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Removal of nitrate nitrogen from water via aerobic denitrification under static and dynamic experimental settings

Daping Xia[1,2,3], Xinyi Wang[1,3,4*], Zhen Wang[2], Shunan Zhang[1]

[1] *School of Resources and Environment, Henan Polytechnic University, Jiaozuo, China*
[2] *School of Energy Science and Engineering, Henan Polytechnic University, Jiaozuo, China*
[3] *Collaborative Innovation Center of Coalbed Methane and Shale Gas for Central Plains Economic Region, Henan Province 454000, China*
[4] *Institute of China Pingmei Shenma Group, Pingdingshan 467000, China*

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A strain of aerobic denitrifying bacteria was isolated from mine water and further investigated for its characteristics of growth and denitrification. As both of optimal C/N ratio and rotational speed were determined through static-sample experiments, batch-fermentor experiments were subsequently conducted respectively with one-time addition and continuous equivalent additions of nitrate nitrogen (NO_3^--N). A phase of exuberant metabolism and rapid denitrification was noted after 20 h of cultivation, the logarithmic growth phase was observed at the 25th hour, and the duration of the entire lifecycle was determined to be 120 h as a whole. The most effective denitrification was achieved during static-sample experiments under such conditions as nitrate concentration of 360 mg/L, 10% bacterial inoculum, initial pH of 7.5, C/N ratio of 10:1, and rotational speed of 100 rpm, as compared to that of batch-fermentor experiments conducted under similar conditions, but only with lower nitrogen gas production. Although the addition of 10% bacterial inoculum led to the highest NO_3^--N removal rate, the addition of 15% bacterial inoculum accomplished an increased NO_3^--N removal rate after pH adjustment. Continuous equivalent additions of NO_3^--N in the denitrification experiments resulted in a more complete use of carbon sources, more stable pH changes, and more effective denitrification, comparing with one-time NO_3^--N addition. The achieved results provide a critical experimental and industrial reference for the bio-removal of nitrate nitrogen in groundwater.

Keywords: Aerobic denitrification, Batch-fermentor experiment, Bio-removal of nitrate nitrogen, Mine water, Static-sample experiment.

INTRODUCTION

Nitrate and nitrite have been universally accepted as environmental contaminants widely present in groundwater, surface water and then being enriched altogether with the nitrogen cycle in ecosystem and consequently consumed by animals and humans [1]. Nitrate and nitrite are toxic and they can be inter-convertible through a series of chemical and biological processes, during which nitrite is reduced to various compounds or oxidized to nitrate, while nitrate is metabolized to nitrite, which is approximately 10-fold more toxic [2]. High levels of nitrate and nitrite in groundwater mainly result from human and animal wastes and excessive use of chemical fertilizers. The other most common sources are including uncontrolled land discharges of municipal and industrial waste wasters, overflowing septic tanks, processed food, dairy and meat products, and decomposition of decaying organic matters buried in the ground [3].

Nitrate released into environment can lead to serious problems, such as eutrophication, deterioration of water quality and potential hazard to human health [4, 5]. Conventional techniques including coagulation, filtration and disinfection are not effective enough to eliminate nitrate and nitrite contaminants from water. Other methods such as reserve osmosis, ion exchange, adsorption, electrodialysis and chemical treatment have also been developed in order to remove nitrate nitrogen, which are only suitable for lower concentration nitrate wastewater [6, 7, 8, 9]. Other than these physical and chemical processes mentioned above, the biological denitrification process is a comparatively promising as well as low-cost technique for removing the nitrogen-source pollutants from groundwater and has been widely used for total nitrogen (TN) removal from water in recent decades [10, 11, 12, 13, 14], which is in fact a so-called "bio-removal" process involving the transformation of nitrate into nitrogen gas (denitrification). Denitrifying bacteria are one of the significant bioresources applicable in groundwater management by oxidizing organic carbon using nitrate as the electron acceptor under anoxic and aerobic conditions [15, 16, 17]. Our Previous study has proved the presence of

To whom all correspondence should be sent:
E-mail: wangxy@hpu.edu.cn

indigenous denitrifying bacteria in mine water of post-mining operations, to great extent indicating the possibility for removing the nitrate/nitrite pollutants from groundwater through the denitrification of the isolated indigenous bacteria [18].

