FINAL REPORT
INTERNATIONAL RESEARCH COLLABORATION

Development of Hybrid Anammox Reactor for High Nitrogen Concentration Removal

Principal Investigator
Tuty Emilia Agustina, PhD
Prof. Dr. Michihiko IKE

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Bio-Environmental Engineering, Osaka University
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Research Title : Development of Hybrid Anammox Reactor for High
Nitrogen Concentration Removal

Principal Investigator:
a. Name : Tuty Emilia Agustina, ST, MT, PhD
b. Sex : Female
c. NIP : 19720809 200003 2001
d. Structural position : III-d
e. Functional position : Lektor Kepala
f. Faculty/Department: Engineering / Chemical Engineering
g. Research Center : Center for Wastewater Treatment Technology
h. Office Address : Jl. Raya Prabumulih Km. 32, Inderalaya, Ogan Ilir (OI),
Sumatra Selatan – 30662
i. Phone/Facs. : (0711) 580303/ 580303
j. Home address : Jl. Tanjung Barangan RT.48 RW.10 No.09 Ilir Barat I
Palembang - 30139
k. Phone/E-mail : (0711) 440910 / 081368920872 /tutycurtin@yahoo.com

International collaborative partner
a. Name : Prof. Michihiko IKE
b. Institution : Bio-Environmental Engineering Laboratory, Graduate
School of Engineering, Osaka University
c. Address : S-4 Building., 2-1, Yamada-Oka, Suita, Osaka 565-0871,
Japan.

Research durations : 3 (three) years

Research members:

Indonesian Team

No.	Name	Institution	Expertise	Time allocated (hrs/week)
1.	Tuty Emilia Agustina, PhD	Dept. Chemical Engineering Univ.Sriwijaya	Advance wastewater treatment	15
2.	Novia, PhD	Dept. Chemical Engineering Univ.Sriwijaya	Anaerobic biochemical processes	10

Japan Team

No.	Name	Instituiton	Expertise
1.	Prof. Dr. Michihiko IKE	Bio-environmental Engineering Osaka University	Biological processes for resource recovery
2.	Dr. Satoshi SODA	Bio-environmental Engineering Osaka University	Modeling for biological process
3.	Masashi TAKEKAWA, M.Eng	Bio-environmental Engineering Osaka University	Bioreactor design and modelling

Inderalaya, December 15, 2015

International Collaborator,

Principal Investigator,

M. Ike

(Prof. Dr. Michihiko IKE)

(Tuty Emilia Agustina, PhD)

Approved by,
Head of Research Institute University of Sriwijaya

Prof. Dr. H. M. Said, M.Sc
NIP 19610812 198703 1003

ABSTRACT

Nitrogen pollution due to the discharge of untreated wastewater containing high nitrogen concentration is recently considered as one of critical environmental problems for water resource protection. Recently, anaerobic ammonium oxidation (anammox) has been recommended as a new biological approach for ammonia removal from wastewater without requiring the oxygen supply and additional carbon source. The anaerobic ammonium oxidation (anammox) process has attracted considerable attention in recent years as an alternative to conventional nitrogen removal technologies. **In this study (Second year),** an innovative hybrid reactor combining fluidized and fixed beds for anammox treatment will be developed. The fluidized bed is mechanically stirred and the gaseous product could be rapidly released from the anammox sludge to prevent washout of the sludge caused by floatation. The fixed bed comprising a non-woven biomass carrier could efficiently catch sludge to reduce washout. This research is intended to promptly establish the anammox reactors with the selected sludge and to optimize the reactor performance for high nitrogen concentration removal.

We divided the proposed work into 3 phases/ years: 1) Enrichment and cultivation of Anammox organisms. 2) Performing bioreactor system treating ammonium in continuous process. 3) Development of anammox process for single-stage nitrogen removal.

Collaborative research activity in **the second phase** mainly focus on alternative strategies to promptly establish the anammox reactors capable of retaining the biomass as effectively as possible and with a highly efficient substrate transfer for treating high ammonium nitrogen concentration (NH_4 > 500 - 1000 mg/l) **Optimal performance in a hybrid nammox reactor with high nitrogen removal rate will be highlight in this phase.**

Chapter 1. Introduction

1.1 Problem statement

Nitrogen pollution from industrial wastewater, domestic wastewater and agricultural wastewater streams is recently considered as one of critical environmental problems for water resource protection. The discharge of untreated wastewater containing high nitrogen concentration is known to be one of the causes of eutrophication and oxygen depletion as a result of an abundance of nitrates and phosphates in surface water (Ahn and Choi, 2006).

Conventionally, ammonium nitrogen from wastewater has been removed by a combination of biological process between nitrification and denitrification. This system requires oxygen supply as electron acceptor during nitrification process. Besides an additional carbon source must sometimes be supplied for anoxic denitrification. Thus, these lead to increase the operational cost of full-scale treatment plant.

