

# 1. Jurnal Nitrogen Removal Tuty EA\_Novia.pdf

19

## Nitrogen Removal by Anammox Biofilm Column Reactor at Moderately Low Temperature

Tuty Emilia Agustina<sup>1\*</sup>, Novia<sup>1</sup>, Gusti Diansyah<sup>2</sup>, Michihiko Ike<sup>3</sup>, and Satoshi Soda<sup>3</sup>

<sup>1</sup>Department of Chemical Engineering, Universitas Sriwijaya, Indonesia

<sup>2</sup>Department of Marine Science, Universitas Sriwijaya, Indonesia

<sup>3</sup>Bio-Environmental Engineering Laboratory, Osaka University, Japan

25

\*Corresponding Author: [tuty\\_agustina@unsri.ac.id](mailto:tuty_agustina@unsri.ac.id)

### Abstract

10

The anaerobic ammonium oxidation (anammox) as a new biological approach for nitrogen removal has been considered to be more cost-effective compared with the combination of nitrification and denitrification process. However, the anammox bioreactors are mostly explored at high temperature (>30°C) in which temperature controlling system is fully required. This research was intended to develop and to apply anammox process for high nitrogen concentration removal at ambient temperature used for treating wastewater in tropical countries. An up-flow biofilm column reactor, which the upper part constructed with a porous polyethylene or non-woven fabric material as a carrier to attach the anammox bacteria was operated without heating system. A maximum nitrogen removal rate (NRR) of 1.05 kg-N m<sup>3</sup> d<sup>-1</sup> was reached in the operation days of 178 with Total Nitrogen (TN) removal efficiency of 74%. This showed the biofilm column anammox reactor was successfully applied to moderate high nitrogen removal from synthetic wastewater at moderately low temperature.

*Keywords: Anammox, biofilm column reactor, ambient temperature, nitrogen removal*

### Abstrak (Indonesian)

Anaerobic ammonium oxidation (anammox) sebagai salah satu pendekatan baru secara biologi untuk menghilangkan nitrogen dianggap lebih murah biayanya dibandingkan dengan kombinasi proses nitrifikasi dan denitrifikasi. Namun, bioreaktor anammox kebanyakan dieksplorasi pada suhu tinggi (> 30°C) dimana diperlukan sistem pengendalian suhu. Penelitian ini bertujuan untuk mengembangkan dan menerapkan proses anammox untuk menghilangkan nitrogen dengan konsentrasi tinggi pada suhu lingkungan yang digunakan untuk pengolahan air limbah di negara tropis. Dalam penelitian ini, dipakai sebuah Reaktor kolom biofilm *up-flow*, dimana pada bagian atas kolom ditempatkan bahan poliester *non-woven* berpori sebagai pembawa untuk menempelkan bakteri anammox, telah dioperasikan tanpa sistem pemanas. Laju penghilangan nitrogen maksimum (NRR) sebesar 1,05 kg-N m<sup>3</sup> d<sup>-1</sup> telah dicapai pada hari operasi ke 178 dengan efisiensi total penghilangan nitrogen (TN) sebesar 74%. Hal ini menunjukkan bahwa reaktor kolom biofilm anammox telah berhasil diterapkan untuk menghilangkan nitrogen dengan konsentrasi cukup tinggi dari air limbah sintetis pada suhu yang cukup rendah.

### INTRODUCTION

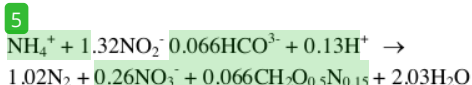
Nitrogen pollution from industrial wastewater, domestic wastewater and agricultural wastewater streams is recently considered as one of critical environmental problems for water resource protection. The discharge of untreated wastewater containing high nitrogen concentration is known to be one of the causes of eutrophication and oxygen depletion as a result of an abundance of nitrates and phosphates in surface water [1].

