METHANE-DEPENDENT DENITRIFICATION APPLICATION FOR UREA AND AMMONIUM CONTAMINATED WASTEWATER TREATMENT

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ABSTRACT

This study was conducted with synthetic wastewater from urea and ammonia (representing nitrogen fertilizer production wastewater) and additional carbon source of methane (CH_4). Methane and air were supplied to microbial liquid phase using a bubble air stone aerator located at the bottom of a bioreactor with methane gas flowrate ranges from 1 to 2 L/h, air flowrate ranges from 3 to 6 L/h. There were three steps in the experiment with the first step served as acclimation period. Removal efficiency in the second step and the third step with total nitrogen concentration range from 100 to 300 mg/L and from 300 to 600 mg/L are 98.11 ± 0.5% and 81.58 ± 1.17%, respectively. The mixed liquor suspended solids (MLSS) at the end of step III increased by 4000 mg/L in compared with initial microorganism density. The optimal C/N ratio of the study was 6.63 at the methane gas and air flowrate of 2 L/h and 6 L/h, respectively. Denitrification rate reached its highest number of 11.4 mgN/L.h while total nitrogen (TN) was supplied at 600 mgN/L. Thereby, aerobic methane oxidation coupled to denitrification, AMO-D technology, is capable of treating wastewater containing high TN concentration (up to 600 mg/L) but poor in organic matter with methane as the additional source of carbon; and microorganisms could grow well in the condition where oxygen and methane were simultaneously supplied for methane oxidation and nitrogen removal.

Keywords: Organic carbon source, methane denitrification, urea, ammonia.

1. INTRODUCTION

Heterotrophic denitrification consists of the respiration process of microorganisms, using nitrates as the final electron acceptor under the lack of air conditions, so that the electron donor or organic carbon source is an important part of the process. In many real cases, the organic carbon source is the limitation of the process and many wastewater treatment plants have to add organic carbon to the denitrification process, especially for wastewaters with high total nitrogen (TN) concentration but poor in organic matters.

The choice of substrate (carbon source) depends on factors such as cost, efficiency, operating principle of the reactor and based on the next treatment step of denitrified water. Methanol is the most used carbon source [1, 2] and is recommended by the US Environmental Protection Agency as the most suitable substrate because it creates low sludge and does not add nitrogen to the system. In our country, molasses is commonly used as a locally available by-products rich in carbon. However, for a large-scale wastewater treatment plant, the cost of buying methanol or molasses can significantly increase the operating costs. Therefore, the

search for an alternative source of carbon with low cost and high efficiency has been one of the priorities of the wastewater treatment industry in the past two decades [3]. Methane is a potential additional carbon source with low cost, suitable for biological denitrification in organic matter-poor wastewater [4]. Currently, many wastewater treatment systems have wastewater or sludge anaerobic digesters but do not effectively utilize the amount of methane generated or the amount of gas is excessive for on-site reuse leading to wasteful disposal and potentially causing fire and explosion. In addition, methane is a greenhouse gas, so the use of methane from anaerobic digestion tanks is an appropriate option to minimize global warming when methane is disposed indiscriminately.

Various experimental models of bioreactor with different operating methods have been carried out to verify the process of aerobic methane oxidation combined with denitrification (AMO-D) and many studies have been achieved positive results [5-10]. In the presence of oxygen, methane is oxidized by aerobic oxidizing bacteria, releasing organic matter. These organic substances are used by denitrifying bacteria that co-exist in the reactor. Soluble organic compounds may include methanol [5], citrate [11], acetate [5, 12], proteins [12], nucleic acids [13], and carbohydrates [14]. Methane oxidizing bacteria, methanotrophs are widely available in nature, so it is easy to find in environments such as soil, swamps, landfills, and mud, especially in environments where methane is produced as much as anaerobic slurry.