Recently, anaerobic ammonium oxidation (anammox) has been recommended as a new biological approach for ammonia removal from wastewater. Anammox is based on the utilization of ammonium nitrogen (NH_4^+-N) as electron donor under anaerobic condition for nitrite (NO_2^- -N) reduction resulting dinitrogen (N_2) gas as the final product. This process is described on below equation (Jetten *et al.*, 2001).

$$NH_4^+ + 1.32NO_2^- 0.066HCO_3^- + 0.13H^+ \longrightarrow$$
$$\rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

The anammox process had attracted worldwide attention for its advantages on nitrogen removal compared to the conventional nitrification and denitrification process. The partial nitrification-anammox process is considered to be more cost-

Chapter 2. Literature review

2.1 Nitrogen cycle

Nitrogen can be present in different forms in aquatic environment. In Figure 1 the general nitrogen cycle is presented. Ammonia assimilation is the biological reaction of transforming nitrogen to cellular material, which is the main biological method for fixing nitrogen. The reaction of converting organic nitrogen to ammonia is called ammonification. Nitrification is the biological oxidation which can convert ammonium to nitrate. The process is performed by micro-organisms and take place in two subsequent steps (Koelmans and de Klein, 2013):

$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$

$2NO_2^- + O_2 \rightarrow \rightarrow 2NO_3^-$

After nitrification, nitrogen is more easily assimilated by plants and transported with water currents. Denitrification is the biological process which can reduce nitrate to Gaseous nitrogen oxides compounds (N_2O or NO), and then form to molecular nitrogen (N_2), which is the primary pathway for the return of nitrogen to the atmosphere.

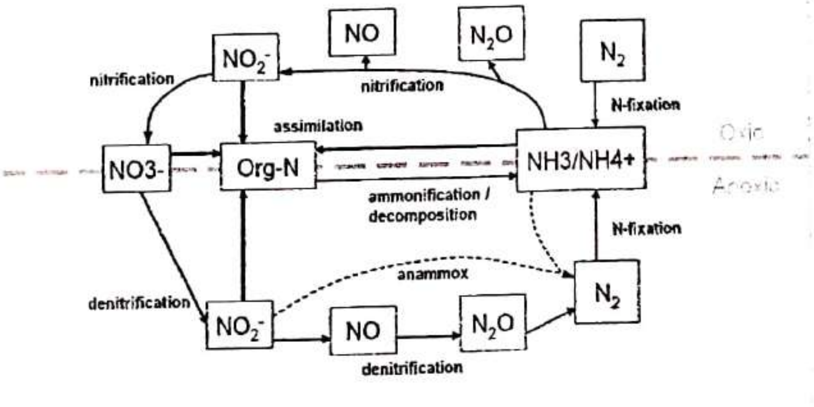

Figure 1. Nitrogen cycle (Koelmans and de Klein, 2013)

Chapter 3. Methodological design

3.1. Research collaboration

The research at Sriwijaya University, Indonesia is part of a project done together with the Laboratory of Bio-environmental Engineering, Osaka University, Japan. The principal researchers at Sriwijaya University will operate the bioreactors while the researchers at Osaka University will focus on microbial analyses and modeling. In this second year research, the researchers from Sriwijaya University will design the hybrid reactor. However, the constructions of the reactor will be done in Osaka University.

The flow chart of the research can be seen in **Figure 4.** In the first part of the project, with duration of one year, has been focused on growing anammox bacteria and increasing the amountof biomass available for the research. Additionally, during continuous culture, different original sludge compositions have been inoculated and evaluated.

The second part of the research will focus on alternative strategies to develop and apply a suitable anammox reactor system capable of retaining the biomass as effectively as possible and with a highly efficient substrate transfer for treating high ammonium nitrogen concentration ($NH_4 > 500 - 1000$ mg/l). For this best operational conditions affecting anammox activity will be introduces into the hybrid anammox reactor combining the fluidized and fixed beds.

After successful application of anammox from previous part, the development of CANON reactor for single-stage nitrogen will be studied and figure out this part is scehdule in the third year of the project.

Chapter 3. Methodological design

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Chapter 4. Results and Discussion

4. Results

4.1 Nitrogen removal

The hybrid anammox reactor has been operated during 111 days. The reactor was continuously fed with ammonium and nitrite with a ratio 1:1.1. The time course of influent and effluent nitrogen concentrations is shown in Figure 6. As can be observed from this figure, during the operation, the influent total nitrogen concentration was increasing up to 685 mg/L (with 340 mg/L NH_4^+-N and 340 mg/L NO_2^-N approximately) in the end of experiments. Meanwhile, hydraulic retention time (HRT) was decreasing from 8 hours in the beginning to 0.8 hours in the end of operation (Figure 7).