1

Excessive use of fertilizers in the agricultural sector and daily human activities are the main sources of nutrient pollution. Nitrogen pollution has become an important problem in many countries around the world, including Indonesia as one of developing countries with a lot of agricultural activity. Indonesia also has many fertilizer industries to support their various crop production. Treatment of nitrogen from industrial wastewater has become an emerging issue for environmental protection in Indonesia.

Conventionally, ammonium nitrogen from wastewater has been removed by a combination of biological process between nitrification and denitrification. This system requires an oxygen supply as electron acceptor during nitrification process. Besides an additional carbon source must sometimes be supplied for anoxic denitrification. Thus, this leads to increase the operational cost of the 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 ( $\text{NH}_4^+$ -N) as an electron donor under anaerobic condition for nitrite ( $\text{NO}_2^-$ -N) reduction resulting dinitrogen ( $\text{N}_2$ ) gas as the final product. This process is described on equation [2] below.



Compared to the conventional nitrification and denitrification process, the partial nitrification-anammox process is considered to be more cost-effective due to less required oxygen demand and no extra additional carbon source [3]. In the partial nitrification and anammox process, half of ammonium is partly oxidized to nitrite and subsequently anammox bacteria oxidize anaerobically the ammonium using nitrite to dinitrogen gas. However, in the practice, an anammox reactor system with highly efficient biomass retention and with high mass transfer system is entailed due to extremely slow growth rate of anammox bacteria with a doubling time of about 11 days [4]. Besides, up to now the reported anammox systems in the literature were mostly operated at 30–37°C [5] [6].

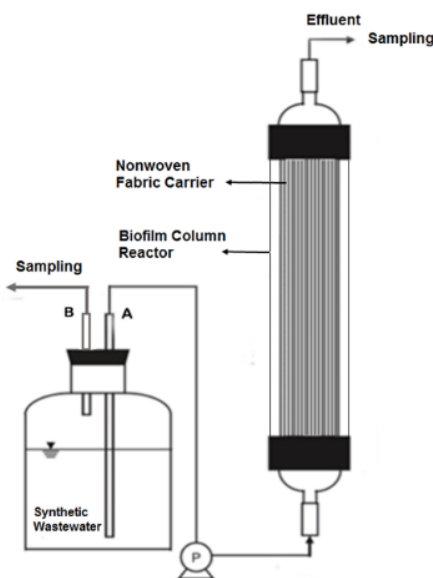
It is therefore important to find a suitable anammox reactor system capable of retaining the biomass as effective as possible and with a highly efficient substrate transfer for treating high ammonium nitrogen concentration at moderately low temperature (25–27°C). This research has opened an operational window for treating fertilizer wastewater with high ammonium concentration applied in tropical countries. In many tropical countries, the availability of sunlight is abundant. It is possible to develop a bioreactor system which equipped with microorganism living in moderate low temperature, without any water heating systems. By using the appropriate bacteria and reducing additional cost caused by heating apparatus, the low cost bioreactor system could be improved. The objective of this research is to develop and to apply anammox process for high nitrogen concentration removal

at ambient temperature used for treating wastewater in tropical countries, such as Indonesia.

## MATERIALS AND METHODS

### Reactor

An up-flow biofilm column reactor with an effective volume of 1.65 L was operated where the upper part was constructed with a porous polyester non-woven fabric material (Ohyapile, Japan) as a carrier to effectively attach the Anammox bacteria. The column in Anammox reactor was inoculated with KSU-1 strain from Osaka University and operated at ambient tropical temperature without any water heating systems (25–27°C). The reactor was always enclosed with a black vinyl sheet to prevent the inhibition of the growth of photosynthetic bacteria [7]. Peristaltic pumps (Watson Marlow, 500 series) were used to control the feed inflow rate. The reactor was run without recycling and the effluent was driven by gravity. Samples were taken from the inflow and overflow lines regularly to analyze nitrogen reduction.



