a)).

Effect of IC concentration

To analyse the effect of IC concentration, the attached growth reactor was operated under no IC addition at various H₂ flow rates, as summarized in Table 1. The denitrification efficiency was compared to the efficiency of the experiment with IC of 70 mg/L (see results from the effect of H₂ flow rate section) and shown in Figure 7. Under no IC addition, the denitrification efficiency was approximately 10 and 35% for H₂ flow rates of 30 and 70 mL/min respectively. Due to no IC and OC addition in this experiment, the denitrification occurred by using internal carbon (i.e., OC from hydrolysis and degradation of bacteria). Again, the higher efficiency of a higher H₂ flow rate (of 70 mL/min) compared to a lower one (of 30 mL/min) confirmed the enhancement of OC production with increased H₂ flow rate and the heterotrophic denitrification occurring in the reactor. Under the condition of IC addition (of 70 mg/L), a high denitrification efficiency of 96% was reached; this suggested that both hydrogenotrophic and heterotrophic denitrification were effective (as summarized in Figure 8).

Comparison of the performance with other reactors

The results of the attached growth reactor in this research were comparable with those obtained in previous studies,
where hydrogenotrophic denitrification was achieved using various reactors (such as a membrane bioreactor, packed bed reactor, biofilm reactor, sequencing batch reactor and submerged membrane reactor). The denitrification efficiency of the attached growth reactor was as high as that of the previous reactors (see Table 2). Although biofilm and sequencing batch reactors (Park et al. 2005; Ghafari et al. 2009) can be operated with ∼100% of NO3-N removal, a long HRT of more than 8 h was required. By contrast, membrane, fluidized bed and packed bed reactors (Chang et al. 1999; Lee & Rittmann 2002; Rezania et al. 2007; Vasiliadou et al. 2009) can achieve high NO3-N removal at a short HRT of less than an hour; however these reactors require high H2 feeding, resulting in an increase in operation cost. With the attached growth reactor, the operational problems of the above-mentioned reactors, such as pore clogging and channelling, and insufficient denitrification due to very short HRT resulting from high upflow velocity (Karanasios et al. 2010) can be solved. Therefore, the attached growth reactor is a suitable system for removing NO3-N from groundwater and/or being combined with a nitrification system for complete nitrogen removal.

Table 2 | Performance of various hydrogenotrophic denitrification reactors

<table>
<thead>
<tr>
<th>Reactor</th>
<th>NO3-N (mg/L)</th>
<th>H2 flow rate (mL/min)/H2 pressure (atm)/DH (mg/L)</th>
<th>HRT (hour)</th>
<th>Efficiency (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane bioreactor</td>
<td>20–40</td>
<td>DH 1.6</td>
<td>0.8</td>
<td>92–96</td>
<td>Mansell &amp; Schroeder (2002)</td>
</tr>
<tr>
<td>Membrane bioreactor</td>
<td>10–70</td>
<td>DH 1.6</td>
<td>9–12</td>
<td>87–100</td>
<td>Mo et al. (2005)</td>
</tr>
<tr>
<td>Membrane bioreactor</td>
<td>10–20</td>
<td>Pressure 0.3–0.5</td>
<td>0.7</td>
<td>40–92</td>
<td>Lee &amp; Rittmann (2002)</td>
</tr>
<tr>
<td>Membrane bioreactor</td>
<td>100</td>
<td>Pressure 0.2–0.5</td>
<td>7.8–14.6</td>
<td>76–99</td>
<td>Terada et al. (2006)</td>
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<tr>
<td>Membrane bioreactor</td>
<td>60–70</td>
<td>Pressure 0.3–0.6</td>
<td>4</td>
<td>~85</td>
<td>Ergas &amp; Reuss (2001)</td>
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<td>Submerged membrane reactor</td>
<td>25</td>
<td>DH 1.6</td>
<td>3</td>
<td>~100</td>
<td>Rezania et al. (2007)</td>
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<tr>
<td>Packed bed reactor</td>
<td>10–60</td>
<td>Flow rate 90</td>
<td>0.2–0.8</td>
<td>85–99</td>
<td>Vasiliadou et al. (2009)</td>
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<td>22</td>
<td>Flow rate 60–120</td>
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<td>Lee et al. (2010)</td>
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<td>Flow rate 5</td>
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<td>~100</td>
<td>Park et al. (2005)</td>
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<td>Sequencing batch reactor</td>
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<td>4–12</td>
<td>~100</td>
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<tr>
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<td>0.9</td>
<td>99–100</td>
<td>Chang et al. (1999)</td>
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<td>Attached growth reactor</td>
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<td>Flow rate 30–70</td>
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</table>
CONCLUSIONS

This research analyses performance of an attached growth reactor to achieve effective NO₃-N removal from groundwater. The reactor was designed for hydrogenotrophic denitrification under continuous H₂ supply. The experimental results suggest that: (1) the denitrification efficiency was enhanced by increasing the H₂ flow rate and HRT (i.e., 70 mL/min of H₂ flow rate and 6.7 h of HRT); and (2) the efficiency was decreased from ~96% to 10–35% when no inorganic carbon (IC) was added to the reactor. Moreover, to achieve high denitrification efficiency, the reactor should be operated under sufficient dissolved hydrogen (DH) of greater than 0.1 mg/L and with excess IC. Further analysis of the denitrification process inside the reactor suggests the occurrence of heterotrophic denitrification using internal organic carbon, produced possibly from bacteria hydrolysis and degradation. Because of its acceptable using internal organic carbon, produced possibly from bacteria hydrolysis and degradation. Because of its acceptable using internal organic carbon, produced possibly from bacteria hydrolysis and degradation. Because of its acceptable using internal organic carbon, produced possibly from bacteria hydrolysis and degradation. Because of its acceptable using internal organic carbon, produced possibly from bacteria hydrolysis and degradation. Because of its acceptable using internal organic carbon, produced possibly from bacteria hydrolysis and degradation. Because of its acceptable using internal organic carbon, produced possibly from bacterial hydrolysis and degradation.

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