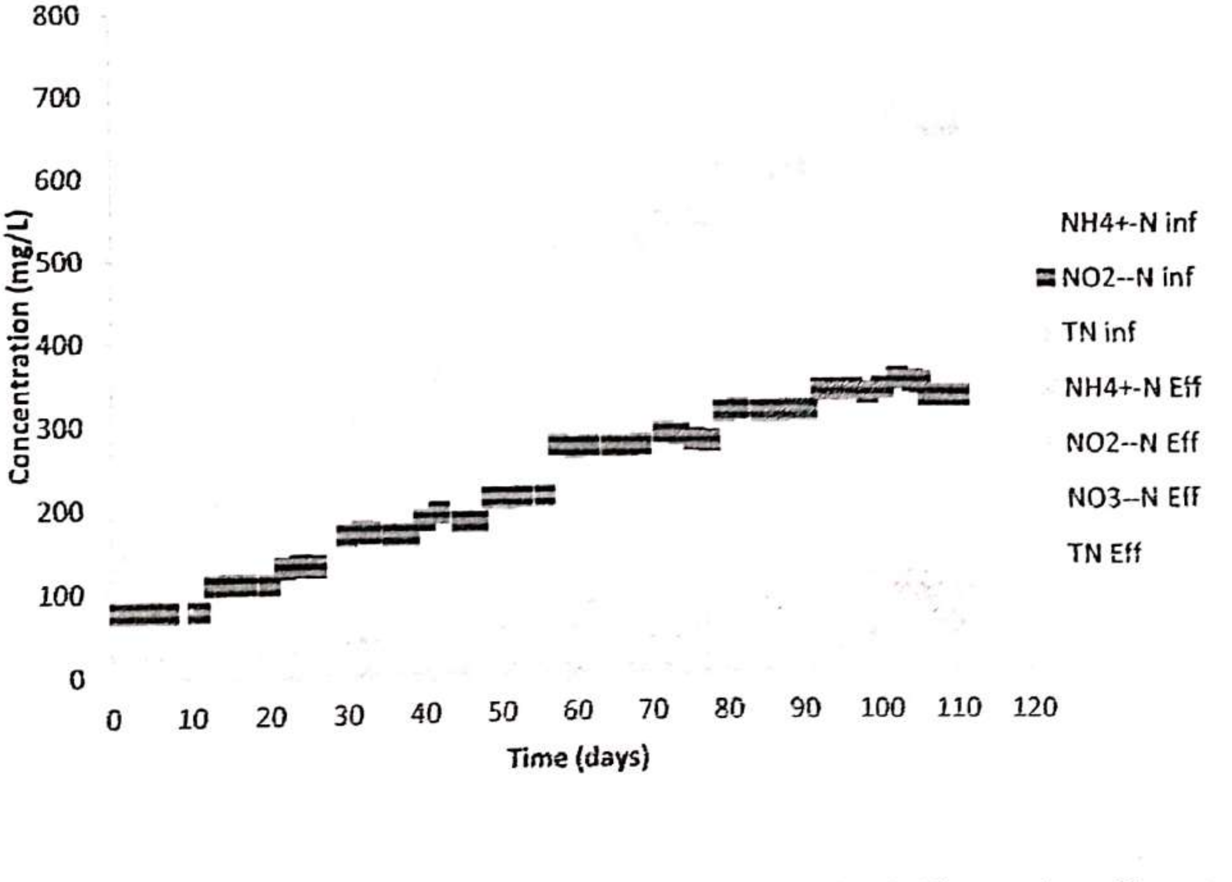

Figure 6. Evolution of the nitrogen concentration in the influent the effluent along the operational period.

Chapter 5. Budgeting

The amount of budget used in this research, during first year of the project depicted in Table 2 bellows:

I. Salary

No	Executants	Volume	Unit	Man hour	Cost
1	Principal Investigator (1 x 12.5 hr x 32 wee	400	OJ	30,000.00	12,000,000.00
2	Research member (1 x 8 hr x 32 week)	256	OJ	22,500.00	5,760,000.00
3	Research assistant (2 x 8 hr x 32 week)	512	OJ	20,000.00	10,240,000.00
4	Administration(1 x 4 hr x 10 week)	40	OJ	15,000.00	600,000.00
				Sub Total	**28,600,000.00**

