An A2O-MBR system for simultaneous nitrogen and phosphorus removal from brewery wastewater

Van Nu Thai Thien¹, Dang Viet Hung²,*⁵, Nguyen Thi Thanh Hoa³

ABSTRACT
Anaerobic/Anoxic/Oxic – Membrane BioReactor (A2O-MBR) system was used to enhance simultaneous removal of nitrogen and phosphorus from brewery wastewater. The A2O unit containing microorganisms with short solids retention time (SRT) was employed mainly for removal of organic matter and phosphorus together with denitrification. The MBR containing microorganisms with long SRT was employed mainly for nitrification of NH₄⁺-N and recirculation of NO₃⁻-N. The model of A2O-MBR system made from polycrylic with the capacity of 49.5 liters was operated with hydraulic retention times decreased from 24, 18 to 12 hours corresponding to organic loading rates increased from 0.50, 0.75 to 1.00 kg COD/m³.day. The results showed that the model not only removed organic matter well but also nearly completely removed both nitrogen and phosphorus. For all three loading rates, chemical oxygen demand (COD) concentration decreased significantly. For loading rate of 0.75 kg COD/m³.day, treatment efficiencies of COD, NH₄⁺-N, total nitrogen (TN) and total phosphorus (TP) of the model were the highest as 95.4, 99.2, 86.7 and 84.6%, respectively. Output values of these parameters were within the limits of Vietnam National Technical Regulation on Industrial Wastewater (QCVN 40:2011/BTNMT), column A. The model of A2O-MBR system was capable of achieving effluents with very low nitrogen and phosphorus concentrations from brewery wastewater.

Key words: A2O-MBR system, brewery wastewater, nitrogen removal, phosphorus removal

INTRODUCTION
By Vietnam Beer Alcohol Beverage Association, Vietnamese people consumed nearly 4.1 billion liters of beer in 2017. Currently, there are approximately 129 brewery production facilities across the country with the installed capacity of 4.8 billion litres of beer. Along with this consumption, serious problems with environmental pollution may be caused by a huge amount of brewery wastewater. This amount of wastewater must be treated before discharge into environment. To brewery wastewater, a combination anaerobic-aerobic treatment system has been used and traditional aerobic biological treatment processes such as activated sludge (suspended growth) or biological filter (attached growth) are often implemented. Nevertheless, these processes have not yet treated thoroughly nitrogen and phosphorus from brewery wastewater to meet QCVN 40:2011/BTNMT, column A. Anaerobic/Anoxic/Oxic (A2O) process commonly used in wastewater treatment is able to remove organic matter together with nitrogen and phosphorus with its own inherent advantages such as short hydraulic retention time (HRT), high pollutant removal efficiency and good shock loading capacity. The process consists of three anaerobic, anoxic, oxic compartments and one settling tank which are arranged in sequence with nitrate circulating flow from the oxic compartment to the anoxic compartment and sludge circulating flow from the settling tank to the anaerobic compartment. In this process, nitrification by nitrifiers occurs in the oxic compartment; denitrification by denitrifiers in the anoxic compartment; absorption of β-polyhydroxybutyrate (PHB) for phosphate release by Phosphorus Accumulating Organisms (PAOs) in the anaerobic compartment and then oxidation of PHB for phosphorus accumulation in the oxic compartment. Excess sludge discharge occurs in the settling tank. However, A2O process is a single sludge process with the only line for excess sludge discharge at the settling tank so there has been limitation to satisfy a proper
SRT for both nitrifiers and PAOs in the oxic compartment of A2O process\textsuperscript{8,9}. On the other hand, nitrifiers need long SRT and PAOs need short SRT. To solve this problem, incorporation of a biological reactor into A2O unit, so-called A2O – Biological Reactor system, for simultaneous nitrogen and phosphorus removal has been attempted in the past decade\textsuperscript{8–10}. The A2O unit containing microorganisms with short SRT is employed mainly for removal of organic matter and phosphorus together with denitrification. The biological reactor containing microorganisms with long SRT is employed mainly for nitrification of NH\textsubscript{4}\textsuperscript{+}-N and recirculation of NO\textsubscript{3}\textsuperscript{-}–N.