However, the entire process of denitrification is so complex [19, 20, 21], for it comprises a series of reductions and electron transports and is influenced by some of the factors responsible for this process such as oxygen, temperature, pH, carbon source, C/N ratio, etc, making the biological denitrification of water usually slow and lasting a quite long period. Thus, a highly efficient denitrification process is needed. Some efforts were made previously concerning the simulation of denitrification [22, 23, 24], basically by introducing a prior single experimental setting, which to some extent did not consider the potential influence of the variations of external experimental conditions on denitrification performances.

In present study, we conducted the static-sample and batch-fermentor experiments simultaneously as pilot scale tests to investigate the denitrification performances of indigenous aerobic denitrifying bacteria isolated from mine water under static and dynamic experimental settings respectively. By using glucose as sole carbon source and KNO_3 as electron acceptor, we further introduced the continuous equivalent additions and one-time additions of nitrate nitrogen (NO_3^--N) separately for the batch-fermentor experiments with the removal rates of NO_3^--N in groundwater as the performing indicators, aiming to investigate the differences of the variations of external experimental settings and the influences on biological denitrafication for the removal of nitrate nitrogen in groundwater. We intend to provide a valuable reference for future study and industrial use regarding the biological treatment of nitrate- and nitrite-polluted groundwater.

MATERIALS AND METHODS

Materials

The aerobic denitrifying bacteria, which were isolated from mine water after being cultivated for 96 h in liquid culture medium, were used as inocula for the following experiments. The medium adopted in this study was composed of glucose, peptone, beef extract, yeast extract, Na_2CO_3, and L(+)-Cysteine, as tabulated in Table 1.

Table 1. Constituents of culture medium for isolated bacteria.

Constituent	Mass (g)
Glucose	5–20
Peptone	10–15
Beef extract	2–3
Yeast extract	5–10
Na_2CO_3	3–4
L(+)-Cysteine	0.2–0.6

Analytical methods

Nitrogen and other gaseous co-products produced during fermentation were examined using gas chromatography (SP-2100A, BFRL). The concentrations of reducing sugar that had not been consumed were determined by ultraviolet spectrophotometer (UV5200, Metash), with the curve of glucose as the standard. The concentrations of nitrate ions were determined using phenol disulfonic acid spectrophotometry. The OD_{600} values (optical density) of the cultivated bacteria were determined by ultraviolet spectrophotometer (UV5200, Metash), with distilled water as the control.

Experimental set-up

A glass fermentor with a working volume of 1 L was employed in this study, and its schematic diagram was shown in Figure 1. The fermentor was equipped with a heating pad for controlling the temperature at 35°C and a magnetic stirring apparatus for maintaining a rotational speed of 100 rpm. Besides, pH and ORP meters and an advanced computing system were also applied for monitoring the experimental process.

Determination of the growth cycle of aerobic denitrifying bacteria

A 250-mL conical flask containing 200 mL of the culture medium was inoculated with 10% of the prepared denitrifying bacteria after the pH was adjusted to around 7.0. The flask was sealed and placed in a shaker for cultivation at (37±1)°C and 100 rpm, and the OD_{600} values were determined at specific intervals.

