COMBINATION OF METHANE OXIDATION AND DENITRIFICATION PROCESS IN A TWO-STAGE BIOREACTOR

Vu Phuong Thu*, Nga Thi Dinh

Hochiminh City University of Natural resources and Environment, 236B Le Van Sy Street, Ward 1, Tan Binh District, Ho Chi Minh City

*Email: thu.vuphuong246@gmail.com

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ABSTRACT

The importance of a combination of methane oxidation and denitrification processes in a two-stage bioreactor was investigated for the removal of nitrate using methane gas. In the configuration I, methane and oxygen were supplied separately to two columns of the two-stage bioreactor, an oxic column and an anoxic column. The nitrate removal efficiency was around 25 % and nitrite presented in the liquid medium, showing that the denitrification process was not complete. In the configuration II, methane and oxygen were supplied together to one column of the two-stage bioreactor, better results were achieved. Nitrate removal efficiency increased to almost 100 %, no nitrite was found in the liquid medium. The methane oxidation and the denitrification processes seemed to be happened simultaneously in one column of the two-stage bioreactor and demonstrated its advantages. Methane utilized concentration in the medium of the methane oxidation column increased from 1 to 2.1 mg/L, which resulted in more soluble organic carbon was created and supplied for denitrifiers. The C/N utilized ratio was lower in the Configuration II showing that the aerobic methane oxidation coupled to denitrification (AMO-D) achieved higher efficiency when methane and oxygen were supplied together.

Keywords: denitrification, methane oxidation, C/N molar ratio, AMO-D, two-stage bioreactor.

1. INTRODUCTION

Denitrification is an anoxic process and is suppressed in the presence of oxygen [1], however, it is an unomissible factor in aerobic methane oxidation couple to denitrification (AMO-D) process, which has been increasingly become an attractive method for denitrification using methane gas [2]. The AMO-D process must be performed in a two-stage process with aerobic methanotrophic bacteria producing metabolites, which are used as hydrogen donor by denitrifier bacteria in anoxic areas [3, 4, 5]. Oxygen is necessary for the oxidation of methane but it may impact on the efficiency of the denitrification process [6]. The separation of the two processes may be an applicable solution. Various experiments were carried out with methane oxidation and denitrification processes simultaneously occurred [7, 8], without considering of the combination and separation of the two processes. This study focused in a new aspect in the
AMO-D process, an evaluation of the combination and separation of the two processes, methane oxidation and denitrification.

Two-stage bioreactor which has two columns connecting by a recirculation line was used to separate the two processes included in the AMO-D process. In this study, each column of the two-stage bioreactor has its own duty to promote anoxic or oxic condition for the denitrification or methane oxidation process. Membrane diffuser was used to supply methane and air to the microbial medium. Packing materials served as a supporter for the microorganisms to grow and then the biofilm growing on the surface of packing materials created anoxic condition for the denitrifiers. The meaning of the combination of methane oxidation and denitrification in the AMO-D process which occurred in a two-stage bioreactor using packing materials was the objective of this study.

2. MATERIALS AND METHODS

2.1. Growth medium and culture

The AMO-D culture was originally enriched from an activated sludge sample taken from an anaerobic digestion reactor in Korea University. Experiments were carried out using a nitrate minerals salts (NMS) medium of the following chemical composition (mg/L): CaCl₂·2H₂O 135; MgSO₄·7H₂O 500; FeSO₄·7H₂O 9.1; KNO₃ 1444. The medium also contained 2 mL/L of phosphate buffer and 1 mL/L of trace element. The phosphate buffer consisted of (g/L) Na₂HPO₄ 10.2 and KH₂PO₄ 24.4. The trace element solution comprised (mg/L): MnCl₂·4H₂O 500, FeSO₄·7H₂O 2486, ZnSO₄·7H₂O 105, NiCl₂·6H₂O 91, CoCl₂·6H₂O 50, Na₂MoO₄·2H₂O 26, H₃BO₃ 50, CuCl₂·2H₂O 212 and 5 mL 35 % HCl.

