BNP test to evaluate the influence of C/N ratio on N₂O production in biological denitrification

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Abstract

With sufficient carbon sources in the influence to the anoxic stage, the total gas production rate in the BNP (Biological Nitrogen potential) system is rapid with a specific gas production of 3.83 mL/g-VSS-hr. The conversion of nitrate to nitrogen gas is accomplished through three steps: nitrate (NO₃⁻) to nitrite (NO₂⁻) to nitrous oxide (N₂O), and nitrogen gas (N₂). The BNP test results indicate that the optimal C/N ratios are 4.5±0.2, 3.1±0.4, and 2.0±0.2 for these three steps with carbon consumptions being 30%, 24%, and 46% of the total denitrification carbon consumption. With different concentrations of the influent nitrate, the optimal C/N ratios are 5.3~5.6, 4.3~4.7, 3.9~4.0, and 2.5~2.7 for 25, 50, 100, and 200 mg/L NO₃⁻-N, respectively. The conversion rate of N₂O under conditions of sufficient carbon source is 0.005%. If the carbon source becomes insufficient, the N₂O conversion rate may increase 100 times to 0.5%.

Introduction

Nitrous oxide (N₂O), one of the greenhouse effect gases, is increasing globally at an alarming rate of 0.31% per year (Czepiel et al., 1996). Industrial combustion emissions contribute most of the atmospheric N₂O but biological nutrient removal systems have also been reported as potential sources of N₂O by many researchers (Bernet et al., 1996; Schulthess and Gujer, 1996; Thorn and Sorensson, 1996). Under an optimum growth condition, the anoxic biological denitrification process converts nitrate and nitrite almost completely into the inert nitrogen gas. If the growth condition deviates from the optimum condition, other types of nitrogen gas including N₂O may be produced. The production of N₂O gas may be affected by the many parameters such as sludge age, hydraulic retention time (HRT), organic loading, types of organic carbon source, dissolved oxygen (DO), pH, and temperature (Brenner and Argaman, 1992). But the C/N ratio seems to affect the production of N₂O more than any other parameters. Hanaki et al. (1992) have reported that the emission of N₂O is highly related to the C/N (or COD/NOx-) ratio in a biological denitrification process. Usually a higher C/N ratio in the influent is favorable to the conversion of nitrate or nitrite into inert nitrogen gas or the production of less N₂O, but too much organic carbon in the influent will cause an increase of the un-used carbon in the denitrified effluent. Thus the BOD or COD in the treated effluent may greatly increase to exceed the effluent limitations.

Experimental design and materials

Preparation of the Denitrification Bacteria Culture

Sludge samples containing denitrifying bacteria are collected from the mixed liquor of an industrial activated sludge wastewater treatment plant receiving the waste effluent from an Acrylonitrile-Butadiene-Styrene (ABS) manufacturing process. The collected mixed liquor samples are inoculated in a 5-L semi-continuous system that is fed twice daily with a basal media solution to culture the denitrifying bacteria. Table 1 lists the composition of the
basic basal solution. The feeding solution consists of 300 mg COD/L of CH₃COONa as the carbon source and 50 mg/L NO₃⁻-N of KNO₃ as the nitrogen source. Sodium acetate, a less expensive carbon source than other commercially available carbon sources, can be recycled from the waste activated sludge (Chiu et al., 1997). The F/M ratio of the laboratory system is controlled at 0.24g-COD/g-VSS/day.

Table 1 Components of basal media used to culture the denitrifying bacteria

<table>
<thead>
<tr>
<th>Trace Element Nutrient Component</th>
<th>Phosphate Buffer Solution</th>
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<tr>
<td>Content</td>
<td>Content</td>
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<tr>
<td>CaCl₂.2H₂O</td>
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</tr>
<tr>
<td>MgCl₂.6H₂O</td>
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<tr>
<td>KCl</td>
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<tr>
<td>MnCl₂.4H₂O</td>
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<tr>
<td>CaCl₂.6H₂O</td>
<td>2.00</td>
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<tr>
<td>H₃BO₃</td>
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</tr>
<tr>
<td>CuCl₂.2H₂O</td>
<td>0.18</td>
</tr>
<tr>
<td>Na₃MoO₄.2H₂O</td>
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</tr>
<tr>
<td>ZnCl₂</td>
<td>0.14</td>
</tr>
<tr>
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</tr>
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</table>

