Study on the Application of Anammox Process Using Polyester Non-woven Biomass Carrier Reactor (PNBCR) for Latex Processing Wastewater Treatment

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ABSTRACT
The research aimed to evaluate the ability of anammox process using polyester non-woven materials as biomass carrier (PNBC) for nitrogen removal in latex processing wastewater. The experiment was operated at nitrogen loading rates (NLR) of 0.5; 1.0 and 2.0 kgN/(m³·day). The average total nitrogen (TN) removal efficiencies were 70% - 78% at all NLRs. The effluent NH4-N concentration was lower than that of the Vietnamese effluent standard limit, level B of QCVN 01:2008/BTNMT (40 mgN/L), at the NLR of 0.5 and 1.0 kgN/(m³·day). The PNBCR was able to run at NLRs higher than 2.0 kgN/(m³·day) and the effluent TN and NH4-N may meet the standard limits if the operation of partial nitritation could achieve the NH4-N:NO2-N ratio of 1.0:1.32 and producing less nitrate. The annamox removal rates obtained at all NLRs tested were in the range of industrial N removal applications. These findings may lead to the development of the PNBCR for latex processing wastewater treatment.

Keywords: anammox, latex processing wastewater, nitrogen removal, polyester non-woven biomass carrier reactor (PNBCR)

INTRODUCTION
Latex processing wastewater contains high total Kjeldahl nitrogen (TKN) and chemical oxygen demand (COD) (Bich et al., 1997). At present, wastewater treatment plants (WWTP) are available in the current latex processing industries in Vietnam. The typical treatment technology used in WWTPs includes primary treatment using rubber trap, dissolved air flotation followed by secondary treatment using upflow anaerobic sludge blanket (UASB) and activated sludge such as oxidation ditches, aerated lagoons and stabilization ponds. However, the effluent of almost all WWTPs has not met the Vietnamese industrial effluent standards due to high ammonia and total nitrogen concentrations in raw wastewater.

In general, the conventional nitrogen removal process such as anoxic/oxic (A/O) or anaerobic/anoxic/oxic (A2O) process requires high energy consumption for nitrification and external carbon sources for denitrification in wastewater having low C/N ratio (COD/TKN < 5) (Uibing et al., 1997) while the range of COD/TKN ratio of raw latex processing wastewater is as low as 3.8 to 5.0.

The recently discovered anaerobic ammonium oxidation (anammox) process is one of the most innovative technological advances for the removal of nitrogen contaminants from wastewater (Kuenen and Jetten, 2000; Schmid et al., 2000). In anammox process,
ammonium is converted into nitrogen gas with nitrite as the electron acceptor (Mulder et al., 1995; Van de Graaf et al., 1996).

\[
\text{NH}_4^+ + 1.32 \text{NO}_2^- + 0.066 \text{HCO}_3^- + 0.13 \text{H}^+ \rightarrow 1.02 \text{N}_2 + 0.26 \text{NO}_3^- + 0.066 \text{CH}_2\text{O}_{0.3}\text{N}_{0.15} + 2.03 \text{H}_2\text{O} \quad \text{(Eq. 1)}
\]

The stoichiometry of the anammox reaction determined by Strous et al. (1998) is shown in Eq. 1. Compared with the conventional treatment system for the removal of ammonium that couples nitrification with denitrification, the anammox process coupled with partial nitritation is economically favorable because it has less oxygen demand, requires no organic carbon and has low sludge production (Schmidt et al., 2003; Khin and Annachhatre, 2004). The bacteria responsible for anammox are very slow-growing organisms, dividing only once every two weeks, which cannot be cultivated using conventional microbiological techniques (Strous et al., 1999).

In 2000, the enrichment and characterization of anammox biofilm culture were carried out successfully on a Polyester Non-woven Biomass Carrier (PNBCR) in Kumamoto, Japan (Furukawa et al., 2002).

This study investigated the application of anammox process using PNBCR for treatment of latex wastewater containing high TKN.

**MATERIALS AND METHODS**

**Laboratory-scale polyester non-woven biomass carrier reactor (PNBCR)**

The PNBCR used in this study was made of acrylic resin, with a working volume of 5 L. The reactor had an inner diameter of 10 cm and water column height of 65 cm (Fig. 1).