2.2. Configuration and operation

A two-stage bioreactor was designed with two acrylic columns; the effective volume of each column was 1 litre. Five hollow membrane fibers located at the bottom center of the bioreactor as membrane diffusers supplying gases to the liquid media. The slits in the membrane were holes with elastic lids, acting as valves cutting off bubbles from the gas stream. Each fiber had the outside and inside diameter of 1.65 mm and 0.73 mm, respectively.

In the Configuration I, methane was supplied from a 99.95 % methane cylinder, nitrogen and air were supplied from pure gas cylinders. The NMS medium inside the reactor was circulated using a peristaltic pump at different circulation rates of 6 L/h and 18 L/h. In the Configuration II, only methane and air were supplied to the first column, the NMS medium inside the reactor was also circulated at circulation rates of 18 and 6 L/h. A half of the medium in the two-stage bioreactor (500 mL) was replaced every day. Plastic packing materials were used to support for attached process. Due to biological growth on the surface of the packing material, an anoxic condition was supposed to be created inside the biofilm. The system was shown in Figure 1a and 1b.

In the initial acclimation period, methane and nitrogen was supplied to the first column at a total gas flowrate of 70 mL/min, methane concentration was controlled at 14.7 %, air was supplied to the second column at the same flowrate of 70 mL/min. Nitrate concentration increased from 20 to 50 mg NO₃-N/L. Following the acclimation period, the operation period was divided into six steps with different gas flowrates, recirculation rates and nitrate concentrations. From Steps I to IV, the first configuration was applied. The second configuration...
was applied to Steps V and VI. The two-stage bioreactor was operated for 82 days overall and the operation was described more clearly in Table 1.

**Table 1.** The operation of the two-stage bioreactor.

<table>
<thead>
<tr>
<th>Days</th>
<th>Step</th>
<th>Gas</th>
<th>Liquid</th>
<th>Methane conc. (%)</th>
<th>Nitrate supply as N(mg/d)</th>
<th>Recirculation rate (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flowrate at 1st column (mL/min)</td>
<td>Flowrate at 2nd column (mL/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>System configuration I</td>
<td>System configuration II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-12</td>
<td>Acclimation</td>
<td>70(CH₄+N₂)</td>
<td>70(Air)</td>
<td>14.7</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>12-18</td>
<td>I</td>
<td>70(CH₄)</td>
<td>70(Air)</td>
<td>14.7</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>18-41</td>
<td>II</td>
<td>70(CH₄)</td>
<td>70(Air)</td>
<td>14.7</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>41-58</td>
<td>III</td>
<td>70(CH₄)</td>
<td>70(Air)</td>
<td>14.7</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>58-73</td>
<td>V</td>
<td>140(CH₄+Air)</td>
<td>0</td>
<td>14.7</td>
<td>50</td>
<td>18</td>
</tr>
</tbody>
</table>

2.3. Analytical methods

![Figure 1](image-url)

**Figure 1.** Two-stage bioreactor: a) the first configuration where methane and air were supplied separately; b) the second configuration where methane and air were supplied together.

The liquid samples taken from two columns once in two days were analyzed for dissolved oxygen concentration, chemical oxygen demand, and optical density at 600 nm. Nitrate and nitrite concentration was analyzed using an ion chromatography (IC) (Metrohm 792, Switzerland).
The mixture of gas taken from a gas sampling port located on the top of the reactor including methane, oxygen, nitrogen and carbon dioxide was collected for gas composition analysis. Methane concentration was analyzed using an infrared detector (GFM series, GASDATA, UK) while the carbon dioxide evolved was directly measured using an infrared CO₂ analyser (LI-820, LI-COR, USA).