Different from the studies accomplished mainly focusing on the combination of methane oxidation and denitrification, using synthetic wastewater with nitrate as the nitrogen source [10, 15-17], this study used wastewater synthesized from urea and ammonium, towards a combination of methane oxidation with urea hydrolysis, nitrification and denitrification together in a bioreactor to confirm the ability of AMO-D technology to treat nitrogen existing in form of NH_2^-, NH_4^+, often found in domestic, livestock wastewater, leachate or nitrogen fertilizer production wastewater. In addition, previous studies on AMO-D technology have rarely been applied to concentrations of TN above 200 mg/L because bacteria are likely to be inhibited when TN concentration is too high. For instance, Rajapakse and Scutt witnessed the efficiency of denitrification process declined when TN concentration increased [17]. Methane oxidation coupled to denitrification has been applied with concentration of TN 20 mgN/L [10], 30 mgN/L [17], 30-40 mg/L [19], 200 mg/L [5, 6, 20] and 100-400 mg N/L [21]. This study tests the capability of AMO-D process with high input TN concentrations up to 600 mg/L to consider the microbiological adaptability as well as nitrogen removal efficiency of the process.

2. MATERIALS AND METHODS

2.1. Growth medium and culture

Synthetic wastewater was used with concentration of urea as N and ammonia as N were in range of 20-600 mgN/L. Nutritional salts were added to facilitate the growth of microorganisms. The wastewater used as the medium in this experiment contained the following composition (mg/L) [6]: $MgSO_4 \cdot 7H_2O$ 500; $CaCl_2 \cdot 2H_2O$ 135; $FeSO_4 \cdot 7H_2O$ 9.1; NH_4OH 375-750; $(NH_2)_2CO$ 86-1286. The medium also contained 2 mL/L of phosphate buffer and 1 mL/L of trace element. The phosphate buffer consisted of (g/L) KH_2PO_4 24.4; Na_2HPO_4 10.2. The trace element solution comprised (mg/L): $FeSO_4 \cdot 7H_2O$ 2486, $MnCl_2 \cdot 4H_2O$ 500, $ZnSO_4 \cdot 7H_2O$ 105, $NiCl_2 \cdot 6H_2O$ 91, $CoCl_2 \cdot 6H_2O$ 50, $Na_2MoO_4 \cdot 2H_2O$ 26, H_3BO_3 50, $CuCl_2 \cdot 2H_2O$ 212 and 5 mL 35% HCl. An activated sludge sample taken from the centralized wastewater treatment system of Vinh Loc industrial park was used as inoculums

for the experiment. The microorganisms were cultivated in a two-liter flask with the nutrient medium to reach a MLSS concentration of 4000 mg/L at the beginning of the experiment.

2.2. Configuration and operation

Bioreactor was designed with an acrylic column with effective volume of 0.5 litre. Methane and air were supplied to the liquid phase of the reactor continuously using a stone diffuser. Methane was supplied from methane cylinder (purity of 99.95%) and air was supplied from an air pump through an air flow meter. Plastic packing media with contact area of 650-750 m^2/m^3 was used to support for attached process. Due to biological growth on the packing material, an anoxic condition was created inside the biofilm. A half of the medium (250 mL) was replaced every day giving a hydraulic retention time of 2 days.

The operation period is divided into 3 steps, with different regimes of gas flowrate and total nitrogen supplied. In the first 2 steps, the nitrogen component in synthetic wastewater was 100% urea, but in Step III they were urea and ammonia in a 1: 1 ratio. In Step I, the total gas flowrate was kept at 5 L/hour, while the TN concentration changed stepwise from 20 to 100 mgN/L. Step I served as acclimation period. In Step II and Step III, TN concentration varied from 100 to 300 mgN/L and from 300 to 600 mgN/L, respectively; methane and air were supplied with different regimes as described in Table 1. The reactor was operated for 78 days overall.

