Greenhouse gas emissions from open-type anaerobic wastewater treatment system in natural rubber processing factory

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1 **ABSTRACT:** We evaluated greenhouse gases (GHGs) emissions from an open-type
2 anaerobic system (OAS) treating ribbed smoked sheets (RSS) wastewater in a processing
3 factory. The emitted biogas comprised 57.7%–60.8% methane, 14.5%–31.5% carbon
4 dioxide, 10.8%–24.7% nitrogen, and 329–423 ppm of nitrous oxide. In the OAS, the
5 chemical oxygen demand (COD) degraded as the methane emission rate increased from
6 influent to effluent. Finally, 94.4% of the total COD was removed. More than 90% of the
7 removed COD converted to methane. Methane emitted from the bottom and the wall
8 surface of the OAS. Methane-producing bacteria was detected by a cloning analysis of the
9 16S rRNA gene. The predominant species differed depending on the degradation
10 characteristics of the RSS wastewater in the OAS. The emission rate of methane and
11 nitrous oxide ranged between 2.58 and 8.85 NL/(m².hr) and between 0.074 and 0.410
12 NL/(m²/hr), respectively. Moreover, 0.153 t-CO_{2eq} of GHGs were emitted from 1 m³ of
13 RSS wastewater treatment. Nitrous oxide accounted for 65% of the total GHGs emissions
14 because of its high global warming potential. Therefore, both nitrous oxide and methane
15 that emitted from the treatment of wastewater from a natural rubber processing factory
16 must be considered as significant contributors to the GHGs.

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18 **Keywords:** GHGs emissions; methane; nitrous oxide; natural rubber processing
19 wastewater; open-type anaerobic system

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1. Introduction

The natural rubber industry is one of the major agriculture-based industries in the Southeast Asia region. More than 60% of the worldwide production of natural rubber comes from Thailand, Indonesia, and Malaysia (Mohammadi et al., 2010). Conversely, Vietnam is one of the world's biggest producers of rubber and ranks fifth in terms of production and fourth in terms of exportation of natural rubber products (Nguyet, 2013). In these countries, mainly three types of natural rubber products, i.e., technically specified rubber (TSR), concentrated latex (CL), and ribbed smoked sheets (RSS), are produced from natural rubber latex and used in downstream rubber products industries (Takaskul and Tekasakul, 2006; Jawjit et al., 2010).

On average, 18–35 m³ of wastewater containing high concentrations of organic matter and nitrogen compounds (especially, ammonia) are discharged during the production of 1 t of natural rubber product (Nguyen and Luong, 2012). These high concentrations occur because a large amount of ammonia is used to preserve natural-rubber latex (Thongnuekhang and Pupetpaiboon, 2004) and acetic acid or formic acid is used to coagulate natural rubber latex to produce blocks of rubber. In natural rubber processing factories throughout the Southeast Asia region, the typical effluent treatment process is an open-type anaerobic–aerobic system that uses a combination of anaerobic, anoxic, and aerated lagoons (Jawjit et al., 2010; Chaiparapatand and Sdoodee, 2007). However, these systems have some disadvantages such as the need for large land areas, long hydraulic retention times (HRT), power consumption for aeration, large discharge amounts of excess sludge, malodorous emissions, and large amounts of greenhouse gases (GHGs) emissions, such as methane,

carbon dioxide, and nitrous oxide (Smitha et al., 2012; Kanyrat and Sumate, 2008). In our study, we evaluated emissions of GHGs from an open-type anaerobic system (OAS) treating wastewater from a natural rubber processing factory.