Weitang Zhang et al. (2013) studied removal of nutrient from domestic wastewater with low COD/N ratio by an A2O–Biological Aerated Filter (A2O-BAF) system. The favorable V\textsubscript{anoxic}/V\textsubscript{oxic} was from 2.5:1 to 6:1. When V\textsubscript{anoxic}/V\textsubscript{oxic} was 6:1, treatment efficiencies of COD, TN and PO\textsubscript{4}\textsuperscript{3–}–P achieved very high values as 89 ± 4, 83 ± 3 and 99 ± 1%, respectively\textsuperscript{11}. Recently, membrane bioreactor (MBR) is an attractive process that has been increasingly used for advanced biological wastewater treatment. With membrane filtration replacing secondary clarification, MBR possesses a number of merits such as biomass enrichment, perfect nitrification, small footprint, ensured sludge-effluent separation, easy manipulation of HRT and SRT, and excellent effluent quality with little organic and solid contents\textsuperscript{12–15}. Thus, MBR was selected as Biological Reactor in the combined system because of the capacity to achieve enhanced nitrification rate and produce high quality effluent\textsuperscript{16,17}.

In this study, an A2O-MBR system was used to evaluate the effects of loading rate on the combined system's simultaneous nitrogen and phosphorus removal performance via continuous flow by treating real brewery wastewater. The role of MBR in the combined system and its contribution to organic matter, nitrogen and phosphorus removal were also investigated.

**MATERIALS AND METHODS**

**Experimental**

The polyacrylic model of A2O-MBR system included Anaerobic/Anoxic/Oxic unit having an approximate dimension of 480 mm L x 150 mm W x 600 mm H with the corresponding working volume of 36.0 liters which was divided by baffles to create three compartments (anaerobic, anoxic, oxic) in ratio of 2:4:2\textsuperscript{11} and MBR having an approximate dimension of 180 mm L x 150 mm W x 600 mm H with the corresponding working volume of 13.5 liters. Total working volume of the model was 49.5 liters. Settling tank had an approximate dimension of 150 mm D x 300 mm H with the working volume of 7.2 liters. In the MBR, a polyethylene hollow-fiber membrane module (0.4 μm pore size, 0.32 m\textsuperscript{2} effective area, Mitsubishi Rayon Co., Ltd, Japan) was immersed. Aeration was provided through fine air diffusers from the bottoms in the oxic compartment and MBR while sludge in the anaerobic and anoxic compartments was suspended by paddle mixers at 50 rpm. Effluent was withdrawn through the membrane module by a suction pump that was designed for intermittent operation with a duty cycle of 8 minutes ON / 2 minutes OFF. To mitigate membrane fouling, backflushing was carried out every 24 hours for 15 min. Dissolved oxygen (DO) concentrations of the oxic compartment and MBR were determined by DO meter and controlled from 2 to 4 mg/L\textsuperscript{18}. Return effluent ratio of 200% and return sludge ratio of 100% were fixed\textsuperscript{9}. Schematic representation of the experimental system was represented in Figure 1.

1/Wastewater tank: 200 liters (PE, Vietnam); 
2/Anaerobic/Anoxic/Oxic unit with three compartments: 36.0 liters (Polyacrylic, Vietnam); 
3/Settling tank: 7.2 liters (Polyacrylic, Vietnam); 
4/MBR with a polyethylene hollow-fiber membrane module: 13.5 liters (Polyacrylic, Vietnam); 
5/Middle tank: 50 liters (PE, Vietnam); 
6/Feed pump: 11 liters/hour (Sandur, India); 
7/Effluent pump: 16 liters/hour (Sandur, India); 
8/Suction pump: 11 liters/hour (Blue & White, United State); 
9/Sludge pump: 11 liters/hour (Sandur, India); 
10/Paddle mixer 1: (IWAKI, Japan); 
11/Paddle mixer 2: (IWAKI, Japan); 
12/Blower 1: 38 liters/min (RESUN, Ap 001, China); 
13/Blower 2: 38 liters/min (RESUN, Ap 001, China); 
14/Sludge valve 1: ∅13 (Copper, Vietnam); 
15/Sludge valve 2: ∅13 (Copper, Vietnam).

