Hydrogenotrophic Denitrification Activity under Intermittent Hydrogen Supply using Micro-Bubble System

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Abstract- **The purpose of the study was to test the performance of a hydrogenotrophic denitrification system (H.D.) for converting nitrate-nitrogen (NO_3-N) to harmless nitrogen gas under intermittent supply using a micro-bubble system as a diffuser. The 4.2 L of laboratory scale experiment was set up and operated under three conditions of H_2 gas supply including continuous supply (condition 1; 14.4 L/d), intermittent supply four times in a day (condition 2; 3.6 L/d) and intermittent supply two times in a day (condition 3; 1.8 L/d) at 10 mL/min of hydrogen flow rate and operating under 60 g-N/m^3·d of nitrogen loading rate (NLR) at a temperature of 32ºC were conducted. At the steady state, the removal efficiency was 97% for condition 1, 94% for condition 2 and 77% for condition 3. Therefore, the amount of H_2 gas was significantly affected by the H.D. activity. The optimum H.D. system was found in condition 2 considering its low cost and high effectiveness. In this case, about 75% of the H_2 gas can be saved compared to the 14.4 L of H_2 gas supplied continuously. Our results demonstrate that the intermittent hydrogen supply using the microbubble system as the diffuser is an effective and low cost method in a H.D. system.**

Keywords- Hydrogenotrophic denitrification (H.D.), Nitrate-nitrogen (NO_3-N), Swilling-Type microbubble, Intermittent H_2 gas supply

1. INTRODUCTION

At present, contamination of nitrate into groundwater and drinking water is a serious problem in developing countries. Nitrate contaminated occurs through uncontrolled discharge sources such as agriculture drainage, septic tank or sewer leakage, animal waste, feedlot runoff and industrial waste water. For example, 60 mg NO_3-N/L was detected in groundwater of Kathmandu, Nepal, which is higher than the drinking water standard value of 11 mg NO_3-N/L and 0.9 mg NO_2-N/L [1]. Consumption of NO_3-N contaminated drinking water can cause methemoglobinemia and stomach cancer [2], indicating the seriousness of the problem of nitrate contamination in drinking water sourced from groundwater in developing countries, which calls for an effective and cost efficient method for improving water quality. There are two types of methods which can be used; abiotic and biological. Biological methods are an alternative technology for nitrate elimination because of low operating condition, capital cost and are friendly to the environment [3]. Hydrogenotrophic denitrification is one of denitrification process to convert nitrate to harmless nitrogen gas (NO_3^- to NO_2^- to NO to N_2O to N_2) under hydrogen oxidation bacteria, using carbon dioxide and bicarbonate as carbon source and hydrogen gas as electron donor [4], as in the equation;

$$NO_3^-+3.03H_2+H^++0.229HCO_3 \rightarrow 0.477N_2 + 3.6H_2O +0.0458C_5H_7O_2N$$

H_2 is a perfect selection for autohydrogenotrophic denitrification because of its cleanness, naturalness, harmlessness to human, low biomass yield and has no residual organic carbon as compared to heterotrophic denitrification. However, the drawback of this method is the low solubility and high cost of H_2 gas. Therefore, the biological degradation of nitrate by direct immobilization of autohydrogenotrophic bacteria is still limited. In recent years, there have been many technologies focusing on improving the performance of H.D. systems such as membrane, fixed bed, fluidize bed and so on. For example, an approach discussed in [5], on the possibility of controlling the process rate by supplying limited amounts of electron donor (H_2) in the membrane biofilm reactor found that the limitation of the hydrogen can inhibit the denitrification rate and growth of the biofilm. It was also mentioned in [6] that continuous H_2 supply is high cost, and can cause a dangerous accumulation of expelled hydrogen beneath the ceiling in laboratory and difficulties in maintaining pH in the system. In [7] it was reported that the denitrification efficiency increased with an increase of H_2 flow rates and a decrease in intermittent H_2 supply periods. Consequently, H_2 gas appears to affect the performance of the denitrification process because the most important factor in controlling denitrification activity is H_2 gas. Bubble size is also an important factor, which relates to DH concentration and the amount of H_2 gas supply. Small sized bubbles can be maintained in the system longer than big bubbles. This therefore can save the amount of H_2 gas used and consequently operational cost.

Our research therefore focused on the biological process and the examination of a method to minimize the amount of H_2 gas supply required resulting in H_2 cost savings. This paper reports on our investigation and discusses the operating efficiency of intermittent hydrogen supply by using a Swilling-type microbubble system in a simple design. Cost-effectiveness of the intermittent hydrogen supply in the H.D. system is also discussed.