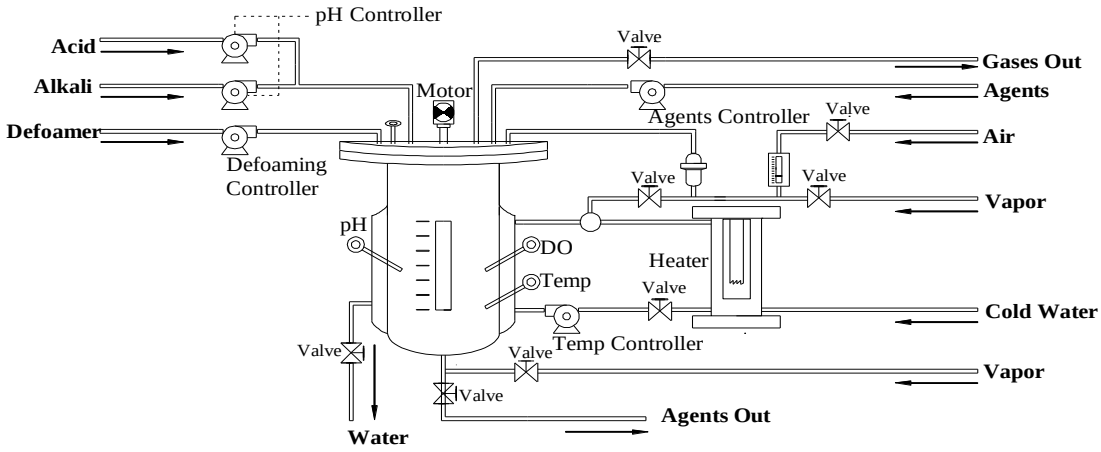

Fig. 1. Schematic diagram of fermentor used in experiments.

Static-sample experiments

The denitrification experiments were performed in a set of simply-connected experimental units (as shown in Figure 2), in which glucose was used as the carbon source and C/N ratios were pre-adjusted to 3:1, 7:1, 10:1, and 20:1. In addition, 200 mL of the culture medium and 360 mg/L NO_3^--N were employed. The experiment was conducted at a constant temperature of 35°C for static cultivation in shakers at 60, 80, 100, and 120 rpm, respectively.

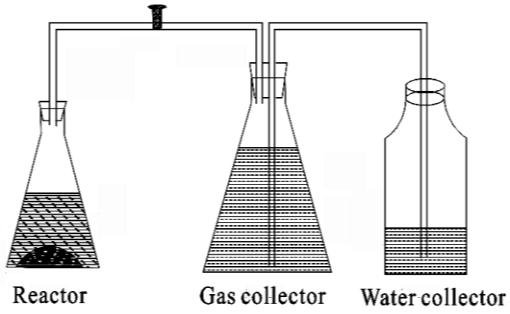

Fig. 2. Static-sample experimental units.

Batch-fermentor experiments

Based on the optimal C/N ratio and rotational speed acquired from static-sample experiments, the batch-fermentor experiments with one-time addition of NO_3^--N were conducted under the same conditions of inoculum, temperature, and pH as those of the static ones. Furthermore, the pH of the cultures with each concentration of the bacterial inoculum was adjusted to 7.5 by adding HCl and the denitrification process was monitored. Furthermore, batch-fermentor experiment with NO_3^--N added as the electron acceptor to the fermentor at specific intervals (continuous equivalent additions) was conducted under the same conditions.

RESULTS AND DISCUSSION

Growth cycle of aerobic denitrifying bacteria

The OD_{600} value of the aerobic denitrifying bacterial culture was 0.291, and 10% of the culture was inoculated into 200 mL of the culture medium and incubated for 72 h. The growth cycle of the inoculated aerobic denitrifying bacteria successively comprised the lag phase, logarithmic growth phase, stable growth phase, and decline phase, as shown in Figure 3. After 10 h of cultivation, the OD_{600} value of the culture, which was nearly 0.2 prior to inoculation, significantly increased during the logarithmic growth phase that occurred at 12–63 h. Then, after 70 h, stable growth phase was observed, during which the amount of bacteria did not exhibit excessive fluctuation.

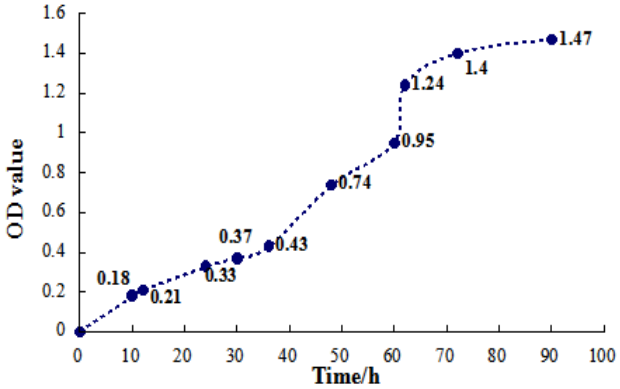

Fig. 3. Growth cycle of isolated bacteria.