BNP System

The evaluation is carried out using a Biological Nitrogen Potential (BNP) system, which is based on the Biological Methane Potential (BMP) with significant modifications for more accurate measurement of the gas produced in the biological process. Using the BNP system, the production potential of N₂O and the optimal C/N ratio for minimizing the N₂O production have been determined. Figure 1 shows the schematic drawing of the BNP system used in this study. A 300-mL BOD bottle, which contains 250-mL of the suspension of well acclimated denitrifying bacteria (B in Figure 1) plus 50-mL headspace (A in Figure 1), is used as the reactor. It is sealed and connected to a pressure-stable bio-gas collecting tube (C and D in Figure 1). The right-hand portion of the tube is a tall slender sealed column used to collect the gas, and the left-hand portion is a relatively large container with vent. The gas from the BNP reactor is trapped in the right-hand column to force the liquid into the left-hand container. The air in the left-hand container can be vented to the atmosphere, thus, a relatively constant pressure in the headspace of the BNP reactor can be maintained. The constant gas pressure will enable more accurate measurements of the gas produced in the reactor. Additionally, sampling of the gas can be carried out without disrupting the operation of the BNP reactor.

The sludge with its initial MLVSS adjusted at the 2,500 mg/L level has been washed three times with de-ionized water before it is added to the BNP reactor. At the beginning of the BNP test, argon gas is purged through the reactor at a rate of 2 L/min for 2 to 3 minutes to drive off all dissolved gases in the system. During the study, the reactor content is mixed constantly with a magnetic stirrer, and the content ORP is continuously monitored. The bio-gas collecting tube is filled with 5% HCl solution to keep the carbon dioxide in gaseous form to be collected with the remaining gases (mostly N₂ and N₂O) in the headspace of the right column. Gaseous samples are periodically withdrawn from the headspace through a sampling port on the top of the column for analyses.
Sampling and Measurement of the Gas Produced

Among all the gaseous samples produced, only N₂, N₂O, and CO₂ are of significance to this study. Nitrogen gas (N₂) has a very low solubility and reactivity thus it exists almost entirely in the gas samples collected from the sampling port. Measurements of N₂O and CO₂, which are highly soluble, must be done separately for the gas samples (collected from A and C, respectively, shown in Figure 2) as well as for the aqueous sample collected from the reactor (B in Figure 2). Quantities of N₂O and CO₂ in the gas sample are analyzed on three 1-mL samples collected through the sampling port. The concentration of CO₂ in the aqueous phase is determined based on the measured quantity of dissolved inorganic carbon (DIC). For the soluble N₂O, after the biological reaction is completed, the dissolved N₂O is purged by bubbling 10-L air through the reactor content. All the gas emitted from the reactor is collected in a bag for analyses. The concentration of N₂O in the 5% HCl solution (part D) is calculated from the measured N₂O concentration in the gas sample collected from part C using a pseudo Henry’s Constant, which has been determined based on laboratory data collected earlier.

All gas samples are analyzed within 3 hours after they have been collected. Analyses of N₂, N₂O, and CO₂ are carried out on a 1-mL gas sample with a gas chromatography (China Chromatograph 8700F) using a 3-metre packing column (Chromosorb 102) with high purity nitrogen as the carrier gas, and an electron capture detector (ECD). The injector temperature and the oven temperature are controlled at 200°C and 50°C, respectively. Commercially available N₂, N₂O, and CO₂ standards are used for obtaining all calibration curves.

Results and discussion

The production of a greenhouse gas, nitrous oxide (N₂O), in the biological denitrification process is closely related to the Carbon/Nitrogen (C/N) ratio. In this study, the influence of C/N ratio on the conversion of nitrate nitrogen to N₂ and N₂O as well as the production of N₂O gas in a biological denitrification system receiving different levels of carbon sources have been investigated.

Optimal C/N Ratio

Based on the report of Schulthess et al. (1995), the conversion of nitrate to nitrogen gas is accomplished through three steps: nitrate (NO₃⁻) to nitrite (NO₂⁻) to nitrous oxide (N₂O), and nitrogen gas (N₂). To examine the nitrogen utilization of every step, three BNP tests
were carried out by adding separately 50 mg/L of nitrate (NO$_3^-$-N), nitrite (NO$_2^-$-N), and nitrous oxide (N$_2$O-N) as the nitrogen source. Table 2 shows the concentration variations of dissolved organic carbon (DOC) and nitrogen sources. The optimal C/N ratios are estimated by minimizing residual DOCs and nitrogen compounds, shown in Figure 2, to lead to a nearly complete conversion of all nitrogen compounds to nitrogen gas.