2. Material and methods

2.1 Survey of greenhouse gas emissions

The survey was conducted twice during October and January at the natural rubber processing factory at the Rubber Research Institute of Vietnam (RRIV) in Binh Duong province, Vietnam. The type of product and capacity were RSS and 1,000 t/year, respectively. In this factory, the RSS wastewater was discharged at 10 m³/t-RSS of product. The effluent treatment system included an OAS, an algal tank, and a polishing tank. The OAS comprised 60 compartments separated by a baffled wall. The OAS body was made of concrete. The surface area of each compartment and the depth of the OAS were 4.5 m² and 1.4 m, respectively. Fig. 1 shows the compartment layout of the OAS. The RSS wastewater flowed from compartment 1 to 60. During the survey, the water temperature of each compartment ranged from 29.1°C to 31.6°C.

The biogas was collected using a collection chamber with a surface area of 0.02 m² made from polyvinyl chloride (PVC) pipes. Fig. 2 shows the schematic diagram of the biogas collection chamber. The two types of chambers were installed for each compartment. Chambers 1 and 2 were used to measure biogas production from the bottom (4.5 m² of total surface area) and wall surface

(12.6 m² of total surface area) of the OAS, respectively. Concentrations of hydrogen, nitrogen, methane, and carbon dioxide were analyzed using a gas chromatograph equipped with a thermal conductivity detector (GC-8A, Shimadzu). Concentration of nitrous oxide was analyzed by a gas chromatograph equipped with an electron capture detector (GC-2014, Shimadzu). The chambers were installed for compartments 1 and 5 near the influent part; for compartments 16, 28, 33, and 45 at the center part; and for compartments 51 and 58 near the effluent part. Emission rates (flux) of both methane and nitrous oxide were calculated using the concentration change of methane and nitrous oxide and the gas phase volume of the chamber. The biogas samples were collected every hour for methane and every 10 min for nitrous oxide. GHGs emissions from each compartment were measured once during October, 2012, and again during January, 2013 (n = 2). Methane emissions rate from the bottom and wall surface were calculated using the following Eq. (1) and Eq. (2):

$$E_b = E_{C1}/A_{C1} \times A_b, \quad (1)$$

$$E_w = (E_{C2} - E_{C1})/(A_{C2} - A_{C1}) \times A_w \quad (2)$$

where E_b is the methane emission rate from the bottom (NL/d); E_{C1} is the methane emission rate of chamber 1 (NL/d); A_{C1} is the surface area of chamber 1 (0.02 m²); A_b is total surface area of the bottom (4.5 m²); E_w is the methane emission rate from the wall surface (NL/d); E_{C2} is the methane emission rate of chamber 2 (NL/d); A_{C2} is the surface area of chamber 2 (0.02 m² × 2 = 0.04 m²);

1 and A_w is the total surface area of the wall (12.6 m²).

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3 *2.2 Chemical analysis*

4 The wastewater samples were collected from the influent, effluent, and each compartment of the
5 OAS ($n = 4$, twice during October and January). The chemical oxygen demand (COD) was
6 measured using an HACH apparatus (DR2700, HACH). Volatile fatty acids (VFA) were
7 determined using a gas chromatograph equipped with a flame ionization detector (GC-1700,
8 Shimadzu). Ammonia, nitrite, and nitrate were analyzed using a high performance liquid
9 chromatograph equipped with a conductivity detector (CDD-10A VP, Shimadzu).

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11 *2.3 Microbial community analysis*

12 To identify the major methane-producing bacteria present in the OAS, we conducted a cloning
13 analysis targeting the Archaeal 16S rRNA gene. DNA was extracted from the sludge harvested
14 from compartments 16 and 33 using an Isoil beads beating kit (Nippon gene, Japan). About 806 bp
15 of 16S rDNA was amplified by a polymerase chain reaction (PCR) using the primers A 109f-m (5'-
16 AM DgC TCA gTA ACA CgT-3') and ARC915R (5'-gTg CTC CCC CgC CAA TTC CT-3'),
17 specific for domain Archaea (Syutsubo et al., 2013).