**Figure 1.** Schematic research equipment of biofilm column reactor

### Feeding Media

The bioreactor's influent was a synthetic wastewater consisted of  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NaNO}_2$ ,  $\text{NaHCO}_3$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and EDTA. All of

The chemicals were supplied by Merck. Concentrations of ammonium and nitrite were increased stepwise over the ranges. The Anammox enrichment medium had a bicarbonate-buffering agent instead of phosphate, given the possible toxicity of the latter.

### Analytical methods

Ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and Nitrite nitrogen ( $\text{NO}_2^-\text{-N}$ ) was determined with the colorimetric method and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) with the ultraviolet spectrophotometric screening method [8]. pH and DO was measured using a pH meter (F55, Horiba Ltd, Japan) and a DO meter (OM-51, Horiba Ltd, Japan).

## RESULT AND DISCUSSION

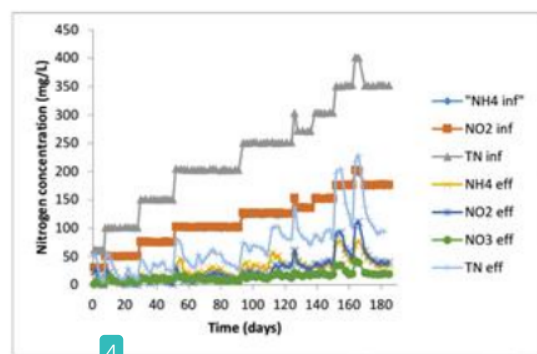


Figure 2. Evolution of the nitrogen concentration in the influent and effluent along the operational period.

The biofilm column in anammox reactor has been operated for 185 days. The reactor was continuously fed with ammonium and nitrite with a ratio of 1:1.1. The time course of influent and effluent nitrogen concentrations shown in Figure 2. As can be observed from this figure, during the operation, the influence of Total Nitrogen concentration had increased up to 350 mg/L (with 174 mg/L  $\text{NH}_4^+\text{-N}$  and 176 mg/L  $\text{NO}_2^-\text{-N}$ ) at the end of the experiment. Meanwhile, Hydraulic Retention Time (HRT) had decreased from 24 hours in the beginning until 6 hours in the day of 145 of total operations (Figure 3).

The reactor was started up with an influent Total Nitrogen concentration of 60 mg/L resulting a feed loading rate of 0.06 kg-N m<sup>-3</sup> d<sup>-1</sup>. In early starting up period, the biomass inoculated into reactor was easy to wash out due to unwell attached of biomass. This shows a slower process during the start-up phase compared to the anammox reactor

studied by Ma et al. [9]. In their study, a hybrid reactor was installed and performed during day 1-39 resulting a rapidly increasing of the Nitrogen Loading Rate (NLR) from 0.35 to 1.2 kg-N m<sup>-3</sup> d<sup>-1</sup> [9]. It is mainly because of temperature difference applied in this project. In our study, the biofilm column reactor was not equipped with a temperature controller, resulting the temperature varied from 25 to 27°C.

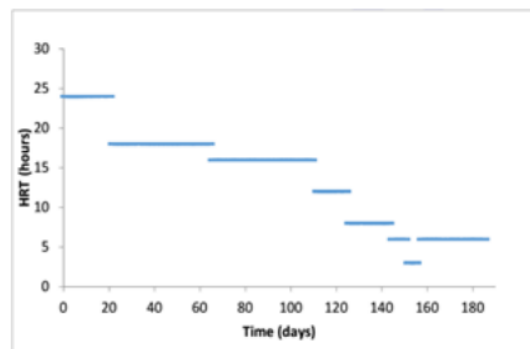
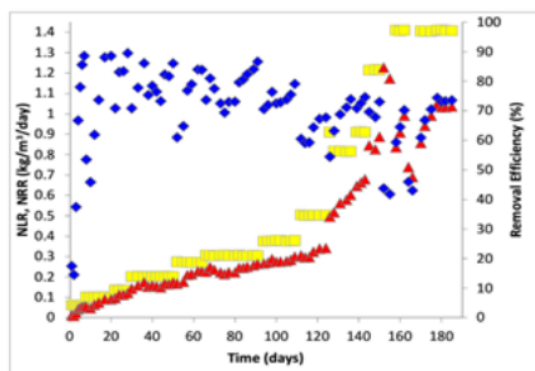