II. Material, supporting instruments and analysis

No	Material, supporting instruments and an	Volume	Unit	Unit cost	Cost
1	Stationery	1	pack	498,000.00	498,000.00
2	(NH4)2SO4	1	5 Kg	2,150,000.00	2,150,000.00
3	NaNO2	4	500 gr	732,000.00	2,928,000.00
4	Nitrogen Gas 6 m3 + tabung	1	buah	2,500,000.00	2,500,000.00
5	EDTA/Tritriplex III	1	250 gr	1,019,000.00	1,019,000.00
6	KH2PO4	1	250 gr	560,000.00	560,000.00
7	Iron II Sulphate	1	250 gr	716,000.00	716,000.00
8	Reactor installation Volume 6 L	1	unit	25,000,000.00	25,000,000.00
9	Silicone Grease Dow corning USA	2	tube	750,000.00	1,500,000.00
10	Tubing Silicone Nalgen	3	meter	150,000.00	450,000.00
11	Regulator N2 (Moris)	1	buah	1,750,000.00	1,750,000.00
12	Mechanical stirrer	1	unit	13,000,000.00	13,000,000.00
13	Analysis of ammonia	90	sampel	40,000.00	3,600,000.00
14	Analysis of nitrate	45	sampel	40,000.00	1,800,000.00
15	Analysis of nitrite	90	sampel	40,000.00	3,600,000.00
16	Waterbath HWS24	1	unit	4,750,000.00	4,750,000.00
17	Tools and cosumable material	1	pack	1,253,000.00	1,253,000.00
	Tax payment				6,427,800.00
				Sub Total	**73,501,800.00**

Chapter 6. Conclusion and Recommendations

6. Conclusions and Recommendations

6.1 Conclusions

The hybrid anammox reactor using porous polyester pile fabric as carrier showed a relatively high adaptability to high NLR and short HRT. A maximum nitrogen removal rate (NRR) of 15.3 kg-N m^{-3} d^{-1} was reached on the operation day 97 with a TN removal efficiency of 77%. The anammox bacteria KSU-1 were detected as the dominant species among detectable members, accounting for 72%.

6.2 Recommendations

For further understand and researches of hybrid anammox reactor, the following studies are required:

Since the hybrid anammox reactor in this study was relatively operated in a shorter experiment compared with previous studies. It will be interesting to run the reactor in longer period of operations in order to observe the maximum performance and activity of the reactor.