3. RESULTS AND DISCUSSIONS

3.1. Nitrate, nitrite concentration and optical density in the two-stage bioreactor

Figure 2 demonstrates the nitrate, nitrite concentration and optical density in the liquid medium of the two-stage bioreactor. As mentioned above, there were six steps in the operational process of the experiment. From Steps I to IV, the gas flowrate supplied to the first column included methane and nitrogen was 70 mL/min while the methane concentration was maintained at 14.7 %. Air was supplied to the second column at a flowrate of 70 mL/min, so that the total gas flowrate was 140 mL/min, yielding a gas retention time (GRT) of 7.1 min.

![Figure 2: The nitrate concentration, nitrite concentration and optical density in the two-stage bioreactor.](image)

During the acclimation period, nitrate concentration was almost decreased to zero. In Steps I and II the nitrate concentration supplied increased stepwise from 50 to 100 and 200 mg NO₃-N/L. The recirculation rate of the medium, between the two columns was set at 6 L/h by a
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The nitrate concentration measured in the medium increased from 0 mg/L at the beginning of Step I to 60 mg/L in Step II, it showed that the nitrate removal in the two-stage bioreactor was not effective. The average removal efficiency in Step II was only 32.2%. To enhance the mixing between the two reactors as well as to examine the effect of recirculation rate in the AMO-D process, the recirculation rate was increased from 6 to 18 L/h in Step III. In the first column, the mass transfer coefficients for oxygen increased from 0.009 to 0.028 (1/min) and the mass transfer coefficients for methane increased from 0.001 to 0.024 (1/min). On the contrary, in the second column, the mass transfer coefficients for oxygen decreased from 0.155 to 0.136 (1/min) and the mass transfer coefficients for methane decreased from 0.134 to 0.117 (1/min). In summary, when the recirculation rate changed, the gases were transferred more quickly, the mass transfer in one column increased then it decreased in the other column. As the result, the nitrate concentration in the reactor did not changed substantially. The optical density was not high and remained around abs 0.8 during the operation of the configuration I.

In the next step, Step IV, the nitrate concentration supplied was decreased to 100 mg/L, so the nitrate concentration in the medium was decreased too, but the removal efficiency was still low at 25%. The C/N molar ratio counted on the amount of methane and nitrate available in the medium of the bioreactor were 2.20, 2.40 and 2.57 in Steps II, III and IV (Figure 3). They were calculated as below:

\[
N_{\text{available}} \text{ (mol/d)} = \frac{[N_{\text{supplied}} + N_{\text{remained}}] \times 0.5L}{14 \times 1000} \quad (1)
\]

\[
C_{\text{available}} \text{ (mol/d)} = \frac{(CH_4^{\text{in}} - CH_4^{\text{out}})}{(\text{mol CH}_4\text{-C/d})} \quad (2)
\]

\[
C/N_{\text{available ratio}} = \frac{C_{\text{available}}}{N_{\text{available}}} \quad (3)
\]

This number was low in compare with those in previous studies [1,9]. The carbon source might not enough for the system then the denitrification performance was not good and microbial density was low.

Figure 3. The C/N available molar ratio.

In general, the low removal efficiency showed that this bioreactor configuration was not suitable for aerobic methane oxidation coupled to denitrification. Methane was supplied to the first column, which was in an anoxic condition, only a small amount of methane dissolved in the medium, and was transferred to the second column for the methane oxidation process by
recirculation. The amount of dissolved methane transferred to the second column was not
enough to produce the necessary soluble organic compound for the denitrification process.
Therefore, the nitrate removal efficiency was not good, the denitrification process in this step
was not complete, and nitrite (NO$_2^-$) ions were found in the medium.