Figure 3. Time course of HRT during reactor operation

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 60 to 350 mg/L. Besides, from days 121 to 145, the HRT was reduced from 12 to 6 hours. Consequently, the NLR was doubled from 0.6 to 1.4 kg-N m<sup>-3</sup> d<sup>-1</sup>. During this period, a relatively high TN removal efficiency average was 75.3%. This value was lower compared with the maximum nitrogen removal based on anammox stoichiometry due to  $\text{NO}_2^-\text{-N}$  limitation. In addition, a maximum Nitrogen Removal Rate (NRR) of 2.05 kg-N m<sup>-3</sup> d<sup>-1</sup> was reached by 178-day operation with a TN removal efficiency of 74%. Values of NRR treated in this research are lower than those previously referred by Ma et al. of 5.72 kg-N m<sup>-3</sup> d<sup>-1</sup> in a UASB reactor treating low strength wastewater at 30°C [10]. Lower and uncontrolled temperature applied in this study could be addressed as the cause of decreased anammox duplicity. However, the value of NRR lower than this experiment was reported by Zekke et al. [11] working in an UASB reactor at 20°C with 0.5 kg-N m<sup>-3</sup> d<sup>-1</sup> of NRR.



**Figure 4.** Time course of total NLR, NRR and Nitrogen removal efficiency ( $\diamond$  : TN removal efficiency;  $\square$  : NLR;  $\Delta$  : NRR)

However, higher total nitrogen removal efficiency achieved was desired. It was reported in a recent study by Reza and Cuenca (2016) that nitrogen removal began 140 days after start-up. In their study about simultaneous biological removal of nitrogen and phosphorus in a vertical bioreactor,  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  concentrations in the effluent were unsteady for approximately 240 days of the reactor operation. The nitrogen removal reached steady state conditions as concentrations were stable and consistent from day 240 until the end of the experiments (day 350) delivering above 90% total nitrogen removal efficiency [12]. The biofilm column reactor has been successfully operated for treating nitrogen from synthetic wastewater at moderately low temperature in this research, but the experimental result has shown total nitrogen removal efficiency of 74% was reached by 178-day operation. It might be because of the operation time was not long enough to allow the bacteria to get the optimum biomass for nitrogen removal. At 178 days of reactor operation probably the steady state condition of nitrogen removal has not been reached. Extended of reactor operation time was needed to increase total nitrogen removal.

## CONCLUSION

Nitrogen removal treatment of synthetic wastewater by using anammox biofilm column reactor was successfully operated. In this study, the application of anammox process for treating the wastewater at moderately low temperatures of 25-27 °C was utilized without any water heating systems. Total nitrogen removal efficiency of 74% in 178 days of reactor operation with a maximum NRR of 1.05

$\text{kg-N m}^{-3} \text{d}^{-1}$  was reached. This biofilm column reactor could be a suitable system for nitrogen removal using anammox biomass at ambient tropical temperature. The results showed that the operating cost of operating anammox biofilm column reactor could be reduced

## ACKNOWLEDGMENT

Authors would like to thank Lembaga Penelitian Universitas Sriwijaya, Indonesia for financial support, and the Bio-Environmental Engineering, Osaka University, Japan. Thanks also addressed to the Waste Treatment Technology Laboratory, Chemical Engineering Sriwijaya University for laboratory support.