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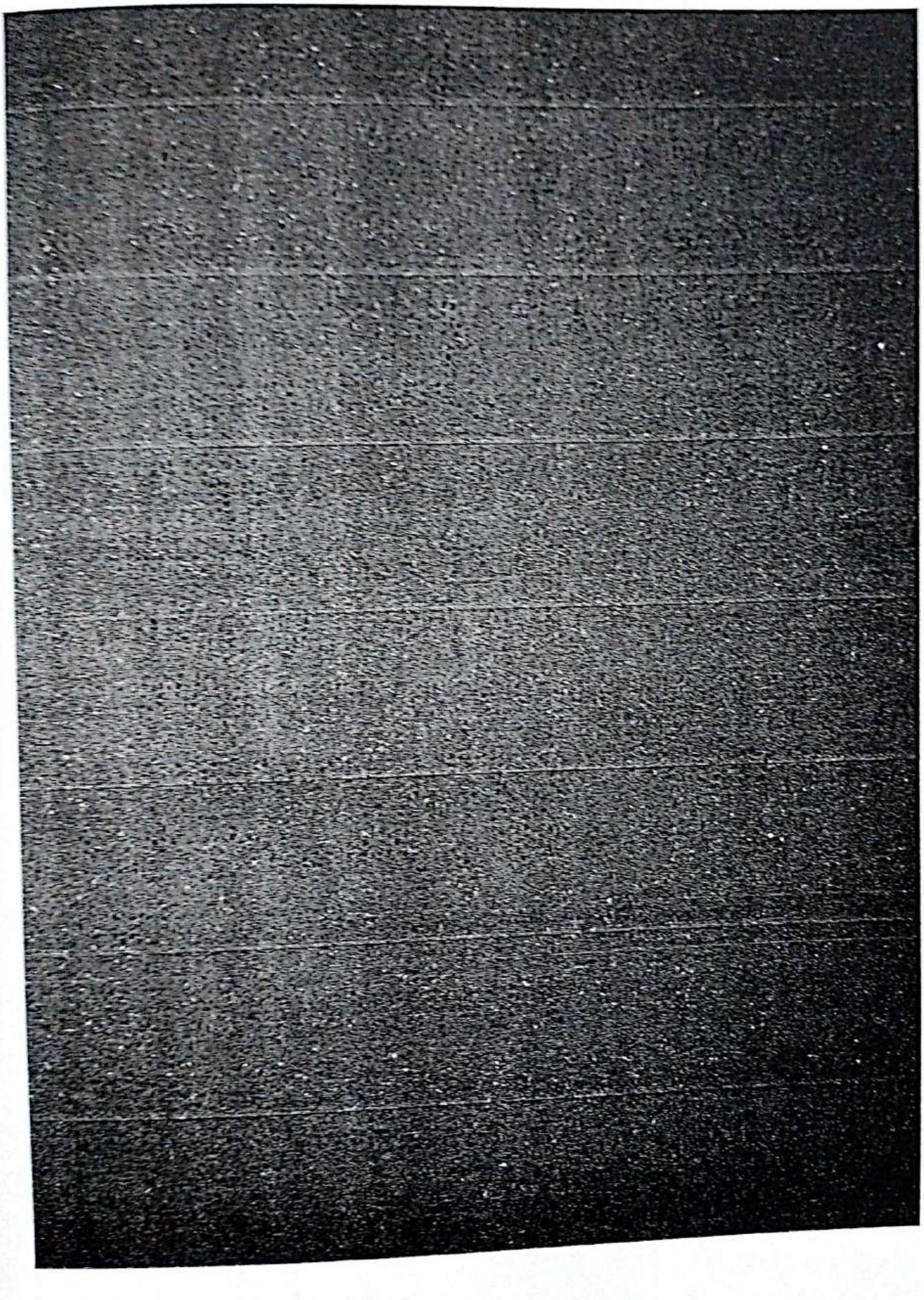
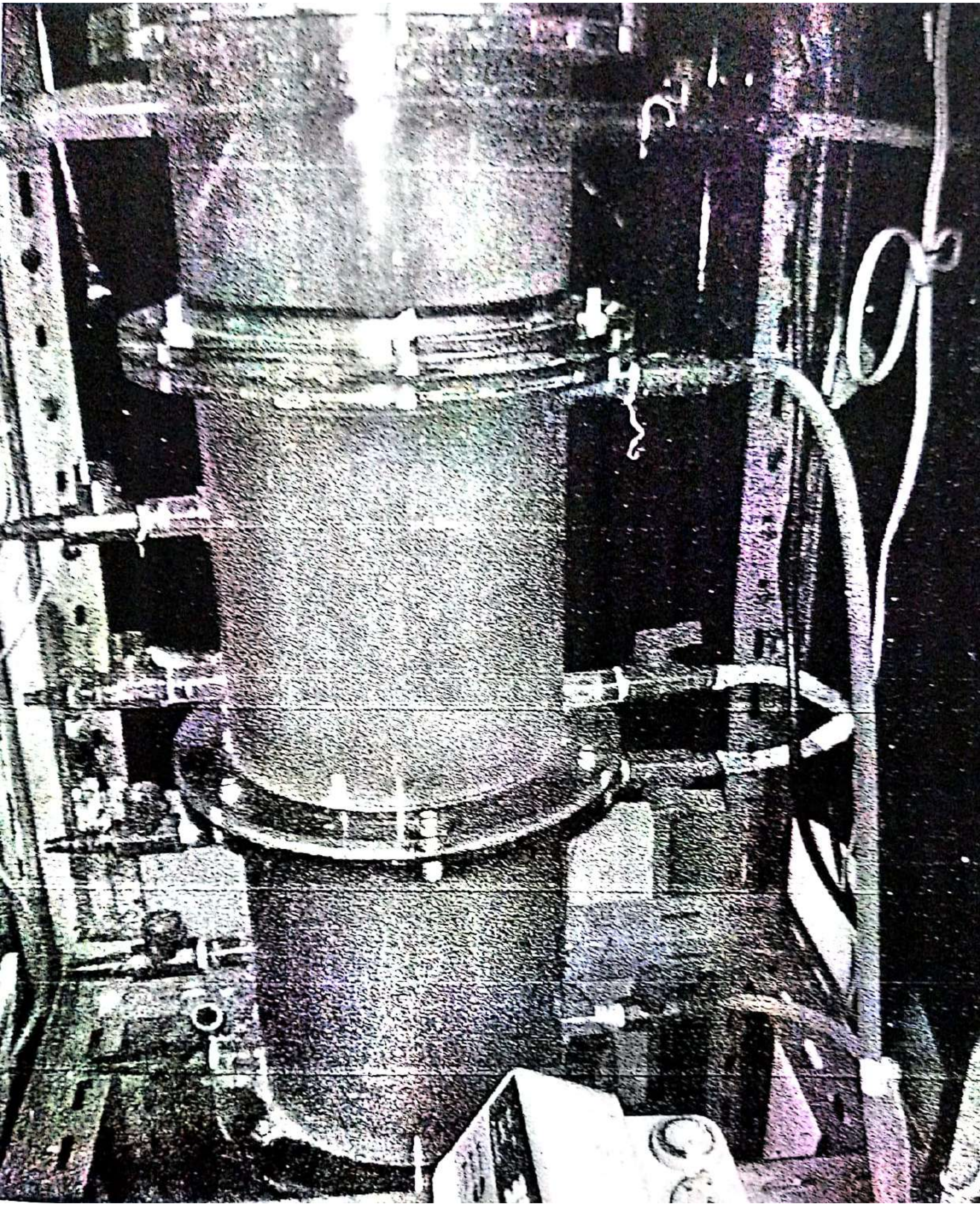
Appendix 1.