18 To capture and clone the PCR fragments, a TOPO-TA cloning kit (Life Science Technologies
19 Ltd., Japan) was used. The nucleotide sequences of the obtained clones were determined by using a
20 3130 genetic analyzer (Applied Biosystems Ltd., Japan). The sequences were compared with the

data stored in NCBI GenBank using BLAST. The 16S rDNA sequences of Archaeal clones have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases under accession numbers LC108794 - LC108829

3. Results and discussions

3.1 Methane emissions and organic matter removal in the OAS

Fig. 3 shows the composition of the biogas collected from compartments 28, 33, and 56 using the water substitution method during the survey in October. The emitted gas from the OAS comprised 57.7%–60.8% methane, 14.5%–31.5% carbon dioxide, 10.8%–24.7% nitrogen, and 329–423 ppm of nitrous oxide. We confirmed that the GHGs emitted from the OAS were not only methane but also nitrous oxide from the nitrogen removal process.

Fig. 4 shows the methane emission rate and the COD concentration of each compartment. The average data of the survey conducted during October and January was indicated in this figure. The methane emission rate ranged from 2.58 NL/ (m².hr) to 8.85 NL/ (m².hr). The maximum and minimum methane emission rates were observed at compartments 33 and 56. RSS wastewater contained 47.1% of solid COD; 65.6% of the soluble COD was VFAs (27.9% of acetate and 38.4% of propionate). From the influent to compartment 5, 45.8% of the total COD (79.1% of the solid COD) was removed. However, methane emitted from these compartments accounted for only 10.3% of the removed COD. At the surface of these compartments, coagulated rubber was

observed. This suggested that the compartments near the influent part functioned as a rubber trap. The concentration of VFAs increased from compartments 1 to 16. After compartment 16, both the total and the soluble COD were linearly degraded as the methane emission rate increased. Finally, 94.4% of total COD was removed by the OAS in 12 days of HRT. This COD removal efficiency was higher than that of other anaerobic treatment systems treating the wastewater from natural rubber processing using a combination of a rubber trap and biological treatment (Chaiprapat et al., 2015; Hatamoto et al., 2012; Boonsawang et al., 2008; Kanyarat and Smate, 2008; Anotai et al., 2006). Furthermore, the HRT of the OAS was shorter than that of the conventional lagoon system (Nguyen and Luong, 2012; Mohammadi et al., 2010). These results indicated that the OAS provides an advantage for organic removal during the treatment of wastewater from a natural rubber processing factory.

3.2 COD mass balance in the OAS

Fig. 5 shows the COD mass balance in the OAS. The COD balance was calculated using the average of the data from the surveys in October and January. The COD balance was calculated for two cases using the source of methane emission. In the first case, the methane emission rate was measured only from the bottom of the OAS at chamber 1. In the second case, chamber 2 was used for measuring the methane emission from both the bottom and the wall surface of the OAS. In the first case, only 30% of the removed COD converted to methane and 70% of the removed COD was accounted for as an unknown part. However, RSS wastewater did not contain enough electron

acceptors for anaerobic degradation of organic matter such as sulphate, nitrate, and other organic ingredients. In contrast, methane emitted from the wall surface of the OAS was replaced to an unknown part in the second case. In the OAS, a higher gas production rate was visually confirmed near the wall surface of each compartment, and sludge was attached to the surface of the wall by coagulated rubber. These occurrences suggested that the methane was emitted from not only the bottom but also from the wall surface of the OAS. Furthermore, we confirmed that almost all of the organic matter was well degraded anaerobically and converted to methane in the OAS.