The biomass carrier consisted of 8 strips of non-woven porous polyester material (4.5 × 41 cm strips, 0.5 cm thickness) for a total one-sided sheet area of 1.476 cm² with a pyridinium-type polymer (Japan Vilene, US patent 5,185,415, 1993). This carrier is designed to enhance the retention of biomass (Fig. 2). The gas was collected and measured using a gas counter.

![Fig. 1 - Schematic diagram of the PNBCR.](image)

![Fig. 2 - Polyester non-woven biomass carrier.](image)
Acclimation of Biofilm
Seed sludge as dry weight (25 g) was taken from a non-woven anammox reactor in the laboratory of Kumamoto University. The seed sludge had been enriched with synthetic wastewater for 72 days using PNBCR. The synthetic wastewater was prepared by adding ammonium and nitrite in the forms of (NH₄)₂SO₄ and NaNO₂ (100 mgN/L), respectively. Other components used in this study were 125 mg/L KHCO₃, 54 mg/L KH₂PO₄, 180 mg/L CaCl₂·2H₂O, 120 mg/L MgSO₄·7H₂O. Trace element solutions were added based on the previous studies (Van de Graff et al., 1996). During acclimatization, the reactor was run at NLR ranging from 0.2 - 1.0 kgN/(m³·day). The nitrogen removal rate (NRR) of 0.76 kgN/(m³·day) was obtained at NLR of 1.0 kgN/(m³·day) during the end of acclimatization, when much of the dark red colored biomass attached on the surface of the PNBCR.

Feed wastewater
The wastewater was taken from the outlet of dissolved air flotation system of Ben Suc latex processing factory in Binh Duong Province, Vietnam. Then, it was treated by an anaerobic reactor using polyvinyl alcohol gel (PVA) carrier (Kururay Co., Ltd, Tokyo, Japan) followed by a swim-bed reactor (SWB) in the laboratory. The partial nitritation process was controlled in a SWB, in which the novel acryl-fiber biomass carrier-biofringe (NET Co., Ltd, Hyogo, Japan) was provided (Fig. 3).

The feed wastewater into PNBCR was the effluent from SWB. The quality of the effluent from the partial nitritation of SWB was unstable. Values of pH, COD, bicarbonate, and nitrate were 7.5 - 8.5, 150 - 300 mg/L, 1,000 - 1,500 mg CaCO₃/L and 3 - 45 mg/L, respectively.

Operational conditions
The feed wastewater was fed continuously in an upflow mode by a dosing pump. The experiment was run under dark conditions using black nylon sheet enclosure. The Na₂SO₃ salt was added into the feed wastewater to reduce the amount of dissolved oxygen (DO) to less than 0.4 mg/L. Influent pH was adjusted to 7.5 with 20% HCl solution. The operational conditions are shown in Table 1.

### Table 1 - Operational parameters for PNBCR.

<table>
<thead>
<tr>
<th>Period</th>
<th>Time (days)</th>
<th>Flow Rate (L/d)</th>
<th>HRT (h)</th>
<th>NLR (kgN/(m³·day))</th>
<th>Influent NH₄-N/NO₂-N (mgN/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
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<td>12.0</td>
<td>10</td>
<td>0.5</td>
<td>100/100</td>
</tr>
<tr>
<td>II</td>
<td>32</td>
<td>25.0</td>
<td>4.8</td>
<td>1.0</td>
<td>100/100</td>
</tr>
<tr>
<td>III</td>
<td>32</td>
<td>12.0</td>
<td>10</td>
<td>1.0</td>
<td>200/200</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>25.0</td>
<td>4.8</td>
<td>2.0</td>
<td>200/200</td>
</tr>
</tbody>
</table>

Fig. 3 - Schematic diagram of the pretreatment of the feed wastewater.
Analytical methods
Parameters of COD, NO₂-N and NO₃-N were measured according to the standard methods (APHA, 1995). The pH and DO were measured using pH meter (HI 8314, Hanna) and DO meter (HI 9146, Hanna), respectively.