Porous polyester pile fabric



Appendix 3. Manuscript

Performance of Hybrid Anammox Reactor Using a Pile Fabric as Biomass Carier for High Nitrogen Removal Rate

Agustina, TE, Diansyah, G, Novia, Takekawa, M, Ma, Y, and Furukawa, K

Tuty Emilia Agustina
Department of Chemical Engineering,
Sriwijaya University, Indonesia

Gusti Diansyah*
Department of Marine Science,
Sriwijaya University, Indonesia
e-mail: diansyah.gusti@gmail.com
*Corresponding author

Novia
Department of Chemical Engineering,
Sriwijaya University, Indonesia

Masashi Takekawa
Graduate School of Science and Technology,
Kumamoto University, Japan

Ma Y
Graduate School of Science and Technology,
Kumamoto University, Japan

Furukawa Kenji
Graduate School of Science and Technology,
Kumamoto University, Japan

***Abstract*—Ammonia removal from wastewater using anaerobic process called anammox which in effect will result in the removal of nitrogen is paramount. Anammox is a biological method of nitrogen removal which is considered to be efficient, cheap and environmentally friendly. This study is aimed to evaluate the feasibility of hybrid anammox reactor for removal of synthetic wastewater containing high ammonia concentration. The anammox hybrid reactor with an effective volume of 6.0 L was operated with two parts, combining fixed bed in the upper part and fluidized bed in the bottom part. The upper part was constructed with a porous polyester pile fabric material to effectively attach the suspended sludge. A maximum nitrogen removal rate (NRR) of 15.3 kg-N m^{-3} d^{-1} was reached on the operation day 97 with a TN removal efficiency of 77%. From the viewpoint of the biomass concentration, the fluidized bed was considerably responsible for 82% of the total nitrogen removal.**

***Keywords*—Anammox, Hybrid reactor, Pile fabric, Nitrogen removal.**

I. Introduction

Nitrogen is known to be one of the causes of eutrophication as a result of an abundance of ammonium in surface water when the effluent of untreated municipal wastewater is discharged into water bodies [1]. In order to reduce fixed nitrogen such as ammonium (NH_4^+) and nitrate (NO_3^-) from wastewater, conventional wastewater treatment systems predominately apply the aerobic nitrification process followed by the anaerobic denitrification process [2]. In this system, supply of oxygen as electron acceptor for nitrification process and supply of extra carbon source for anoxic heterotrophic denitrification are fully required and result in the increasing of the operational cost.

A new process in the nitrogen cycle called anaerobic ammonium oxidation (Anammox) was discovered in the mid -1990's [3]. This process yields the dinitrogen gas from ammonium oxidation and nitrite reduction under anaerobic condition [4]. Some of the advantages of the Anammox process in comparison to conventional nitrification/denitrification include reduced oxygen demand (thus less energy input), no need for external carbon addition (CO_2 is used instead as carbon source for bacteria growth), negligible sludge production, lower space requirement and hence higher cost-effectiveness [5; 6]

In the present research, we worked with similar reactor with that of Ma et al. [7] to investigate the performance of anammox process for high nitrogen removal rate in an hybrid reactor using a pile fabric as biomass carrier.

II. Materials and Methods

A. Seed anammox sludge

The anammox sludge which contains KSU-1 and KU-2 strains [8] was used for inoculation. The enriched anammox sludge was originated from an up-flow fixed bed reactor fed with synthetic wastewater [8].

B. Reactor and experimental setup

The schematic overview of the hybrid anammox reactor is depicted in Figure 1. The anammox hybrid reactor with an effective volume of 6.0 L was designed with two parts, combining fixed bed in the upper part and fluidized bed in the bottom part. The upper part was constructed with a porous polyester pile fabric material (Ohyapile, Japan) to effectively attach the suspended sludge. In the fluidized bed, a mechanical stirrer (Z-2200. Tokyo Rikakikai, Japan) was installed to increase mass transfer between biomass and wastewater and at the same time to release the dinitrogen gas from the sludge.

The hybrid anammox reactor was operated at 32°C controlled thermostatically with a thermostated jacket. A pH controller was introduced to preserve the pH at around 7.0-7.4 by adding the HCl 0.1M. To keep the reactor in anaerobic condition, the dissolved oxygen (DO) concentration in the influent was consistently maintained below 1.0 mg/L in which the influent was flushed with nitrogen gas. Besides, the reactor was enclosed with a black vinyl sheet to prevent the light interference that possibly reduces 30-50% of ammonium removal rate [4]. The feeding solution in the influent tank was filled to the reactor from the bottom in an up-flow mode by a peristaltic pump (RP-2000, Rikakikai Japan).

A. Feeding Media

In this study, synthetic wastewater which contains NH_4^+-N and NO_2^- -N was made and continuously filled into the hybrid anammox reactor. $(NH_4)_2SO_4$ and $NaNO_2$ were used as the ammonium and nitrite sources with molar ratio 1.0-1.2. During the experiments, 150-350 mg/L of NH_4^+-N and 150-350 NO_2^- -N was used as influent concentration of synthetic medium. In addition, the other components consisting of $KHCO_3$ 125-500 mg/L, KH_2PO_4 54 mg/L, $FeSO_4.7H_2O$ 9 mg/L and EDTA were also added to the synthetic medium.