Table 1. The operation of the system with 3 steps

Step	Day	Nitrogen content in synthesis wastewater	TN concentration inlet (mgN/L)	Total gas flowrate (L/h)	Methane gas flowrate (L/h)	Air flowrate (L/h)
1	0-30	Urea	20-100	4	1	3
2	30-54	Urea	100-300	4-8	1-2	3-6
3	54-78	Urea and ammonium	300-600	8	2	6

The system was showed in Figure 1.

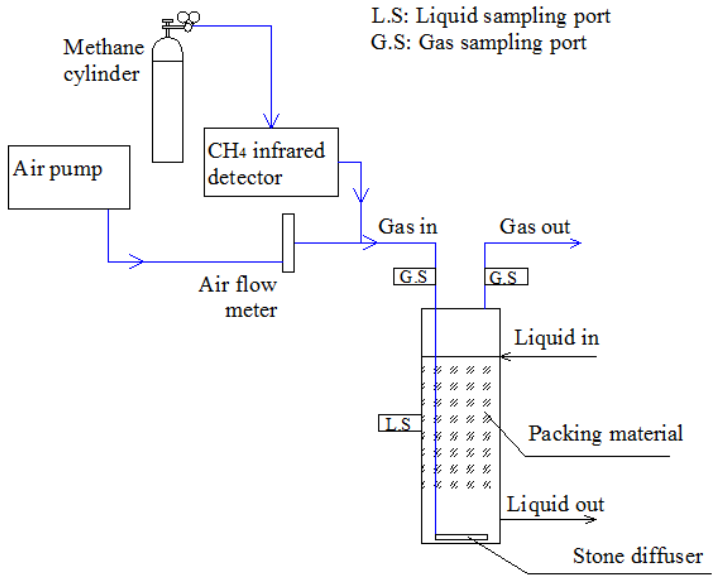

Figure 1. AMO-D bioreactor with methane and air supplied together.

2.3. Analytical methods

The liquid samples taken from reactor once in two days were analyzed for pH, total nitrogen (TN) concentration and optical density at 600 nm. 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 analysis. Methane concentration was measured using an infrared detector (HC/CH_4 RI – 415).

3. RESULTS AND DISCUSSION

3.1. Optical density

In experimental model, the bacteria concentration gradually increased in Step I while having a lot of fluctuation in the two next steps. In the first step, the microbial density went up to an optical density (OD) of 0.8 abs (at 600 nm) when total nitrogen concentration inlet rose from 20 to 100 mg/L (Fig. 2). The rapid increasing of TN concentration in Step II (100-300 mgN/L) and step III (300-600 mgN/L) affected the growth of microorganism in the reactor, bacteria concentration went up and down in the OD range from 0.6 to 1.25 abs. The change of nitrogen concentration in the mixed liquor made bacteria require a period of time to adapt with new environment, thererfore sometime the bacteria concentration decreased. However, in general, optical density increased from step I to step III, even when the TN concentration inlet climbed to 600 mgN/L. At the end of the experiment, MLSS concentration was 8000 mg/L, which double to the intial concentration of the model. It can be said that microorganism could grow well in the condition where methane was supplied as a carbon source for assimilation and denitrification in a reactor with very high nitrogen concentration.