## REFERENCES

- [1] Y.-H. Ahn, H.-C. Choi, Autotrophic nitrogen removal from sludge digester liquids in upflow sludge bed reactor with external aeration, *Process Biochem.* vol. 41, pp. 1945–1950, 2016.
- [2] M. Strous, J.J. Heijnen, J.G. Kuenen, M.S.M. Jetten, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, *Appl. Microbiol. Biotechnol.* vol. 50, pp. 589–596, 1998.
- [3] C. Trigo, J.L. Campos, J.M. Garrido, R. Mendez, Start-up of the Anammox process in a membrane bioreactor, *J. Biotechnol.* vol. 126, pp. 475–487, 2006.
- [4] M. Strous, J.G. Kuenen, M.S.M. Jetten, Key Physiology of Anaerobic Ammonium Oxidation, *Appl. Environ. Microbiol.* vol. 65, pp. 1–3, 1999.
- [5] W.R.L. van der Star, W.R. Abma, D. Blommers, J.W. Mulder, T. Tokutomi, M. Strous, C. Picioreanu, M.C.M. van Loosdrecht, Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam, *Water Res.* vol. 41, pp. 4149–4163, 2007.
- [6] W.R. Abma, W. Driessen, R. Haarhuis, M.C.M. Van Loosdrecht, Upgrading of sewage treatment plant by sustainable and cost-effective separate treatment of industrial wastewater, *Water Sci. Technol.* vol. 61, pp. 1715–1722, 2010.
- [7] L. Zhang, J. Yang, Y. Ma, Z. Li, T. Fujii, W. Zhang, N. Takashi, K. Furukawa, Treatment capability of an up-flow anammox column reactor using polyethylene sponge strips as biomass carrier, *J. Biosci. Bioeng.* vol. 110, pp. 72–78, 2010.

- [8] American Public Health Association (APHA), Standard method for the examination of water and wastewater, 22nd ed., American Water Work Association and Water Environment Federation, Washington DC, 2012.
- [9] Y. Ma, D. Hira, Z. Li, C. Chen, K. Furukawa, Nitrogen removal performance of a hybrid anammox reactor, *Bioresour. Technol.* vol. 102, pp. 6650–6656, 2011.
- [10] B. Ma, Y. Peng, S. Zhang, J. Wang, Y. Gan, J. Chang, S. Wang, S. Wang, G. Zhu, Performance of anammox UASB reactor treating low strength wastewater under moderate and low temperatures, *Bioresour. Technol.* vol. 129, pp. 606–611, 2012.
- [11] I. Zekker, E. Rikmann, T. Tenno, K. Kroon, A. Seiman, L. Loorits, H. Fritze, T. Tuomivirta, P. Vabamäe, M. Raudkivi, others, Start-up of low-temperature anammox in UASB from mesophilic yeast factory anaerobic tank inoculum, *Environ. Technol.* vol. 36, pp. 214–225, 2015.
- [12] M. Reza, M.A. Cuenca, Simultaneous biological removal of nitrogen and phosphorus in a vertical bioreactor, *J. Environ. Chem. Eng.* vol. 4, pp. 130–136, 2016.

# 1. Jurnal Nitrogen Removal Tuty EA\_Novia.pdf

---

## ORIGINALITY REPORT

---

30%

SIMILARITY INDEX

---

## PRIMARY SOURCES

---

1 Maryam Reza, Manuel Alvarez Cuenca. "Simultaneous biological removal of nitrogen and phosphorus in a vertical bioreactor", *Journal of Environmental Chemical Engineering*, 2016 102 words — 5%

[Crossref](#)

---

2 Yongguang Ma, Daisuke Hira, Zhigang Li, Cheng Chen, Kenji Furukawa. "Nitrogen removal performance of a hybrid anammox reactor", *Bioresource Technology*, 2011 62 words — 3%

[Crossref](#)

---

3 S.K. Toh, R.I. Webb, N.J. Ashbolt. "Enrichment of Autotrophic Anaerobic Ammonium-Oxidizing Consortia from Various Wastewaters", *Microbial Ecology*, 2002 50 words — 2%