In Step V, the configuration of the two-stage bioreactor was changed, methane and air were
supplied together and the combined gas was supplied directly to the first column. The total gas
flowrate supplied, including methane and air, was 140 mL/min, yielding a gas retention time of
7.1 min. The methane concentration was still remained at 14.7 %. The nitrate concentration in
the two-stage bioreactor decreased dramatically and reached nearly zero after 6 days. The
denitrification in this step was complete, so that no nitrite was found. The C/N available molar
ratio increased to 6.71 and mass transfer coefficient 0.153 (1/min) in the first column. The C/N
ratio was in range of the ratio shown in previous studies, from 2.78 to 12, the carbon available
might be enough for the biological process. Besides, the high removal efficiency was due to the
combination of the two processes, methane oxidation and denitrification, in the two-stage
bioreactor. Methane supplied to the reactor was oxidized directly by methanotrophs, releasing
soluble carbon source which was used by coexisting denitrifiers. This reasonable combination
created a stable operation for the reactor and achieved high nitrate removal efficiency. The
optical density suddenly went up to abs 1.5 at the beginning of the operation of the second
configuration. It showed that the second configuration created convenient condition for the
growth of the bacteria.

It can be said that the configuration of the reactor had an important factor to the process.
Methane and air should be supplied together for high efficiency in the methane oxidation as well
as the denitrification. In Step VI, the recirculation rate was decreased to 6 L/h to examine once
again the effect of the recirculation rate on the AMO-D process. The nitrate concentration in
the two-stage bioreactor in these steps did not change substantially. Besides, the decrease of
recirculation rate made the second column develop an absolute anoxic condition. Some bacteria
decayed, and the biomass concentration was decreased so that the optical density decreased.

In the two last steps, the second configuration was applied, and the denitrification rate
increased dramatically. The denitrification rate reached 2.08 mg NO$_3$-N/L/h as well as almost all
nitrate was removed from the medium. The recirculation rate was changed in Steps III and VI to
determine the affecting factors for this process. The results in Figure 2 showed that the
recirculation rate appeared to be not affecting the AMO-D process. Nitrate removal efficiency
was still good as long as the gas was supplied enough and reasonably.

3.2. C/N utilized molar ratio and carbon dioxide relationship

A C/N utilized molar ratio was calculated based on the molar of methane as C and nitrate
as N, which were used in the two-stage bioreactor. The C/N utilized ratio from Step I to Step VI
was high, the averaged number was 11. It meant the nitrate removal efficiency using methane
gas in the Configuration I was low. With the second configuration, the C/N molar ratio
decreased to 4. The relationship between the C/N molar ratio and the amount of CO$_2$ was
measured in the gas outlet of the bioreactor was described in Figure 4.

As shown in this figure, when the amount of CO$_2$ created was not high, the denitrification
process did not get high efficiency so that the C/N molar ratio was high. When the system was
changed to the second configuration, more CO$_2$ was created and C/N molar ratio decreased.

Methane is not a soluble gas; therefore the mass transfer of methane from the first column
to the second column was the limiting point in the first configuration of the two-stage bioreactor
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Even though, the methane oxidation was good in the first column of the second configuration, the second column still did not work well, which reduced the total removal efficiency in the whole system.

![Graph showing CO₂-outlet (mol/d) vs C/N utilized molar ratio (mol C/mol N)](image)

**Figure 4.** The C/N utilized molar ratio and carbon dioxide relationship.

4. CONCLUSIONS

The two-stage bioreactor was designed to examine the combination and separation of methane oxidation and denitrification processes in an AMO-D system. Methane and oxygen should be supplied together to promote a mutual effect between methane oxidation and denitrification processes and enhance the overall efficiency. When methane and oxygen was supplied separately, the methane concentration in the oxidation column was only 0.033 mg/L, the removal efficiency was low at 25%. When the two processes were combined in the Configuration II, more methane and oxygen were utilized; therefore the removal efficiency was higher. More carbon dioxide was created then lower C/N utilized molar ratio was achieved. Besides, C/N available ratio should be high enough for the operation of the system. In the Configuration I, the C/N available was around 2.39 and it might not enough for the combined process.

REFERENCES