2. METHODOLOGY

2.1 Cultivation of Microorganism and Synthetic Medium

The mix-culture of denitrification bacteria was enriched on non-woven fabric from sludge taken from wastewater treatment plant in Kofu city, Yamanashi prefecture, Japan. This sludge was cultivated about 600 days at a temperature of 35℃. 50 mL/min of H_2 supply flow rate and 0.17 kg/m^3/d of nitrogen loading rate (NLR). Synthetic medium was prepared according to the concentration of groundwater at Kathmandu, Nepal. The chemicals for mixing were (g/L); 0.36 of $NaNO_3$, 0.122 of $NaHCO_3$, 0.094 of $MgSO_4$, 0.081 of $CaCl_2$, 0.03 of $Na_2HPO_4.12H_2O$ and 0.012 of KCl. NO_3-N was maintained at a concentration of 60 mg N/L in all experiments. Synthetic water was supplied with argon gas for removing oxygen gas and to keep the DO concentration lower than 0.3 ± 0.1 mg/L to maintain it under anaerobic condition.

2.2 Reactor Setup and Operating Condition

A laboratory scale H.D. reactor was constructed at University of Yamanashi, Japan. The reactor was made of acrylic sheet in rectangular shape with a dimension of 11.5 x 16 x 16 cm (length x width x height) with working volume of 4.2 L. The top part of the reactor was unsealed to avoid an explosion of H_2 gas and plastic beads were put in to prevent O_2 from dissolving into the system and also to maintain dissolving hydrogen (DH) and temperature in the system. The temperature in the reactor was maintained at 32 ± 0.5℃ by thermostat and continuously stirred using a magnetic bar at 150 rpm during all the experiments. To start, about 39 g or 0.8 g/L of biomass concentration of the enriched H.D. sludge on the non-woven fabric was collected and put into the reactor. Swilling-Type micro bubbles were then put in the reactor, as the diffuser. Synthetic medium and H_2 gas were supplied into the system with flow rate of 0.175 L/h and 10 mL/min, respectively. The schematic diagram of experimental set up is shown in figure1.

This system used a Swilling-Type micro-bubble system diffuser, which can generate fine bubbles, of about 50 μm in size. Bubbles from this diffuser were found to be smaller than bubbles from an air stone system. Consequently, DH can be maintained for a longer time in the system than in a normal diffuser. It was also found that the H.D. performance from the micro-bubble operation was higher than from a normal operation with a small amount of H_2 supply (comparison of diffuser efficiency results is not shown in this paper). These results may be explained by having microbubbles which are maintained for a long time in reactor.

However, with a more sufficient H_2 supply, there will be more residual H_2 released to the atmosphere, which can cause high cost and less utilization of the H_2 gas. Hence, this experiment was divided into three conditions in order to minimize the H_2 supplied amount for the H.D. activity. The operating condition in each experiment is shown in the Table 1. In three conditions from Table 1, condition 1 was continuous supply of H_2 gas, condition 2 was intermittent supply of H_2 gas four times per day (1.5 hour of H_2 gas supply and 4.5 hour of stop H_2 gas supply) and condition 3 was intermittent supply of H_2 gas two times per day (1.5 hour of H_2 gas supply and 10.5 hour of stop H_2 gas supply).

3. ANALYTICAL METHODS

The analytical method was separated into two parts; liquid and gas part. In the first part, liquid samples from influent and effluent were collected and filtrated with a 0.45 μm membrane filter and kept into the freezer until nitrate and nitrite concentration were analyzed. Nitrate and nitrite concentration were analyzed according to APHA method [8] of ultraviolet spectrophotometric screening method (nitrate concentration) and Colorimetric method (nitrite concentration) by using an UV spectrophotometer (UV-1800, japan). pH was measured with a pH meter (Horiba, B712) after the sample was filtrated using a membrane filter. In the second part; the gaseous hydrogen concentration was analyzed by using a Micro-Gas Chromatography (Varian 490 GC, Netherlands). The carrier gas is helium of 99.99% purity. For H_2 gas quantification, samples were put in an icebox before being analyzed to remove the vapor; each sample used 10 ml of H_2 gas and 3 minute for analyzing.