[Crossref](#)

---

4 Trigo, C.. "Start-up of the Anammox process in a membrane bioreactor", *Journal of Biotechnology*, 20061201 41 words — 2%

[Crossref](#)

---

5 Zhang, L.. "Treatment capability of an up-flow anammox column reactor using polyethylene sponge strips as biomass carrier", *Journal of Bioscience and Bioengineering*, 201007 39 words — 2%

[Crossref](#)

---

6 Sunja Cho, Naoki Fujii, Taeho Lee, Satoshi Okabe. "Development of a simultaneous partial nitrification and anaerobic ammonia oxidation process in a single reactor", *Bioresource Technology*, 2011 26 words — 1%

[Crossref](#)

---

7 Lai Minh Quan, Do Phuong Khanh, Daisuke Hira, Takao Fujii, Kenji Furukawa. "Reject water treatment by improvement of whole cell anammox entrapment using polyvinyl alcohol/alginate gel", *Biodegradation*, 2011 22 words — 1%

[Crossref](#)

---

8 Yang, J.. "Anammox treatment of high-salinity wastewater at ambient temperature", *Bioresource Technology*, 201102 22 words — 1%

[Crossref](#)

---

9 140.116.228.7 20 words — 1%

Internet

---

10 Mumtazah Ibrahim, Norjan Yusof, Mohd Zulkhairi Mohd Yusoff, Mohd Ali Hassan. "Enrichment of anaerobic ammonium oxidation (anammox) bacteria for short start-up of the anammox process: a review", *Desalination and Water Treatment*, 2015 17 words — 1%

[Crossref](#)

---

11 Qaisar Mahmood. "Comparison of anoxic sulfide biooxidation using nitrate/nitrite as electron acceptor", *Environmental Progress*, 07/2007 17 words — 1%

[Crossref](#)

---

12 Toh S., Ashbolt N.. "Adaptation of anaerobic ammonium-oxidising consortium to synthetic coke-ovens wastewater", *Applied Microbiology and Biotechnology*, 2002 15 words — 1%

[Crossref](#)

---



13 Yang, J.. "High-rate nitrogen removal by the Anammox process with a sufficient inorganic carbon source", *Bioresource Technology*, 201012 15 words — 1%

Crossref

---

14 Helio López. "Start-up and enrichment of a granular anammox SBR to treat high nitrogen load wastewaters", *Journal of Chemical Technology & Biotechnology*, 03/2008 14 words — 1%

Crossref

---

15 Masahiro Tatara, Shu Ishikawa, Yoshiyuki Ueno. "Continuous nitrogen removal by a single-stage reactor packed with ring-laced string medium", *Journal of Bioscience and Bioengineering*, 2017 14 words — 1%

Crossref

---

16 Helio López, Sebastià Puig, Ramon Ganigué, Maël Rusalleda, Maria D Balaguer, Jesús Colprim. "Start-up and enrichment of a granular anammox SBR to treat high nitrogen load wastewaters", *Journal of Chemical Technology & Biotechnology*, 2008 13 words — 1%

Crossref

---

17 Chong-jian Tang. "Effect of substrate concentration on stability of anammox biofilm reactors", *Journal of Central South University of Technology*, 02/2010 12 words — 1%

Crossref

---

18 Meng, Fangang, Guangyi Su, Yifang Hu, Hui Lu, Li-Nan Huang, and Guang-Hao Chen. "Improving nitrogen removal in an ANAMMOX reactor using a permeable reactive biobarrier", *Water Research*, 2014. 12 words — 1%

Crossref

---

19 Amin Mojiri, John L. Zhou, Harsha Ratnaweera, Akiyoshi Ohashi et al. "Performance optimization of 11 words — 1%

a chitosan/anammox reactor in nitrogen removal from synthetic wastewater", Journal of Environmental Chemical Engineering, 2021