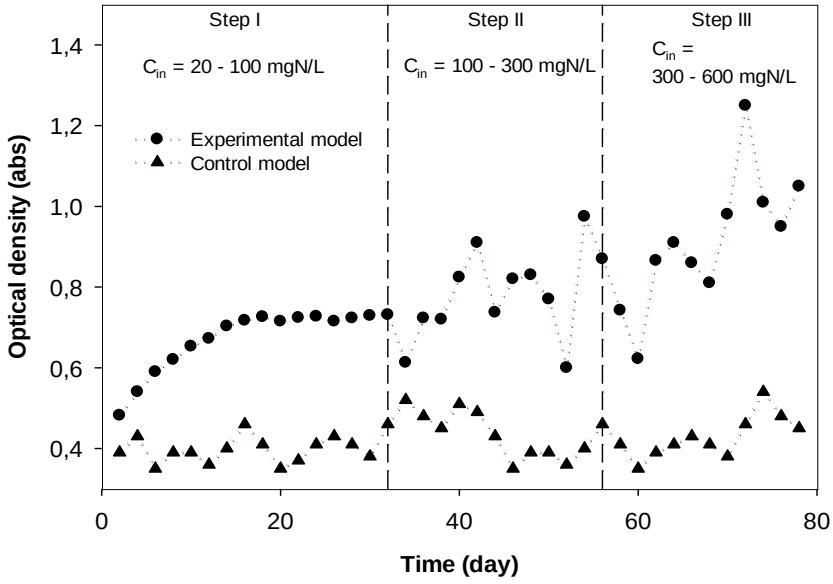

Figure 2. Microbial density in AMO-D bioreactor.

Besides, in control model, optical density fluctuated around 0.4 and seemed not to be increased due to the lack of carbon source. It proves that the methane supplied in the experimental model was oxidized to soluble carbon compounds by methanotrophs, which

created subtrates for the assimilation and lead to the growth of microorganism better than in control model which was not supplied by methane.

3.2. Denitrification rate

In term of denitrification rate, in experimental model the rate went up almost together with the increasing of nitrogen supplied to the reactor in each step. However, in some specific days, total nitrogen concentration had significant increases and denitrification rate went down (Figure 3).

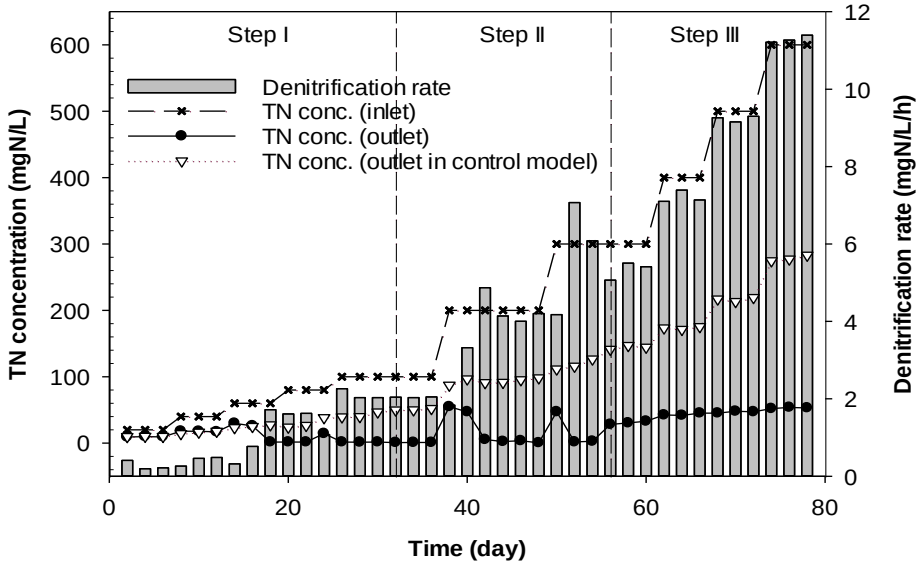

Figure 3. TN concentration and denitrification rate.

In Step I, when TN concentration inlet rose stepwise from 20 to 100 mg/L, denitrification rate fluctuated from 0.4 mgN/L.h to 2.26 mgN/L.h. Denitrification rate in step II was higher than Step I and got the average value about 4 mgN/L.h. The rate reached its highest number of the whole experiment in Step III, which was 11.4 mgN/L.h. Nitrogen was supplied with high concentration and good performance of microorganism for the nitrogen removal made the denitrification rate grow in Step III.

On the other hand, TN concentration outlet (experimental model) in the two first steps was lower than in the last step. In acclimation period - step I since the bacterial concentration was low, TN concentration outlet was relatively high, around 10-20 mgN/L but since day 18, when optical density was almost stable above 0.7, TN concentration outlet dropped to 1.4 mg/L and stayed at this level until the end of Step I.