**System operating conditions**

The wastewater treatment experiment was conducted in four phases in the laboratory at room temperature (∼ 25°C). In the short initial phase, so-called phase 0, seed sludge was given to 50% volume of the model with MLSS concentration about 5000 mg/L. Influent wastewater with average COD concentration of 500 mg/L diluted with tap water was pumped into the model. Organic loading rate was increased little by little from 0.1 to 0.3 kgCOD/m\textsuperscript{3}.day. The phase 0 ended when COD removal efficiency remained stable at above 80%. There was no sludge discharged except sampling to keep large amounts of biomass.
In the next three phases according to overall treatment performance in relation to the different loading rates, denoted as 1, 2 and 3, respectively, raw wastewater was pumped continuously with wastewater flow rates increased from 49.5 to 99.0 liters/day corresponding to HRTs decreased from 24 to 12 hours and organic, nitrogen, phosphorus loading rates increased from 0.5 to 1.0 kgCOD/m$^3$.day, 0.08 to 0.16 kgTN/m$^3$.day, 0.014 to 0.028 kgTP/m$^3$.day, respectively as in Table 1. Excess sludge was discharged from the A$_2$O unit and MBR to maintain SRTs from 5 to 7 days and from 45 to 60 days, respectively. Trans-membrane pressure (TMP) was used as an indicator of membrane fouling and monitored continuously by a data logging manometer. When TMP reached 40 kPa, membrane washing was performed physically and chemically following the guidelines of the manufacturer. In the phases 0, 1, 2 and 3, the membrane module was physically washed on a daily basis for 15 min. During the entire period of experiment, the TMP was maintained below 40 kPa. Therefore, the membrane module was not cleaned chemically.

**Wastewater source**

Brewery wastewater came from the outlet of the UASB reactor of Wastewater Treatment Plant at Nguyen Chi Thanh – Saigon Beer Manufacturing Factory, Ho Chi Minh City, Vietnam. The main characteristics of influent wastewater were presented in Table 1. Seed sludge for the model of A$_2$O-MBR system was taken from one of the two SBRs of this wastewater treatment plant. Seed sludge was light brown, well-settled with sludge volume index of 98 and MLVSS/MLSS ratio of 0.74.

**Analytical methods**

The samples were collected at the input and output positions of the experimental system. They were also collected in three compartments of the A$_2$O unit. For each loading rate, the model was operated for 45 days to achieve a steady-state condition and the samples were collected over a 3-day period during these days. For determination of the overall treatment performance in terms of organic and nutrient removals, the parameters of wastewater such as COD, suspended solid (SS), Total Kjeldahl Nitrogen (TKN), NH$_4^+$-N, NO$_2^-$-N, NO$_3^-$-N and TP were analyzed according to Vietnam National Standards together with Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, and WEF) at Research Institute for Aquaculture No.2 in Ho Chi Minh City. The value for TN was based on the sum of TKN, NO$_2^-$-N and NO$_3^-$-N. pH and DO were measured by pH (Mettler Toledo MP220, Switzerland) and DO (YSI 5000, United States) meters, respectively. The results below were based on average value and standard deviation by using Microsoft Office Excel software.

**RESULTS - DISCUSSION**

**Organic removal efficiency**

COD concentrations at different positions in the model were revealed in Figure 2 for loading rates of 0.50, 0.75 and 1.00 kgCOD/m$^3$.day. The results showed that COD concentration decreased significantly in the anaerobic and anoxic compartments. The decline could be attributed mainly by the dilution and uptake. About 40% of COD was utilized in the anaerobic compartment by PAOs and 40% of COD was consumed in the anoxic compartment by denitrifiers. It changed slightly in the oxic compartment and the MBR. The additional organic removal was attributable to the step of membrane filtration which is beneficial to keep a higher COD removal efficiency. Accumulation of PO$_4^{3-}$-P by PAOs happened mostly in the anoxic compartment. Nitrification of NH$_4^+$-N by nitrifiers happened mostly in the MBR. Before wastewater flowed into the MBR, large amount of COD in wastewater was removed. It
Table 1: THE EXPERIMENTAL CONDITION IN DIFFERENT PHASES FOR THE MODEL OF A-O-MBR SYSTEM

| Phase | Duration (day) | COD (mg/L) | NH\(_4\)\(^+\)-N (mg/L) | TP (mg/L) | Organic loading (kgCOD/m\(^3\).day) | HRT (h) |
|-------|----------------|------------|--------------------------|-----------|-----------------------------------|---------|
| 1     | 1 - 45         | 523 ± 48   | 67 ± 9                   | 13 ± 3    | 0.50                              | 24      |
| 2     | 46 – 90        | 505 ± 43   | 70 ± 10                  | 15 ± 3    | 0.75                              | 18      |
| 3     | 91 - 135       | 518 ± 47   | 69 ± 10                  | 14 ± 3    | 1.00                              | 12      |

Figure 2: Change of COD concentration at various loading rates.

Figure 3: COD removal efficiencies at various loading rates.
was considered to be advantageous for the nitrification because of non-inhibitory effects. Therefore, the growth of nitrifiers was favourable and the nitrification was enhanced as well. COD removal efficiencies at various loading rates of the model were represented in Figure 3. For loading rates of 0.50, 0.75 and 1.00 kg COD/m³.day, average COD removal efficiencies of the model were 94.1, 95.4 and 92.3%, respectively. It could be seen that COD removal efficiency reached the highest value at the proper loading rate of 0.75 kgCOD/m³.day. For these three loading rates, output values of COD were within the limits of QCVN 40:2011/BTNMT, column A. COD removal at different loading rates depended on nitrogen and phosphorus removal mutually through treatment performance.