3.3 Nitrous oxide emission and ammonia removal in the OAS

Fig. 6 shows the nitrous oxide emission rate and ammonia concentration in each compartment during the survey in January. The nitrous oxide emission rate ranged from 0.10 NL/(m².hr) to 1.19 NL/(m².hr). The maximum nitrous oxide emission rate was observed with an increase of ammonia concentration in compartment 5. In addition, the concentrations of nitrate and nitrite in each compartment were below 1.55 mgN/L and 0.50 mgN/L, respectively. We considered that ammonia was oxidized to nitrate and nitrite at the surface of the OAS; therefore, nitrate and nitrite promptly were consumed by denitrification. Finally, 18.1% of the ammonia was removed in the OAS, and the nitrous oxide emission factor became 0.0263 kgNO₂-N/kg-N. This emission factor was much higher than 0.005 kgNO₂-N/kg-N, which is the emission factor for the direct emissions from wastewater treatment plants applied by IPCC (2006) and similar to the emission factor for full-scale biological nutrient removal wastewater treatment plants (Daelman et al., 2013; Foley et al., 2010; Kampshreur

et al., 2008). Both a low dissolved oxygen concentration in the nitrification stage and a low COD/N ratio in the denitrification stage caused high nitrous oxide emissions (Kampshreur et al., 2009). Therefore, our results suggested that a high nitrous oxide emission resulted because of a low COD/N ratio of RSS wastewater and oxygen limitation at the surface of the OAS.

3.4 Total GHGs emissions

Fig. 7 shows the composition of emitted GHGs from places near the influent part, the center part, and the effluent part of the OAS during the survey in January, 2013. Amounts of both methane and nitrous oxide were converted to carbon dioxide using a global warming potential of 25 and 298, respectively (IPCC, 2007). The emission rates (flux) from 1 m³ of treated RSS wastewater for methane, nitrous oxide, and total GHGs were calculated as 0.054 t-CO_{2eq}/m³, 0.099 t-CO_{2eq}/m³, and 0.153 t-CO_{2eq}/m³, respectively. These emission rates were higher than the emission rates from the aerobic wastewater treatment system in CL processing factories (Jawjit et al., 2013). Furthermore, 1.53 t-CO_{2eq}/t-product of the GHGs emissions from the OAS was higher than GHGs emissions from production of CL, TSR and RSS (Usubharatana and Phungrassami, 2014; Jawjit et al., 2013; Jawjit et al., 2010). The maximum ratio of nitrous oxide was confirmed in the vicinity of the influent; it accounted for approximately 80% of the total emitted GHGs. Conversely, methane gas accounted for more than 60% of the total GHGs emitted near the effluent part. Consequently, nitrous oxide accounted for 65% of the total GHGs emissions from the OAS.

3.5 Microbial community analysis

Fig. 8 shows the phylogenetic position of the obtained clones. The description of each clone in the tree includes the number of the compartment and the clone number. As a hydrogen-utilizing methanogen, clones (OAS 16-1) closely related to *Methanospirillum hungatei* were the most frequently detected (39.3% of total clones) in compartment 16. From compartment 1 to 16, the concentration of acetate, propionate, and other VFAs increased 6.5%, 11.7%, and 23.9%, respectively. These results indicated that methane-producing bacteria mainly utilized hydrogen and carbon dioxide produced by hydrolysis, acidogenesis, and hydrogen-producing acetogenesis of RSS wastewater in these compartments. Moreover, clones (OAS 16-2) closely related to *Methanosaeta concilii*, which is known as an acetate-utilizing methanogen, were detected (19.6% of total clones) in compartment 16. In addition, some of clones (such as OAS 16-3) related to genus *Methanosarcina* were detected.

In compartment 33, the number of the clones (such as OAS 33-3, OAS 33-5) closely related to the genus *Methanosaeta* increased significantly (43.1% of total clones). Conversely, the number of clones that were related to the order *Methanomicrobiales*, which is known as a hydrogen-utilizing methanogen, decreased. Therefore, we confirmed that a microbial community formed according to the degradation characteristics of RSS wastewater in the OAS.

4. Conclusion

In our study, emissions of GHGs from an open-type anaerobic wastewater treatment system of a

natural rubber processing factory were evaluated. The characteristics of both the degradation of RSS wastewater and the emission of GHGs were changed by the flow of wastewater through the OAS.