Crossref

20 Jiachun Yang, Li Zhang, Kechen Xu, Yongzhen Peng. "Using combined multiple techniques to characterize refractory organics during anammox process with mature coal chemical wastewater as influent", Environmental Science and Pollution Research, 2018

11 words — 1%

Crossref

21 Liu, S.. "Enhanced anammox consortium activity for nitrogen removal: Impacts of static magnetic field", Journal of Biotechnology, 20081125

9 words — < 1%

Crossref

22 Wan Yang, Shilong He, Ming Han, Bingbing Wang, Qigui Niu, Yujia Xu, Yi Chen, Haibo Wang. "Nitrogen removal performance and microbial community structure in the start-up and substrate inhibition stages of an anammox reactor", Journal of Bioscience and Bioengineering, 2018

9 words — < 1%

Crossref

23 [theses.whiterose.ac.uk](http://theses.whiterose.ac.uk)

Internet

9 words — < 1%

24 A. Monballiu, E. Desmidt, K. Ghyselbrecht, H. De Clippeleir, S.W.H. Van Hulle, W. Verstraete, B. Meesschaert. "Enrichment of anaerobic ammonium oxidizing (Anammox) bacteria from OLAND and conventional sludge: Features and limitations", Separation and Purification Technology, 2013

8 words — < 1%

Crossref

25 Kurniabudi, Deris Stiawan, Darmawijoyo, Mohd Yazid bin Idris, Alwi M. Bamhdi, Rahmat Budiarto.

8 words — < 1%

"CICIDS-2017 Dataset Feature Analysis with Information Gain for Anomaly Detection", IEEE Access, 2020

[Crossref](#)

26 Lotti, T., R. Kleerebezem, Z. Hu, B. Kartal, M.K. de Kreuk, C. van Erp Taalman Kip, J. Kruit, T.L.G. Hendrickx, and M.C.M. van Loosdrecht. "Pilot-scale evaluation of anammox-based mainstream nitrogen removal from municipal wastewater", Environmental Technology, 2014.

[Crossref](#)

27 Melati Ireng Sari, Tuty Emilia Agustina, Elda Melwita, Tine Aprianti. "Color and COD degradation in photocatalytic process of procion red by using TiO<sub>2</sub> catalyst under solar irradiation", AIP Publishing, 2017

[Crossref](#)

28 Winkler, M.K.H.. "Integration of anammox into the aerobic granular sludge process for main stream wastewater treatment at ambient temperatures", Water Research, 20120101

[Crossref](#)

29 Zheng, Zhaoming, Zebing Li, Jing Ma, Jia Du, Guanghui Chen, Wei Bian, Jun Li, and Baihang Zhao. "The nitrification performance of biofilm reactor for treating domestic wastewater under high dissolved oxygen", Journal of Environmental Sciences, 2016.

[Crossref](#)

30 Gao, F.. "The contrast study of anammox-denitrifying system in two non-woven fixed-bed bioreactors (NFBR) treating different low C/N ratio sewage", Bioresource Technology, 201206

[Crossref](#)

31 Ivar Zekker, Ergo Rikmann, Anni Mandel, Kristel Kroon, Andrus Seiman, Jana Mihkelson, Taavo

Tenno, Toomas Tenno. "Step-wise temperature decreasing cultivates a biofilm with high nitrogen removal rates at 9°C in short-term anammox biofilm tests", Environmental Technology, 2016

Crossref

32 Yuhai Liang, Dong Li, Xiaojing Zhang, Huiping Zeng, Jie Zhang. "Performance and influence factors of completely autotrophic nitrogen removal over nitrite (CANON) process in a biofilter packed with volcanic rocks", Environmental Technology, 2014

6 words — < 1%

Crossref

EXCLUDE QUOTES OFF

EXCLUDE SOURCES OFF

EXCLUDE BIBLIOGRAPHY ON

EXCLUDE MATCHES OFF