**Nitrogen removal efficiency**

Nitrogen concentrations at different positions in the model were revealed in Figures 4 and 5 and Figure 6 for loading rates of 0.50, 0.75 and 1.00 kgCOD/m³.day, respectively. The results showed that NH₄⁺-N and TN concentrations decreased significantly in the anaerobic and anoxic compartments. The decline could be attributed mainly by the dilution of the return sludge flow in the anaerobic compartment and denitrification by denitrifiers in the anoxic compartment. It also showed that TN at the oxic compartment and MBR was mostly NH₄⁺-N and NO₃⁻-N, respectively. Due to membrane separation, a sufficiently long SRT necessary to prevent the washout of nitrifiers was applied in the MBR to improve the nitrification capability of activated sludge. Small amount of NH₄⁺-N was metabolized for the growth of microorganisms in the system and the remaining was almost completely transformed by the nitrification in the MBR. Very low NO₃⁻-N concentration in the anoxic compartment indicated that the denitrification happened as much as possible in this compartment. It was fully reasonable with the change of COD stated above. Removal efficiencies of nitrogen at various loading rates of the model were represented in Figure 7. For loading rates of 0.50, 0.75, 1.00 kgCOD/m³.day, average NH₄⁺-N and TN removal efficiencies of the model were 99.1 and 83.7, 99.2 and 86.7, 98.7 and 82.5%, respectively. Nitrogen removal efficiency also reached the highest values at the proper loading rate of 0.75 kg COD/m³.day. For all three loading rates, output values of NH₄⁺-N and TN were within the limits of QCVN 40:2011/BTNMT, column A. COD and nitrogen removal decreased when loading rate increased.

**Phosphorus removal efficiency**

Phosphorus concentrations at different positions in the model were revealed in Figure 8 for loading rates of 0.50, 0.75 and 1.00 kgCOD/m³.day. The results showed that TP concentration increased to the maximum level in the anaerobic compartment when PAOs released phosphate by utilizing 40% of COD in wastewater as mentioned above. Conditions that favor the growth of PAOs and anaerobic phosphorus release could be provided. TP concentration decreased in the anoxic compartment by the dilution of the return effluent flow from the MBR. In addition, TP concentration also decreased significantly in the anoxic compartment due to its uptake by Denitrifying Phosphorus Accumulating Organisms (DPAOs), which could use nitrate and/or nitrite rather than oxygen as an electron acceptor when exposed to an anoxic environment. In the oxic compartment, TP was further accumulated by PAOs to reach complete biological phosphorus removal. Yongzhi Chen et al., 2011 also showed that DPAOs played an important role in removing almost entirely phosphorus from wastewater when treating domestic wastewater by an A₂O-BAF system. Phosphorus removal efficiencies at various loading rates of the model were represented in Figure 9. For loading rates of 0.50, 0.75 and 1.00 kgCOD/m³.day, average TP removal efficiencies of the model were 74.6, 84.6 and 73.5%, respectively. Phosphorus removal efficiency also reached the highest values at the proper loading rate of 0.75 kgCOD/m³.day. For all three loading rates, output values of TP were within the limits of QCVN 40:2011/BTNMT, column A. In relation to the results obtained above, the more COD removal or cell growth is, the more phosphorus removal is.

**Membrane fouling**

Membrane fouling in MBR was inevitable. The TMP in the MBR of the model was monitored continuously to evaluate the membrane fouling during the entire running period. The TMP was in the range of 10 – 33 kPa and the flux was from 6.4 to 12.8 L/m².h (LMH). The membrane fouling rate in the MBR correlates well to the MLSS concentration during 135 days of operation. The MLSS concentration initially increased from around 5600 mg/L to nearly 6000 mg/L on day 60 and was maintained for the remaining days of running. When the flux was 6.4 LMH in the phase 1, the TMP was in the range of 10 – 16 kPa for 45 days. During the phase 2, the flux was kept at 9.6 LMH. The TMP increased gradually with
time to 26 kPa on day 90. After the phase 2, the flux increased again to 12.8 LMH in the phase 3. The TMP increased almost linearly and reached about 33 kPa on day 135. As mentioned above, the membrane fouling could be alleviated to a certain degree by the intermittent operation of the membrane (2 min rest in every 10 min operation), air bubbling and backflushing.