DNA extraction and PCR amplification
A sludge sample was taken from PNBCR at day 139. The granular sludge sample was first ground with a pestle under liquid nitrogen. Meta-genomic DNA was extracted using an ISOIL kit (Wako, Osaka, Japan) according to the manufacturer’s instructions. The amplification of 16S rRNA gene was performed with Phusion High-Fidelity DNA polymerase (FINNZYMES Finland) using conserved eubacterial primers 6F (forward primer: 5’-GGAGTTAGATTGCTCTGACG-3’) (Tchelet et al., 1999) and 1492r (reverse primer: 5’-GGTAGTTGCTGACGAT-3’) (Lane, 1991). Polymerase Chain Reaction (PCR) was carried out according to the following thermocycling parameters: 30 s initial denaturation at 98°C, 25 cycles of 10 s at 98°C, 20 s at 51°C, 35 s at 72°C, and 5 mins final elongation at 72°C. The amplified products were electrophoresed on a 1% agarose gel and the excised fragments were purified using a Wizard SV Gel and PCR Clean-Up System (Promega, USA).

Cloning and sequencing of 16S rRNA gene
The purified fragments were ligated into the EcoRV site of pBluescript II KS+ (Stratagene, USA), and E. coli DH 5α was transformed using the constructed plasmids. White colonies including the inserts were randomly chosen and the plasmids were extracted by the alkaline method. The nucleotide sequences were determined with a 3130xl genetic analyzer and BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, USA). Operational taxonomic units (OTUs) were defined by a 1% distance level in the nucleotide sequences. The sequences determined in this study were compared with the sequences in the non-redundant database using the basic local alignment search tool (BLAST) program available on the National Center for Biotechnology Information web site.

RESULTS AND DISCUSSION
Removal of nitrogenous compounds
After acclimatization was finished, the PNBCR was run continuously during four periods (I, II, III, IV) with the feed wastewater, which was the effluent of the partial nitritation. The ammonia and nitrite concentrations ranged from trace to 200 mgN/L. Therefore, ammonium (NH₄Cl) or nitrite (NaNO₂) was added into the feed wastewater to achieve the ratio of NH₄-N to NO₂-N equal to 1:1. Influent NH₄-N and NO₂-N concentrations were adjusted to 100 mgN/L each during periods I and II and 200 mgN/L during periods III and IV. In periods I and II, the effluent of the partial nitritation was between 100 mgN/L and 200 mgN/L, the dilution ranged from trace to 1 time.

The time courses of concentrations of nitrogenous compounds in the influent and effluent are shown in Fig. 4. The experiment started at loading rates of 0.5 kgN/(m³·day). The highest NH₄-N (80%) and NO₂-N removal (90%) efficiencies has been obtained since day 125. The effluent concentrations of NH₄-N and total nitrogen (TN) were less than 19.3 ± 3.1 mgN/L and 68.0 ± 6.5 mgN/L (n = 3), respectively. The effluent NH₄-N
concentrations met the Vietnamese effluent standards QCVN 01:2008/ BTNMT, level B (40 mgN/L) (QCVN). However, TN concentration did not meet the standard limit of QCVN (60 mgN/L) due to high influent NO$_3$-N concentration from partial nitritation. In order to meet the QCVN standard, control of effluent nitrate concentration of the partial nitritation is necessary. Overall, the average TN removal efficiency was about 75 - 78% (Fig. 4).

At higher NLRs of 1.0 and 2.0 kgN/(m$^3$.day), the effluent NH$_4$-N and TN did not meet the standard limit of QCVN. Figure 5 illustrates that the increase of both influent ammonium and nitrite concentrations leads to the increase of effluent ammonia nitrogen concentration while the effluent nitrite concentration does not change. Indeed, at NLRs

![Fig. 4 - Time courses of influent and effluent concentrations of nitrogenous compounds.](image)

![Fig. 5 - Effluent concentration of nitrogenous compounds at various NLRs.](image)
with influent NH$_4$-N concentration of 200 mg/L, the effluent NH$_4$-N concentration was 48 ± 5.5 mgN/L, whereas the effluent NO$_2$-N concentration was only 11 ± 2.6 mgN/L, which were not much different with those (12 ± 2.7 mgN/L) at low NLRs with influent NH$_4$-N concentration of 100 mgN/L. High effluent ammonia and low nitrite were due to the insufficiency of influent nitrite to achieve the ratio of NH$_4$-N to NO$_2$-N of 1.0 - 1.32 (Van de Graaf et al., 1996). Therefore, if the ratio of NH$_4$-N and NO$_2$-N concentrations in the influent to the anammox reactor was 1.0 - 1.32, the effluent NH$_4$-N concentration might be lower than the Vietnamese standard (40 mgN/L).