According to each nitrogenous concentration; nitrogen loading rate (NLR) and nitrogen removal rate (NRR) were calculated by using equation 1 and 2

Calculation equation;

$$\text{NLR} = \frac{\{\text{Inf. } NO_3\text{-N [g/L] x Q [L/d]}\}}{\text{V [m}^3]} \qquad (1)$$

$$\text{NRR} = \frac{\{\text{R.}NO_3\text{-N+ }NO_2\text{-N [g/L] x Q [L/d]}\}}{\text{V [m}^3]} \qquad (2)$$

Where NLR is the nitrogen loading rate (g. N/m^3.d), NRR is the nitrogen removal rate (g. N/m^3.d), Inf. is the influent NO_3-N concentration (g/L), R. is the removal amount (g/L), Q is the water flow rate (L/d) and V is the reactor volume (m^3)

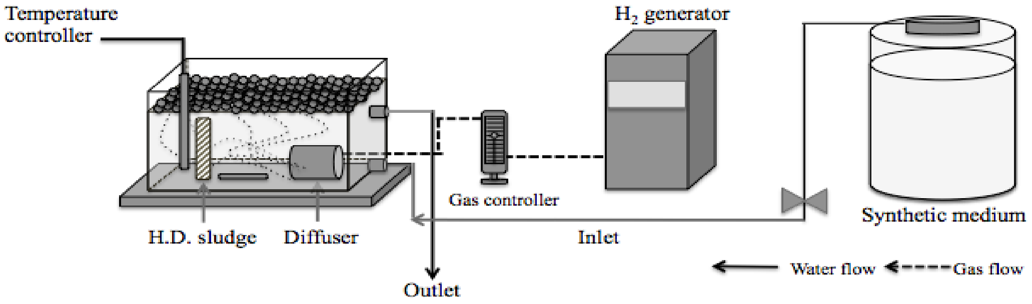


Figure 1 Schematic diagram of experimental setup

Table 1 Operating condition of H.D. reactor.

Con.	Biomass Conc. (g/L)	Influent Conc. (mg/L)	NLR (gN/m^3.d)	H$_2$ flow rate (mL/min)	Total H$_2$ supply (L/d)	Type of H$_2$ supply
1	0.8	60	60	10	14.4	Continues supply (24 h)
2	0.8	60	60	10	3.6	Intermittent 4 time per day (1.5 h/4.5h)
3	0.8	60	60	10	1.8	Intermittent 2 time per day (1.5 h/10.5 h)

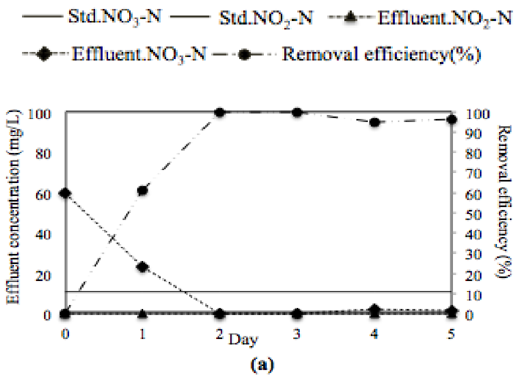
Note: Working volume is 4.2 L, HRT is 24 h and Temperature is 32℃

4. RESULTS AND DISCUSSION

4.1 Effect of H_2 Gas on Hydrogenotrophic Denitrification Activity

This experiment examined the effect of H_2 amounts on H.D. activity by decreasing the amount of H_2 gas supplied under intermittent supply periods. This experiment followed operating condition as presented in table1. The H.D. efficiencies achieved under these three conditions in terms of effluent concentrations (NO_3-N and NO_2-N) and percentage of nitrogenous removal efficiency are shown in figure 2 (a to c).

The results show that the amount of H_2 had a significant effect on the denitrification activity; the H.D. efficiency was decreasing by decreasing amounts of H_2 gas supply. The maximum nitrogen removal rate (NRR) was found to be 58.65 gN/m^3.d, 55.7 gN/m^3.d and 46.23 gN/m^3.d from 60 gN/m^3/d of NLR of conditions 1, 2 and 3, respectively. The corresponding removal efficiencies were 97.75%, 94.45% and 76.84% of condition1-3, respectively. As well, the effluent concentration of NO_3-N and NO_2-N were found to be lower than the potable standard values of 11 and 0.9 mg/L, respectively except in condition 3. Condition 1 and 2 were suitable for H.D. activity because the H_2 gas amount and DH concentration were sufficient for the H.D. bacteria reaction in the reactor. In this way, the removal efficiency for condition 1 reached 97% and for condition 2, 94%, within two to four days of operation. In contrast, condition 3 was found not suitable for H.D. activity due to the limitation of H_2 gas amount. Stopping the supply of H_2 gas for a long in the system can cause low dissolved hydrogen (DH) concentration in the system, which is not enough for H.D. bacteria reaction in the reactor. Incomplete denitrification or low efficiency of H.D. system occurred in condition 3 and the effluent concentration of NO_3-N and NO_2-N were also found higher than potable standard value of 11 mgNO$_3$-N/L and 0.9 mgNO$_2$-N/L, respectively.