In the OAS, methane and nitrous oxide were emitted as GHGs via removal of organic matter and ammonia, respectively. Relating to the organic matter removal in the OAS, approximately 80% of the solid COD in the RSS wastewater was removed by hydrolysis, acidogenesis and coagulation of rubber particle from compartment 1 to 16. After compartment 16, both the total COD and the soluble COD were linearly degraded as the methane emission rate increased. Finally, 94.4% of the total COD was removed and converted to methane in the OAS. In addition, methane emission was confirmed not only from the bottom but also from the wall surface of the OAS. On the contrary, 18.1% of ammonia in the RSS wastewater was removed and nitrous oxide was emitted in the OAS.

The emission rate of both the methane and the nitrous oxide ranged between 2.58 and 8.85 NL/(m².hr) and between 0.10 and 1.19 NL/(m².hr), respectively. The maximum emission rates of both the methane and the nitrous oxide were observed at the center part of the OAS. The total rate of GHGs emissions from 1 m³ of treated RSS wastewater was 0.153 t-CO₂/m³ and nitrous oxide comprised 65% of the total emitted GHGs. Furthermore, the GHGs emissions (in t-CO_{2eq}/t-product) from the OAS was higher than GHGs emissions from production of natural rubber products. These results suggested that both nitrous oxide and methane emitted from open-type wastewater treatment systems in natural rubber processing factories must be considered as significant contributors to the GHGs that cause the greenhouse effect.

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Acknowledgement

This research was conducted by ESCANBER (Establishment of Carbon-Cycle-System with Natural Rubber) project supported by the JST/JICA, SATREPS (Science and Technology Research Partnership for Sustainable Development), Japan.

REFERENCES

Anotai, J., Tontisirin, P., Churod, P., 2007. Integrated treatment scheme for rubber thread wastewater: Sulfide precipitation and biological processes. J. Haz. Mat. 141, 1-7.

Boonsawang, P., Laeh, S, Intrasungkha, N., 2008. Enhancement of sludge granulation in anaerobic treatment of concentrated latex wastewater. Songklanakarin J. Sci. Technol. 30(1), 111-119.

Chaiparapatand, S., Sdoodee S., 2007. Effects of wastewater recycling from natural rubber smoked sheet production on economic crops in southern Thailand. Resour. Conserv. Recycl., 51, 577-590.

Chaiprapat, S., Wongchana, S., Loykulnant, S., Kongkaew, C., Charnnok, B., 2015. Evaluating sulfuric acid reduction, substitution, and recovery to improve environmental performance and biogas productivity in rubber latex industry. Process Saf. Environ., 94, 420-429.

Daelman, M.R.J., van Voorthuizen, E.M., van Dongen, L.G.J.M., Volcke, E.I.P., van Loosdrecht, M.C.M., 2013. Methane and nitrous oxide emissions from municipal wastewater treatment- results from a long-term study. Wat. Sci. Technol., 67(10), 2350-2355.

1 Foley, J., de Haas, D., Yuan, Z., Lant, P., 2010. Nitrous oxide generation in full-scale biological
2 nutrient removal wastewater treatment plants. *Wat. Res.* 44, 831-844.

3 Hatamoto, M., Nagai, H., Sato, S., Takahashi, M., Kawakami, S., Choeisai, P.K., Syutsubo, K.,
4 Ohashi, A., Yamaguchi T., 2012. Rubber and methane recovery from deproteinized natural
5 rubber wastewater by coagulation pre-treatment and anaerobic treatment. *Int. J. Environ. Res.*
6 6(3), 577-584.

7 IPCC., 2007. Changes in atmospheric constituents and in radiative forcing, in: Solomon, S., Qin, D.,
8 Manning, M, Marquis, M., Averyt, K. Tignor, M.M.B., Miller, H.L., Jr., Chen, Z. (Eds.), IPCC
9 Fourth Assessment Report: Climate Change 2007, 1, Cambridge University Press, pp.210-216.