B. Analytical method

Ammonium nitrogen (NH_4^+-N) was quantified based on the indophenol reaction with ortho-phenylphenol (OPP). Nitrite nitrogen (NO_2^--N) was determined with the colorimetric method (APHA, 1995) and nitrate nitrogen (NO_3^--N) with the ultraviolet spectrophotometric screening method (APHA, 1995). pH and DO was measured using a pH meter (F55, Horiba Ltd, Japan) and a DO meter (OM-51, Horiba Ltd, Japan). For MLSS and MLVSS determination, the centrifuged sludge sample was dried at 105°C and 550°C respectively.

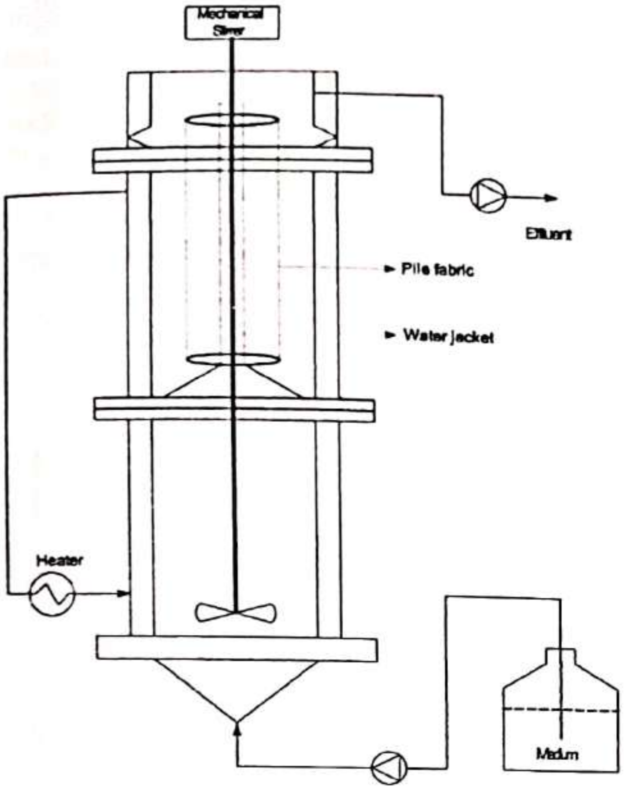


Figure 1. Schematic of hybrid anammox reactor used for continuous treatment.

III. Results

a. Nitrogen removal

The hybrid anammox reactor was operated during 111 days. The reactor was continuously fed with ammonium and nitrite with a ratio 1:1.1. The time course of influent and effluent nitrogen concentrations is shown in Figure 2. As can be observed from this figure, during the operation, the influent total nitrogen concentration was increasing up to 685 mg/L (with 340 mg/L NH_4^+-N and 340 mg/L NO_2^--N approximately) in the end of experiment. Meanwhile, hydraulic retention time (HRT) was decreasing from 8 hours in the beginning to 0.8 hours in the end of operation (Figure 3).

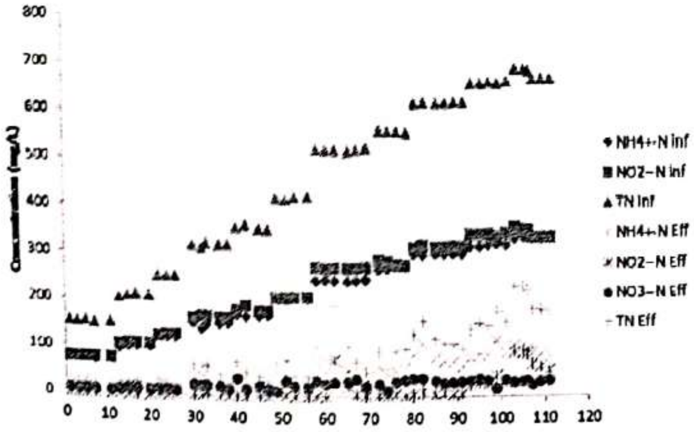


Figure 2. Evolution of the nitrogen concentration in the influent the effluent along the operational period.

The reactor was started up with an influent total nitrogen (TN) concentration of 150 mg/L resulting a feed loading rate of 0.5 kg-N m^{-3} d^{-1}. In Ma et al. (2011), an internal circulation and a continuous stirring at 30 rpm were installed and performed during days 1-39 resulting a rapidly increasing of the nitrogen loading rate (NLR) from 0.35 to 1.2 kg-N m^{-3} d^{-1}. This indicates that applying the internal circulation during start up period is important to increase biomass retention. However, in our study, during start-up period, increasing the influent TN concentration and decreasing HRT increased the NLR during days 1-26. The NLR during this period was increased from 0.5 to 2.0 kg-N m^{-3} d^{-1} with maximum removal efficiency was 90 % indicating a better adaptation of anammox bacteria than earlier phase.