At NLRs with influent TN concentrations of 200 mgN/L (period I and II) and 400 mgN/L (period III and IV), the NO$_3$-N concentrations produced from anammox process were 22.2 ± 3.6 mgN/L (n = 22) and 40.2 ± 5.3 mgN/L (n = 18), respectively. Thus, the ratio of NO$_3$-N produced to influent TN for all NLRs was 10.6 ± 3.2% (n = 9). This ratio is close to the empirical ratio of 10% (Van de Graaf et al., 1996). This shows that the control of oxygen penetration was good enough to inhibit nitrification.

**TN removal rates (TNRRs)**

The TNRR increased significantly from 0.05 kgN/(m$^3$·day) at the beginning (day 1 - 2) and up to 1.56 kgN/(m$^3$·day) at the final days of the experiment (day 135 - 139). Figure 6 illustrates that the anammox activity was enhanced rapidly at high NLR of 2.0 kgN/(m$^3$·day). This demonstrates that the PNBCR was able to run at NLRs higher than 2.0 kgN/(m$^3$·day). Some previous studies obtained high TN efficiency at high NLRs. For example, the nitrogen removal rate obtained was as high as 9.5 kgN/(m$^3$·day) for the first full-scale anammox reactor (Van der Star et al., 2007), while for a lab-scale anammox gas-lift reactor, it was up to 8.9 (Sliekers et al., 2003) and 11.5 kgN/(m$^3$·day) for an anaerobic biological filtrated (ABF) fixed bed reactor filled with porous polyester non-woven fabric carriers (Isaka et al., 2007). Tsushima et al. (2007) has reported a nitrogen removal rate of 26.0 kgN/(m$^3$·day).

In general, TNRRs of the conventional nitrification-denitrification process for industrial wastewater treatment were within the range of 0.05 to 4.0 kgN/(m$^3$·day) (Jetten et al., 2002). While, TNRR of PNBCR in this study were 0.74 ± 0.01 kgN/(m$^3$·day) and 1.54 ± 0.01 kgN/(m$^3$·day) at NLR of 1.0 and 2.0 kgN/(m$^3$·day), respectively (Fig. 6). This confirmed that the use of PNBCR for latex wastewater treatment is feasible and comparable with the conventional nitrogen removal processes.

![Fig. 6 - Time courses of TN removal rates.](image_url)
Overall, at all NLRs tested, the ratios of TNRR, NO\textsubscript{2}-N consumption rate and NO\textsubscript{3}-N production rate to NH\textsubscript{4}-N consumption rate were close to the stoichiometric ratio shown in Eq. 1. At NLR of 2 kgN/(m\textsuperscript{3}·day), this ratio was 2.00:1.25:0.26, which was the closest (Fig. 7). Moreover, the nitrate production rate is stable on the ammonium consumption rate in this study. This indicates that the more stable anammox treatment performance was obtained for latex wastewater. The anammox reaction ratios will change depending on the culture conditions such as temperature, substrate composition and salt concentration (Changliang, 2006). Thus, the latex wastewater may contain minerals and trace elements enough for the growth of anammox. Bich \textit{et al.} (1997) reported that Mg, Fe, Ca, K and Cu concentrations of the raw latex wastewater were 25.9 mg/L, 3.6 mg/L, 7.1 mg/L, 61.0 mg/L and 3.2 mg/L, respectively.

Ratios of T-N removal, NO\textsubscript{2}-N consumption and NO\textsubscript{3}-N production rates to NH\textsubscript{4}-N removal rate during the operational time are shown in Fig. 7 and summarized in Table 2. A comparison between the ratios for latex wastewater treatment and the ratios of anammox processes by various researchers is listed in Table 2. In this study, the ratios changed from 1:1.15:0.32 to 1:1.25:0.26 at four various periods (Fig. 7a, b, c, d). This change shows that the values were gradually improved and are close to the reported ratios. In view of the study by Feng \textit{et al.} (2007) and Van der Star \textit{et al.} (2007), the

![Fig. 7 - Ratios of TNRR, NO\textsubscript{2}-N consumption and NO\textsubscript{3}-N production rates to NH\textsubscript{4}-N consumption rate.](image-url)
ratios were very similar to the reported ratios, which indicated that nitrifiers and denitrifiers were rare. However, Ruscalleda et al. (2008) considered that anammox bacteria could coexist with denitrifiers and play an important role in treating low C/N ratio wastewater with high quantities of slowly biodegradable organic matters, such as digested liquor, latex wastewater and landfill leachate. Consequently, a slight proportion of denitrifiers would have a positive impact on the nitrogen removal in treating various types of wastewater such as this latex processing wastewater.