In Step II, day 38, TN concentration inlet jumped from 100 to 200 mg/L then the outlet leaped from 0.9 to 54.8 mgN/L and remained high in day 40. A change of air flow rate from 3 L/h to 6 L/h showed a remarkable variation of TN concentration outlet and denitrification rate which reached 4.9 mgN/L.h in day 42. The rate stayed higher than 4 mgN/L.h with the same TN concentration inlet of 200 mg/L in these following days.

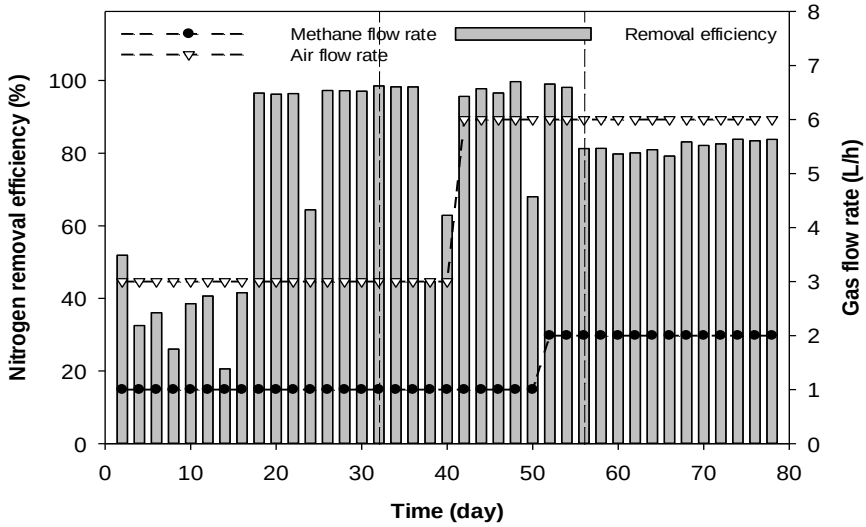

Figure 4. Removal efficiency with different gas flow rate.

Due to the sudden change of TN concentration to 200 mg/L, bacteria needed time to adjust to new conditions. Besides, mass of nitrogen to be removed was high and it required a better mass transfer of methane to supply more soluble carbon for the denitrification process. An increase in gas flowrate as well as mass transfer rate made a clear change. The mass transfer appeared to be clearly important for the denitrification using methane because of its low solubility [21]. After 4 days, TN concentration outlet went back to low value, with 5.3 mgN/L (day 42) and 0.31 mgN/L (day 48).

In day 50, there was a change in TN concentration inlet, from 200 mgN/L to 300 mgN/L, the TN concentration outlet went up again to 47.1 mgN/L. Total gas flow rate was gently increased to 8 L/h to adapt with the ability of mass transfer and supply carbon source for bacteria (Figure 4). The removal efficiency rose to 99% after two days (day 51).

The content of synthetic wastewater supplied in Step 3 including urea and ammonia was different from its in Step 1 and Step 2, which contained only urea as nitrogen source provided. Denitrification rate declined from 6.1 (day 54) to 5.1 (day 56), as a result, removal efficiency dipped from 98.1% (day 54) to 81.3% (day 56). The change of content in wastewater affected to microorganism and made the slight reducing of removal efficiency. When TN concentration inlet grew to 400, 500 and 600 mgN/L in step III, denitrification rate jumped to around 7.2 mgN/L.h, 9.2 mgN/L.h and 11.3 mgN/L.h, respectively. The average efficiency in Step 3 was 81,8% and TN concentration outlet was around 43 mgN/L in average. To compare with the QCVN 40:2011/BTNMT - National technical regulation on industrial wastewater, the value of pollution parameter total nitrogen of industrial wastewater in columm B was 40 mg/L, so that the TN concentration outlet in the experimental model nearly met the standand. In this step, total nitrogen supplied went up to 600 mgN/L, the high concentration could restrict the microorganism and make the denitrification rate drop, this issue was mentioned in a research “Bio-removal of nitrogen from wastewater - A review” [23]. To increase the denitrification rate and met the national requirement, the parameter of pH, C/N ratio and microbial density should be optimized in a following research.