Determination of the optimal C/N ratio and rotational speed

As can be observed in Table 2, the removal rate of NO_3^--N was used as an indicator for obtaining the optimal C/N ratio and rotational speed. It reached an appreciable level of 87.30% when the C/N ratio was adjustedto 10:1, the temperature was

maintained at 35°C, and the initial pH was controlled at 7.5. The rotational speed had a major effect on the dissolved oxygen level, and higher rotation speed generally resulted in greater consumption of dissolved oxygen. In the present study, the removal rate of NO_3^--N reached a maximum of 88.13% when the rotational speed was 100 rpm, whereas relative lower removal rates were achieved at 60 and 120 rpm, respectively.

Table 2. Optimal C/N ratio and rotational speed determined by static-sample experiments.

C/N ratios				Rotational speeds (rpm)			
3:1	7:1	10:1	20:1	60	80	100	120
Removal rates of NO_3^--N							
42.96 %	69.50 %	87.30 %	74.50 %	73.25 %	84.11 %	88.13 %	69.34 %

Static-sample and batch-fermentor experiments

The batch-fermentor and static-sample experiments were compared on the basis of optimal C/N ratio and rotational speed. Considering the experimental reality and feasibility, the dosages of all the required reagents for batch-fermentor experiments were noted to be 5-fold higher than those needed for static-sample experiments. With regard to the nitrogen gas production rate and NO_3^--N removal rate, batch experiments yielded a slightly higher nitrogen gas production rate, but lower NO_3^--N removal rate, as compared to static-sample experiments (Figure 4).

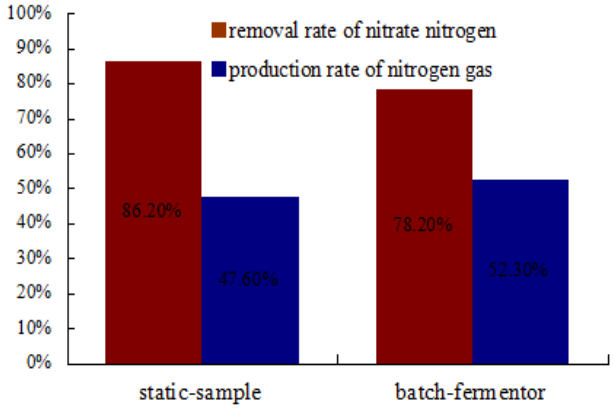

Fig. 4. Comparisons of static-sample and batch-fermentor.

Batch-fermentor experiments with various bacterial inoculum concentrations

Batch-fermentor experiments with different bacterial inoculum concentrations, such as 50, 100, 150, and 200 mL/L, respectively, 1000 g of KNO_3, and a constant OD_{600} value of 0.219 were performed. As shown in Figure 5, the highest NO_3^--N removal rate was achieved at a bacterial inoculum concentration of 100 mL/L, because a modest addition of bacterial inoculum neither resulted in the condition of "rich consumers but poor nutrients" nor "rich nutrients but poor consumers," both of which did produce lower NO_3^--N removal rates. Furthermore, when 1 mol/L HCl was used for adjusting the pH to 7.0 in the post-reactive solution with an inoculum concentration of 150 mL/L (Table 3), both the glucose consumption rate and NO_3^--N removal rate increased at varying degrees possibly owing to the improvement in the growth environment and further enhancement in the metabolic activities of the bacteria in the culture.