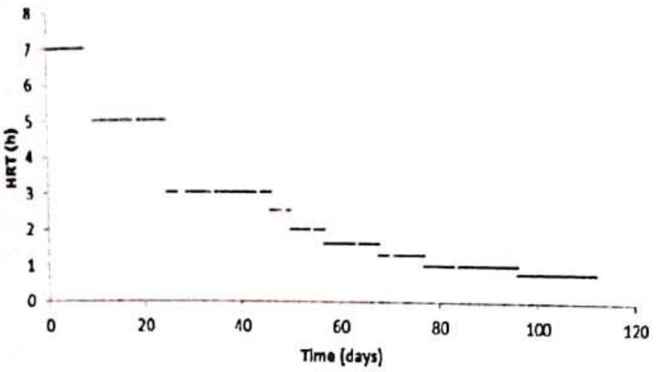


Figure 3. Time course of HRT during reactor operation.

As can be seen in Figure 4, as the capacity of nitrogen removal of the system going up after a successful start-up, both the ammonium and nitrite concentrations in the influent flows were gradually increased from 100 to 350 mg/L. Besides, from days 22-96, the HRT was reduced from 5 to 0.8 hours. Consequently, the NLR increased from 1.2 to 20.5 kg-N m^{-3} d^{-1}. During this period, a relatively high TN removal efficiency was obtained with the average was 81.5±6%. This value was lower compared with the maximum nitrogen removal based on anammox stoichiometry due to NO_2^-N limitation. In addition, a maximum nitrogen removal rate (NRR) of 15.3 kg-N m^{-3} d^{-1} was reached on the operation day 97 with a TN removal efficiency of 77%.

At the end of operation, the total biomass concentration in fluidized bed (lower part) and on the polyester pile fabric carrier in the upper part reached 19.4 g-VSS/L. A 15.9 g-VSS/L of anammox sludge was measured in the fluidized part, while the anammox biomasss concentration attached on the polyester pile fabric carrier was 3.5 g-VSS/L. This showed, from the viewpoint of the biomass concentration, that the fluidized bed was considerably responsible for 82% of the total nitrogen removal.

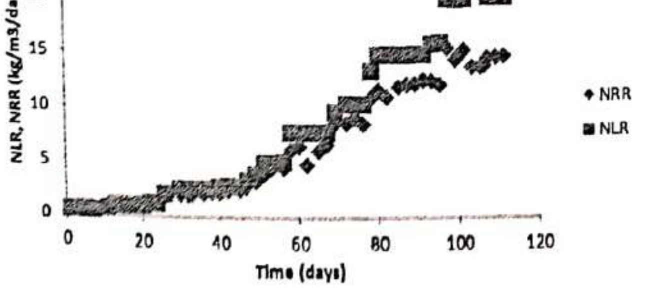


Figure 4. Time course of total NLR, NRR and nitrogen removal efficiency (◊ : NRR; □ : NLR)

IV. Discussion

In the present study, the hybrid reactor was successfully applied to perform high nitrogen loading rate. The combination between fixed and fluidized beds in this reactor was employed to increase conversion capacity of the reactor. In the upper part, the fixed bed using a porous polyester pile biomass carrier was installed to effectively retain the suspended biomass and to reduce the washout. Additionally, the fluidized bed was mechanically stirred to increase the mass transport between anammox sludge and wastewater. It is also confirmed by Mulder et al. [9] that factors including biological conversion capacity, mass transport and biomass concentration are crucial in order to reach high conversion capacity of a bioreactor.

The NRR treated in this study are comparable to those previously obtained by other studies. Sliekers et al. (2003) reported a maximum NRR of 8.9 kg-N m^{-3} d^{-1} was reached using a gas-lift anammox reactor. Besides, a study from Quan et al. [10] succeeded to obtain the NRR of 8.2 kg-N m^{-3} d^{-1}. In this study, the polyvinyl alcohol (PVA) gel was used as biomass carrier entrapped anammox sludge in a stirred tank reactor (STR). Moreover, values of NRR higher than this study were referred by Tsushima et al. [11] and Ma et al. (2011) of 26.0 kg-N m^{-3} d^{-1} in an up-flow fixed bed glass biofilm column reactor and of 20.7 kg-N m^{-3} d^{-1} in a hybrid anamox reactor. The maximum NRR of 20.7 kg-N m^{-3} d^{-1} from Ma et al. [7] was reached in a longer reactor operation on day 333 compared with our study on day 156. Additionally, the porous polyester non-woven fabric used by Ma et al. [7] as biomass carrier could catch more effective the suspended sludge and has a higher surface area than the porous polyester pile fabric used in our study.

V. *Acknowledgment*

The authors are grateful to Professor Kenji Furukawa and Furukawa Laboratory for providing the research. Also to Sriwijaya University Research Institute for financial support.

VI. *References*

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