In control model, without methane, nitrogen removal efficieny varied from 30 to 48%. The average removal efficiency in Step I, II and III were 39.6%, 35.3% and 35.8%, respectively. Nitrogen concentration oulet was very high and peaked at 156.9 mgN/L with

the nitrogen concentration inlet of 600 mgN/L. It was easy to see that methane played a very important role in the denitrification process.

3.3. C/N molar ratio

The C/N molar ratio counted on the amount of methane and nitrate available in the medium of the bioreactor changed with the variation of inlet nitrogen concentration between 100 and 600 mgN/L (Figure 5). They were calculated as below:

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

$$C_{available} \text{ (mol/d)} = (CH_{4\ in} - CH_{4\ out}) \text{ (mol } CH_4\text{-C/d)} \quad (2)$$

$$C/N \text{ available ratio} = C_{available} / N_{available} \quad (3)$$

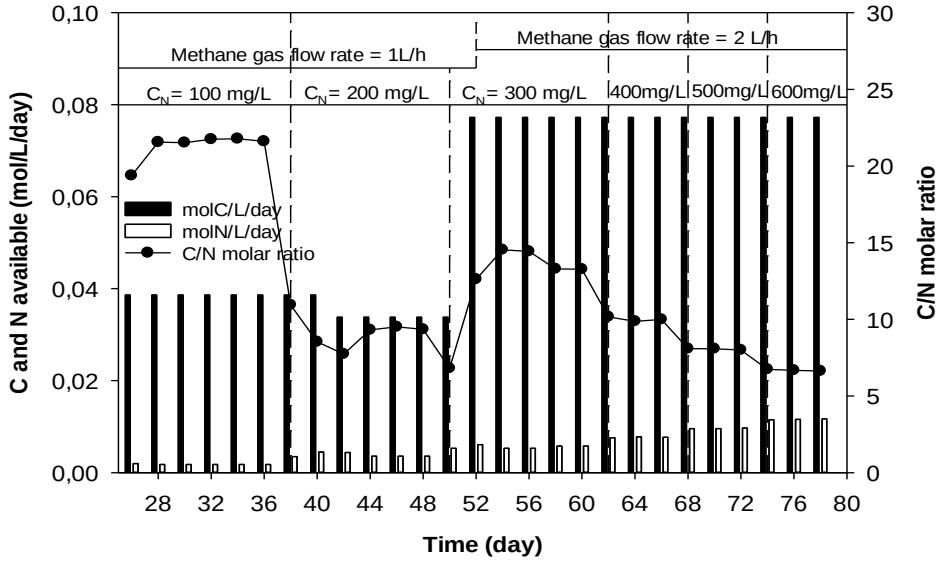

Figure 5. C/N available ratio

At TN concentration of 100 mg/L, the C/N ratio almost higher than 20 due to the excess of methane supplied while nitrogen was limited in the liquid. The ratio suddenly plunged to 11 when inlet nitrogen concentration went up to 200 mgN/L. At this concentration, the lowest C/N ratio was 7.74, nearly equal to the average C/N ratio in previous study (7.4) [22]. From the 52nd day, methane flow rate rose to 2 L/h and made an increase in C/N ratio. In the following days, C/N ratio declined with a gradual rising of inlet nitrogen concentration and reached its best number of 6.63 in day 78. The C/N ratio was in range of the ratio shown in previous studies, from 4.0 to 12.7, the carbon available might be enough for the biological process.