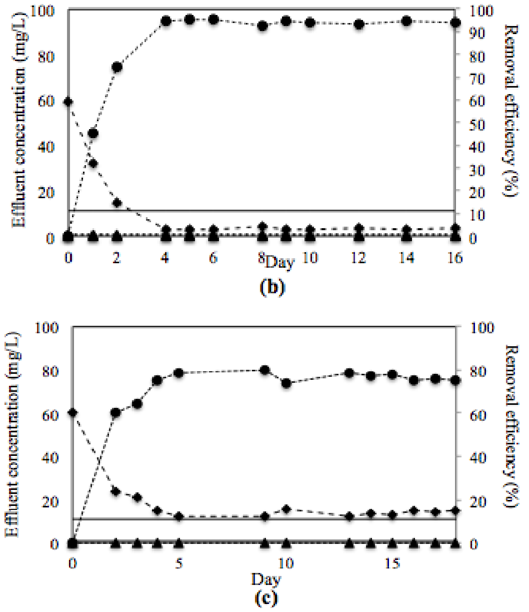


Figure 2 H.D. activity (NO3-N, NO2-N, removal efficiency) at various amount of H_2 supply of (a) continues H_2 supply, (b) supplying intermittent H_2 four times in a day and (c) supplying intermittent H_2 two times in a day

So, H_2 gas amount and DH concentration seemed to have a significant effect on nitrate and nitrite reduction amount and H.D. bacteria activity. Hence, the amount of H_2 gas supply and duration of supply in the system were significant.

4.2 Cost-Effective Analysis on H.D. System

Cost-effective analysis about three conditions of H_2 gas supply was evaluated based on performance of H.D. system as nitrogen removal rate (NRR) and total cost for H_2 supply. The summarized data are shown in table 2. The results found that supplying intermittent H_2 gas four times and two times within a day can save about 75% and 87.5% corresponding to 0.22 USD/d and 0.25 USD/d, when compared to continuously supply. Based on these results, condition 1 and 2 are suitable for H.D. bacteria activity while condition 3 was not, due to the limitation of H_2 gas amount. Moreover, the maximum NRR in condition 2 was of high efficiency, which is almost the same as the continuous H_2 supply. In consequence, the optimum condition of H.D. system was found in condition 2 (3.6 L/d of H_2 supply) under low cost (save H_2 cost) and high effectiveness of H.D. system, making it a preferred option for developing countries.

Table2 Cost-effective analysis between nitrogen removal rate and amount of H_2 supply in each condition

Conditions		**NLR (g N/m^3.d)**	**NRR (g N/m^3.d)**	**Total H_2 supply (L/d)**	**H_2 Cost (Nepal Rs/d, [USD/d])**
1	Continues H_2 supply	60	58.65	14.40	29.82 [0.29]
2	Intermittent H_2 supply 4 times per day (1.5 h/4.5h)	60	55.70	3.60	7.46 [0.07]
3	Intermittent H_2 supply 2 times per day (1.5 h/10.5 h)	60	46.23	1.80	3.73 [0.04]

Note: H_2 gas cost is 2,071 Nepal Rs/m^3 of H_2 gas, 1 USD equal to 102.176 Nepal Rs.

5. CONCLUSIONS

This research analyses the performance of H.D. system under different conditions of H_2 gas supply to achieve the optimal NO3-N removal. The results suggest that intermittent supply of H_2 gas by using Swilling Type microbubble system is a promising method to minimize and H_2 gas supply and therefore cost. However, the amount of H_2 had a significant effect on the denitrification activity; the H.D. efficiency was decreasing by decreasing amounts of H_2 gas supply. In all terms of high efficiency, low H_2 supply amount and low expense, condition 2 in which 3.6 L/d of H_2 gas amount (supplying intermittent four times per day) was found to be appropriate. Particularly, condition 2 can reach the same removal efficiency while 75% of H_2 gas corresponding to 0.22 USD/d can be saved if 14.4 L of H_2 gas is continuous supplied (94% of condition two and 97% of condition one). Further studies are necessary to determine the optimization of H.D. system under simple design and operation, low cost and to determine its suitablility for developing countries. Other parameters such as the minimum amount of H_2 gas consumption, effect of reactor configuration, bubble source or diffuser and so on should be considered.

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