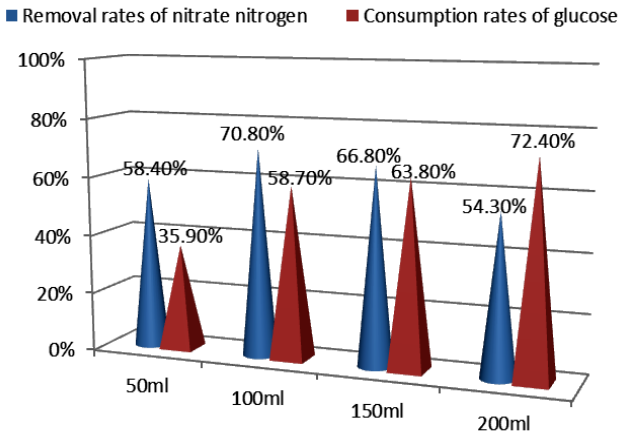

Fig. 5. Effects of various bacterial inocula concentrations.

Table 3. Effects of pH adjustment in culture containing 150 mL/L bacterial inoculum concentration.

150mL bacteria liquid	Consumption rate of glucose	Removal rate of NO_3^--N
Pre-adjustment of pH	63.80%	66.80%
Post-adjustment of pH	81.24%	75.07%

Continuous equivalent additions of NO_3-N

The major difference between reactions performed with one-time addition of NO_3^--N and continuous equivalent additions of NO_3^--N was the time at which NO_3^--N was added. In other words, in reactions performed with "continuous" equivalent NO_3^--N additions, nitrates were added at specific intervals during the experimental process, whereas in reactions conducted with one-time NO_3^--N addition, nitrates were added at the beginning of the batch experiments. Fig. 6 shows that during the first 4 days (96 h) of continuous NO_3^--N additions, the concentration of the residue in the solution was at a relatively low but rather stable level; however, it slightly fluctuated in the last 2 days, particularly reaching a final removal rate of 88.74% at the 120th h. Therefore, the most effective denitrifying period

during reactions conducted with continuous additions of NO_3^--N could be the first 4 days. Besides, a low pH of 6.8–8.7 was maintained throughout the entire reaction process, indicating the lack of accumulation of volatile fatty acids (VFAs) possibly due to neutralization with the OH^- ions produced during the denitrification process.

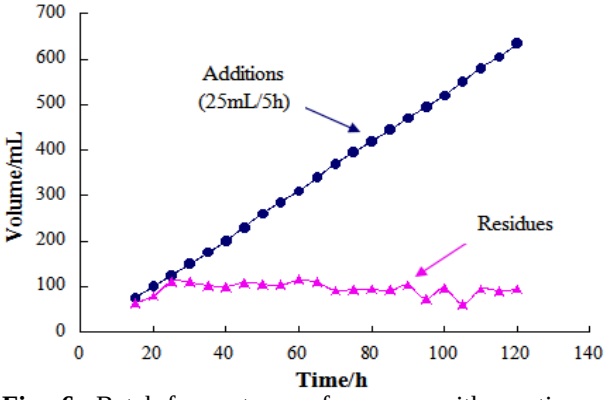

Fig. 6. Batch-fermentor performance with continuous equivalent additions of NO_3—N.

CONCLUDING REMARKS

The C/N ratio of 10:1 and rotational speed of 100 rpm were determined to be optimal through static-sample experiments. An optimal rotational speed can be predominant for maintaining a dissolved oxygen level suitable for the growth of bacteria.

The accumulated denitrification rate of the batch-fermentor experiments was 78.2%, which was 8% less than that of static-sample experiments. The total gas production was 600 mL, of which 68.73% was determined as nitrogen gas. After the adjustment of pH of the culture media to 7.0 with the addition of 1 mol/L HCl, 150 mL/L inoculum achieved the highest denitrification rate of 80.65% among the various bacteria inocula concentrations; however, an inoculum concentration of 100 mL/L exhibited the highest denitrification rate before the pH adjustment.

Furthermore, we concluded that the continuous equivalent additions of NO_3^--N exhibited a 7.7% higher denitrification rate for the batch-fermentor experiments prior conducted, as compared with that performed with one-time addition of NO_3^--N, which was evidenced by the lack of VFA accumulation during the entire process as the pH value reached nearly 7.0, indicating the function of the continuous equivalent additions of NO_3^--N could lead to a higher biological denitrification efficiency.

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