4. CONCLUSIONS

The study determined the ability to treat nitrogen-containing wastewater existing in the form of urea and ammonium with high concentration and proportion corresponding to wastewater producing nitrogen fertilizer by aerobic methane oxidation combined with denitrification (AMO-D). In Step II and Step III, efficiency of the denitrification process were 98.11 ± 0.5% and 81.58 ± 1.17%, respectively. At the end of Step 3, MLSS content in the tank increased by 4000 mg/L compared to the initial amount of sludge put into the tank,

which proved that bacteria could adapt to the experimental conditions. In term of nitrogen removal efficiency, the highest number was achived (99.69%) at inlet TN concentration of 200 mgN/L, its outlet was 0.31 mgN/L. However, in term of denitrification rate and C/N ratio, the optimal number in the study was gained at the highest inlet TN concentration of 600 mgN/L, which were 11.4 mgN/L.h (denitrification rate) and 6.63 (C/N ratio). In reported studies, denitrification rate varied from 0.7 mgN/L.h [19] to 22.9 mgN/L.h [7] and C/N ratio was in range from 4.0 to 12.7 mol-CH_4-C/mol-NO_3-N [24]. These indicated that AMO-D technology could handle wastewater containing high nitrogen content (up to 600 mg/L) with methane as external carbon source and microorganisms could grow well under condition that oxygen and methane were supplied simultaneously for methane oxidation and denitrification. The technology should be applied in actual wasterwater in future studies to confirm the capable of being applied to nitrogen fertilizer production wastewater, leachate or livestock wastewater.

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TÓM TẮT

ÁP DỤNG PHƯƠNG PHÁP LOẠI BỎ NITƠ VỚI NGUỒN CƠ CHẤT LÀ MÊTAN ĐỂ XỬ LÝ NƯỚC THẢI Ô NHIỄM URÊ VÀ AMONI Ở NỒNG ĐỘ CAO

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Nghiên cứu được tiến hành với nước thải tổng hợp từ urê và amoni (đại diện cho nước thải sản xuất phân đạm), sử dụng mêtan làm nguồn cacbon bổ sung cho quá trình xử lý nitơ trong nước thải bằng phương pháp sinh học. Mêtan và không khí được cung cấp vào bể phản ứng bằng đá sủi bọt khuếch tán khí đặt dưới đáy mô hình với lưu lượng khí mêtan 1-2 L/h, lưu lượng không khí từ 3 đến 6 L/h. Nghiên cứu chia làm ba giai đoạn với giai đoạn 1 đóng vai trò giai đoạn thích nghi. Hiệu suất xử lý trong giai đoạn 2 (khi TN = 100-300 mg/L) và giai đoạn 3 (khi TN = 300-600 mg/L) lần lượt là 98,11 ± 0,5% and 81,58 ± 1,17%. Ở cuối giai đoạn 3, nồng độ hỗn hợp chất rắn lơ lửng (MLSS) trong bể tăng thêm 4000 mg/L so với mật độ vi sinh ban đầu. Tỷ lệ C/N tối ưu của nghiên cứu là 6,63 với lượng khí mêtan và không khí cung cấp lần lượt là 2 L/h và 6 L/h. Tốc độ khử nitơ đạt giá trị cao nhất là 11,4 mgN/L.h khi nồng độ TN trong nước thải cấp vào bể là 600 mgN/L. Có thể thấy công nghệ oxy hóa mêtan hiếu khí kết hợp khử nitơ (AMO-D) có khả năng xử lý được nước thải chứa hàm lượng nitơ cao (đến 600 mg/L) nhưng nghèo chất hữu cơ với nguồn cơ chất đầu vào là khí mêtan và vi sinh vật có thể sinh trưởng tốt trong điều kiện được cung cấp đồng thời oxy và mêtan cho quá trình oxy hóa mêtan và khử nitơ.

Từ khóa: Nguồn cacbon bổ sung, khử nito, oxy hóa mêtan, urê.