10 IPCC., 2006. Wastewater treatment and discharge, in: Eggleston, H.S., Buendia, L., Miwa, K.,
11 Nagara, T., Tanabe, K. (Eds.), 2006 IPCC Guidelines for National Greenhouse Gas
12 Inventories, 5, IGES, Japan, pp.6.24-6.27.

13 Jawijt, W., Pavasant, P., Kroeze, C., 2015. Evaluating environmental performance of concentrated
14 latex production in Thailand. *J. Clean. Prod.* 98, 84-91.

15 Jawijt, W., Kroeze, C., Rattanapan, S., 2010. Greenhouse gas emissions from rubber industry in
16 Thailand. *J. Clean. Prod.* 18, 403-411.

17 Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M., van Loosdrecht, M.C.M.,
18 2009. Nitrous oxide emission during wastewater treatment. *Wat. Res.* 43 (17), 4093-4103.

19 Kampschreur, M.J., van der Star, W.R.L., Wielders, H.A., Mulder, J.W., Jetten, M.S.M., van
20 Loosdrecht, M.C.M., 2008. Dynamics of nitric oxide and nitrous oxide emission during full-

scale reject water treatment. Wat. Res. 42 (3), 812-826.

Kanyrat, S., Sumate, C., 2008. Effect of pH adjustment by parawood ash and effluent recycle ratio on the performance of anaerobic baffled reactor treating high sulfate wastewater. Bioresour. Technol. 99, 8987-8994.

Mohammadi, M., Man, H.C., Hassan, M.A., Yee, P.L., 2010. Treatment of wastewater from rubber industry in Malaysia. African J. Biotechnol. 9, 6233-6243.

Nguyen, N.H., Luong, T.T., 2012. Situation of wastewater treatment of natural rubber latex processing in the Southeastern region, Vietnam. J. Vietnamese Environ. 2, 58-64.

Nguyet, A.V., 2013. Natural rubber industry in Vietnam. VietinbankSc Industry Report. 1-15.

Smitha, H.S.S, Rahavendra, M.P., Shruthi, S., Girish, K., 2012. Bioremediation of rubber processing industry effluent by *Arthrobacter* sp.. Int. J. Res. Environ. Sci. Technol. 2, 31-34.

Syutsubo, K., Onodera T., Choeisai, P., Khodphuvieng, J., Prammanee, P., Kaewpradit, W., Kubota, K., 2013. Development of appropriate technology for treatment of molasses-based wastewater. J.Environ. Sci. Health: Part A. 48, 1114-1121.

Tekasakul, P., Tekasakul, S., 2006. Environmental problem related to natural rubber production in Thailand. J. Aerosol Res. 21, 122-129.

Thongnuekhang, V., Puetpaiboon, U., 2004. Nitrogen removal from concentrated latex wastewater by land treatment. Songklanakarin J. Sci. Technol. 26, 521-528.

Usubharatana, P., Phunggrassami, H., 2014. Carbon footprint of concentrated latex: A case study in Southern Thailand. J. Seluck Univ. Nat. Appl. Sci., 459-465.

FIGURES

Fig. 1. The compartment layout of the OAS.

Wastewater flowed according to the numbering of the compartments.

Fig. 2. The schematic diagram of the biogas collection chambers.

Fig. 3. Biogas composition of compartments 28, 33, and 56.

Fig. 4. Methane emission rate and COD concentration of each compartment.

Fig. 5. COD mass balance in the OAS.

Fig. 6. Nitrous oxide emission rate and ammonia concentration of each compartment.

Fig. 7. Composition of emitted GHGs from near the influent part, the center part, and the effluent part of the OAS.

OAS 16-: Clones of compartment 16 OAS 33-: Clones of compartment 33

Bold font: Dominant clones of each compartment

Fig. 8. Phylogenetic position of obtained clones among domain Archaea (16S rRNA gene).