The BNP test results indicate that the optimal C/N ratios are 4.5±0.2, 3.1±0.4, and 2.0±0.2 for these three steps. The data show nitrate (NO$_3^-$-N), nitrite (NO$_2^-$-N), and nitrous oxide (N$_2$O-N) could serve as the nitrogen source of denitrification and imply the carbon consumption is 30%, 24%, and 46% of the total denitrification carbon consumption, separately. With different concentrations of the influent nitrate, the optimal C/N ratios are 5.3~5.6, 4.3~4.7, 3.9~4.0, and 2.5~2.7 for 25, 50, 100, and 200 mg/L NO$_3^-$-N, respectively.

N$_2$O Conversion

The N$_2$O converted from nitrate nitrogen during the denitrification process exists in the BNP test system as: (1) dissolved N$_2$O in the solution of the reactor, (2) N$_2$O gas in the headspace of the reactor, and (3) collected N$_2$O gas in the bio-gas collecting tube. During the denitrification process, most of the N$_2$O produced is dissolved in the aqueous phase of

![Figure 2](https://iwaponline.com/wst/article-pdf/42/3-4/23/428274/23.pdf)

**Figure 2** Optimal C/N ratio determined by residual carbon and nitrate under initial NO$_3^-$-N concentration of 50 mg/L

<table>
<thead>
<tr>
<th>C/N Ratio</th>
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<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
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<tr>
<td>COD (mg/L)</td>
<td>0</td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>400</td>
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<tr>
<td>NO$_3^-$ as the N source</td>
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<tr>
<td>Initial NO$_3^-$-N (mg/L)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Residual NO$_3^-$-N (mg/L)</td>
<td>40.9</td>
<td>23.3</td>
<td>3.5</td>
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<td>ND</td>
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<tr>
<td>Initial DOC (mg/L)</td>
<td>4</td>
<td>38</td>
<td>76</td>
<td>122</td>
<td>156</td>
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<tr>
<td>Residual DOC (mg/L)</td>
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<td>ND</td>
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<td>22</td>
<td>61</td>
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<td>50</td>
<td>50</td>
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<tr>
<td>Residual NO$_2^-$-N (mg/L)</td>
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<tr>
<td>Initial DOC (mg/L)</td>
<td>7</td>
<td>36</td>
<td>72</td>
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<td>153</td>
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<td>Residual DOC (mg/L)</td>
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<td>ND</td>
<td>11</td>
<td>43</td>
<td>64</td>
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<td>N$_2$O as the N source</td>
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<td>Initial N$_2$O-N (mg/L)</td>
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<td>50</td>
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<td>Residual N$_2$O-N (mg/L)</td>
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<td>0.6</td>
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<td>Initial DOC (mg/L)</td>
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<td>78</td>
<td>125</td>
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<tr>
<td>Residual DOC (mg/L)</td>
<td>ND</td>
<td>ND</td>
<td>29</td>
<td>73</td>
<td>83</td>
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</table>
the reactor content. Although a small portion (less than 3%) of the total N₂O may leave the reactor as gas, the summation of all these three forms of N₂O is defined as the “N₂O Potential”. Figure 3 shows a summary of the N₂O conversion rates under different C/N ratios. The N₂O conversion rates are decreasing with the increasing of the C/N ratio. The conversion rate of N₂O under conditions of sufficient carbon source is less than 0.005%. If the carbon source becomes insufficient, the N₂O conversion rate may increase 100 times to 0.5%.

Conclusion
The BNP system can maintain a relatively constant pressure in the headspace thus making the measurement of gas production more accurate and allowing frequent sampling without interrupting the operation. Based on the residual DOC and nitrogen compound method, the optimal C/N ratios are 4.5±0.2, 3.1±0.4, and 2.0±0.2 for three steps of denitrification (NO₃\(^{-}\)-N → NO₂\(^{-}\)-N → N₂O-N → N₂). When the C/N ratio of the BNP system is maintained at the optimal level, the conversion rate of N₂O is observed to be less than 0.5%. If the C/N ratio is higher than the calculated optimal C/N ratio, more N₂O is produced. These results verify the reliability of the BNP system and the validity of the calculated C/N ratio for N₂O produced.

References