Gas production
Gas production was measured during period IV. At TNRR of 1.56 kgN/(m³·day) (equivalent to 0.77 kgNH₄-N/(m³·day)), nitrogen gas production rate was 3.15 ± 0.08 L/d. This amount of nitrogen is the same as that produced from the stoichiometric reaction (Eq. 1). This result indicated the dominant existence of anammox strains in the reactor.

DNA analysis
An analysis of the microbial community by 16S rRNA was used to investigate the dominant species in the anammox PNBCR. Table 3 shows the main results of homology search for 16S rRNA gene sequences. 19 clones (OTU1) (45.2%) had 99 - 100% sequence identities with Candidatus Kuenenia stuttgartiensis and uncultured anoxic sludge bacterium KU-2, which was detected in the anammox reactors used by Furukawa et al. (2002) and are known to be anammox bacterium. Additionally, three of the 42 clones (OTU2) had 99% sequence identities with uncultured bacterium clone. Asahi BRW1 which was detected in the anammox reactors of Asahi Breweries Ltd., and one of the 42 clones (OTU3) had 98% sequence identities with planctomycete KSU-1 which was also detected in the anammox reactors used by Furukawa et al. (2002), which are also known to be anammox bacteria. These bacterial species were responsible for the anammox reaction of the PNBCR. Other clones seemed to belong to Chloroflexi (OTU4, OTU5 and OTU6), Chlorobi (OTU7 and OTU8), Bacteroidetes (OTU9 and OTU10), Proteobacterium (OTU11, OTU12 and OTU13). Because these clones had relatively low sequence identity with the BLAST database, it was difficult to identify their genus. However, Qiao et al. (2008) reported that Chloroflexi bacteria coexisted with KSU-1 in a fixed-bed anammox reactor treating synthetic inorganic wastewater. Cho et al. (2010) investigated the microbial community in an anaerobic upflow granular bed anammox reactor treating synthetic inorganic wastewater and reported that Chloroflexi bacteria were present on the surface of anammox granules. Moreover, OTU11 had 99% sequence identities with uncultured bacterium clone: A, which was detected in the anammox reactors used by Furukawa et al. (2002). The microbial communities of this study and the previous studies appear to have similarity in the coexistence of anammox and other bacteria. Thus, the function of Chloroflexi and other bacteria which are frequently detected in anammox reactors should be investigated.
Table 3 - Microbial community in the anammox reactor.

<table>
<thead>
<tr>
<th>OTU</th>
<th>Taxon</th>
<th>Accession</th>
<th>Identity</th>
<th>Number of clones</th>
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<td>1</td>
<td>Uncultured anoxic sludge bacterium KU-2</td>
<td>AB054007</td>
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<td></td>
<td>Candidatus Kuenenia stuttgartiensis</td>
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<td>Uncultured ammonia-oxidizing bacterium</td>
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<td>Planctomycete KSU-1</td>
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CONCLUSIONS
The results of this study gave the following conclusions:
- The total nitrogen removal rate of the anammox process in PNBCR for latex processing wastewater was 1.54 ± 0.01 kgN/(m³·day), corresponding to 75 - 78% at NLR of 2.0 kgN/(m³·day).
- The annamox removal rates could be obtained at all NLRs tested were in the range of industrial nitrogen removal applications. The effluent TN and NH₄-N concentrations could meet the Vietnamese standard limits if the operation of partial nitritation achieved the NH₄-N:NO₂-N ratio of 1.0:1.32.
- The PNBCR is feasible and comparable with the conventional nitrogen removals treating latex processing wastewater due to its NLR as high as 2.0 kgN/(m³·day).

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level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. 
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