**CONCLUSIONS**

In this study, the model of A₂O-MBR system was operated well and treatment efficiencies of nitrogen and phosphorus at three loading rates were high. It was capable of achieving effluents with low nitrogen and phosphorus concentrations from brewery wastewater. For a loading rate of 0.75 kg COD/m³/day, treatment efficiencies of COD, NH₄⁺ -N, TN, TP of the model were the highest as 95.4, 99.2, 86.7, 84.6%, respectively. Output values of these parameters were within the limits of QCVN 40:2011/BTNMT, column A. Making a short SRT for A₂O unit and a long SRT for MBR helps A₂O-MBR system remove simultaneously nitrogen and phosphorus from wastewater.

**LIST OF ABBREVIATIONS**

A₂O-MBR: Anaerobic/Anoxic/Oxic – Membrane BioReactor  
SRT: Solids Retention Time  
COD: Chemical Oxygen Demand  
EBPR: Enhanced Biological Phosphorus Removal  
TN: Total Nitrogen
COMPETING INTERESTS
None of the authors reported any conflict interest related to this study.

AUTHORS’ CONTRIBUTIONS
Van Nu Thai Thien: writing the draft of the research paper.
Dang Viet Hung: designing and conducting the experiments.
Nguyen Thi Thanh Hoa: sampling and analysis of wastewater.

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Figure 8: Conversion of TP concentration at various loading rates.

Figure 9: TP removal efficiencies at various loading rates.

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Figure 10: Variation of MLSS concentration during the operational period.

Figure 11: Variation of TMP during the operational period.

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Nghiên cứu hệ thống A_2O-MBR để loại bỏ đồng thời thành phần nitơ và phốt pho có trong nước thải sản xuất bia

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TÓM TẮT
Hệ thống xử lý nước thải sử dụng phương pháp sinh học kị khí, thiếu khí, hiếu khí đồng thời và kết hợp màng siêu lọc (A_2O-MBR) đã được sử dụng nhằm tăng cường khả năng xử lý cùng lúc thành phần nitơ và phốt pho có trong nước thải sản xuất bia. Cụm A_2O chứa vi sinh vật với thời gian lưu bùn ngắn chịu trách nhiệm loại bỏ hữu cơ, phốt pho và khử nitrat. Bể MBR chứa vi sinh vật với thời gian lưu bùn dài chịu trách nhiệm nitrat hóa amoni và tuần hoàn nitrat. Mô hình A_2O-MBR được làm bằng mica với thể tích chứa 49,5 lít đã được vận hành với thời gian lưu nước giảm dần từ 24, 18 đến 12 giờ tương ứng với tải trọng hữu cơ tăng dần từ 0,5; 0,75 đến 1,00kgCOD/m³ ngày. Kết quả cho thấy mô hình không chỉ xử lý tốt thành phần hữu cơ mà còn loại bỏ gần hoàn toàn nitơ và phốt pho. Ở cả 03 tải trọng, nồng độ COD đã giảm đáng kể ở các ngăn kị khí và thiếu khí của cụm A_2O, cho thấy phân lê vi khuẩn đã được sử dụng ở các ngăn khí khí và thiếu khí, tuần hoàn với các quá trình tách phốt pho và khử nitrat. Nitrat hóa trong bể MBR đã xảy ra gần như hoàn toàn, với hiệu quả loại bỏ amoni là trên 98%. Khử nitrat trong ngăn thiếu khí đã được thực hiện nhờ nitrat thế. Các điều kiện cần thiết cho sự phát triển của vi sinh vật tích lũy phốt pho (PAOs) để loại bỏ phốt pho bởi tạng cầu sinh học (EBPR) có thể đã được cung cấp. Ông tạng trong 0,75kgCOD/m³ ngày, hiệu quả xử lý COD, NH₄⁺-N, TN và TP của mô hình là cao nhất, tương ứng với 95,4; 99,2; 86,7 và 84,6%. Giá trị đầu ra của các thông số này là nằm trong giới hạn cho phép của QCVN 40:2011/BTNMT, có thể đạt được từ các ngăn thiếu khí của nước thải sản xuất bia với nồng độ nitơ và phốt pho rất thấp. 

Từ khóa: Hệ thống A2O-MBR, nước thải sản xuất bia, loại bỏ nitơ, loại bỏ phốt pho

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Lịch sử
• Ngày nhận: 18-1-2019
• Ngày chấp nhận: 14-5-2019
• Ngày đăng: 20-6-2019

DOI: https://doi.org/10.32508/stdjsee.v3i1.507

Bản quyền
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