

PAPER • OPEN ACCESS

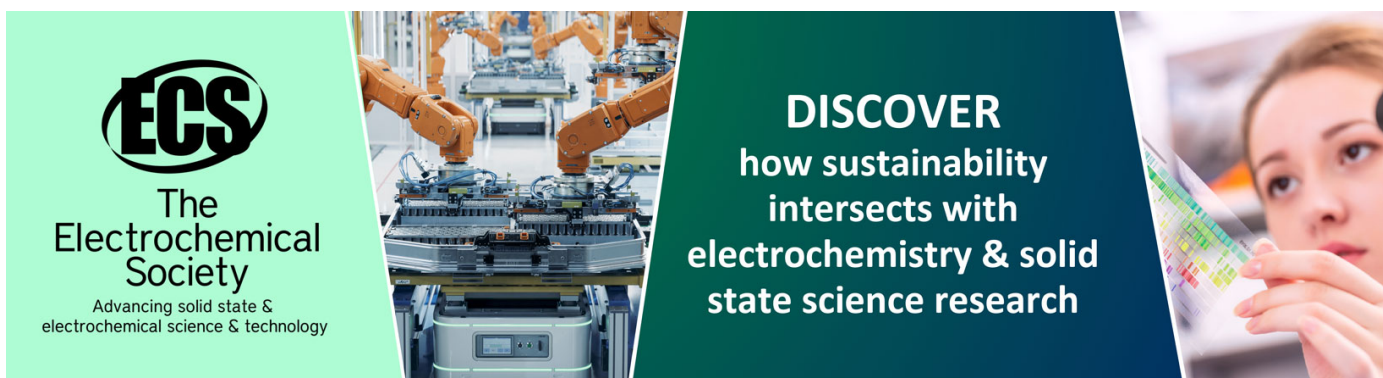
Effect of C/N ratio variations on Muara Karang sediment in oxidizing high concentrated ammonia and identification of nitrifying bacteria using VITEK 2

To cite this article: S A Ilham *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **308** 012023

View the [article online](#) for updates and enhancements.

You may also like

- [Effect of C/N ratio variations on the capability of microbes from Muara Karang river sediment in the production of biogas and identification using VITEK 2](#)
L M Widadri, W Mangunwardoyo and H Ambarsari
- [Fishing industry comprehensive health standard on fish market: The case of Muara Angke](#)
Darmawan, Nurjali and T Nugroho
- [Environmentally friendly fish processing center in Muara Angke, Jakarta](#)
B R Condro and U Siahaan



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

Effect of C/N ratio variations on Muara Karang sediment in oxidizing high concentrated ammonia and identification of nitrifying bacteria using VITEK 2

S A Ilham¹, W Mangunwardoyo¹, and H Ambarsari²

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, 16424, Indonesia

²Center for Environmental Technology - Agency for Assessment and Application of Technology, Serpong, Tangerang Selatan, Banten 15314, Indonesia

Email: sarahaudadi@gmail.com; wibowo.mangun@ui.ac.id; hanies.ambarsari@bppt.go.id

Abstract. Oxidation of ammonia in the environment is known as nitrification. The process itself is a two-step process which oxidizes ammonia into nitrite then nitrate. A research was conducted to study the effect of C/N ratio variations in oxidizing high concentrated ammonia (100 ppm) by the bacteria isolated from the sediments collected from Muara Karang. The variations of C/N ratio implemented in the systems were 5:1, 10:1, 15:1, and 20:1 with glucose as the carbon source and ammonium sulphate as the nitrogen source. Parameters measured during the research were ammonia and nitrate concentrations. Identification of bacteria from the sediments was performed using VITEK 2 identification kits. The results revealed a significant difference ($\alpha=0.05$) in ammonia concentrations between treated groups, whilst no differences were noted in nitrate ($\alpha=0.001$) concentrations. Potential nitrification bacteria from the systems were identified as *Acinetobacter ursingii* (88%), *Pseudomonas aeruginosa* (99%), and *Shewanella putrefaciens* (91%).

1. Introduction

Excessive concentration of ammonia in bodies of water can be reduced with a process known as nitrification by nitrifying bacteria capable of oxidizing ammonia to nitrite and nitrate in aerobic condition. The high value of ammonia could indicate the low dissolved oxygen level because nitrification process requires oxygen to occur. Most living organisms could not cope with low level of oxygen. The nitrification process itself mainly occurs in the sediments [1,2].

Carbon and nitrogen possess significant roles in nitrification process. The right ratio of carbon and nitrogen would encourage biomass formation and enhance ammonia-oxidizing performance. Increased C/N ratio would promote growth, while decreased C/N ratio would cause accumulation of nitrogen source without sufficient amount of bacteria to utilize it. Several researches regarding nitrification revealed different optimal C/N ratios depending on the nitrification bacteria species. It was known from several previous researches that the optimal C/N ratio in *Agrobacterium* is 8-9:1, 10:1 in *Alcaligenes faecalis*, 15:1 in *Bacillus* sp., and 8:1 in *Pseudomonas putida* [3,4]. In spite of the aforementioned studies that indicated a tendency towards low optimal C/N ratio, further experiments were still needed to be carried out to learn the optimum C/N ratio for sediment from Muara Karang in oxidizing high concentrated ammonia. The research was conducted to determine the optimum C/N



ratio with glucose as the carbon source in oxidizing ammonia and to identify the potential ammonia-oxidizing bacteria. This identification of isolates was performed using VITEK 2 identification method.

Identification of microorganisms by VITEK 2 is performed by the principle of isolate's various reaction towards specific substrate. The identification cards in VITEK 2 are distinguished into several types such as Gram-negative card, Gram-positive card, yeast and yeast-like organisms card, as well as anaerobic and coryneform bacteria card. Isolate has to reach enough turbidity on each type of identification using DensyCheck™ before it could be identified. The results of test reaction appear as “+” or “-”. The data then are compared to the respective database to determine a quantitative value of proximity to the database taxa [5].

2. Materials and Methods

2.1. Muara Karang sediment

Sediments were taken from three sites in Muara Karang, Pluit, North Jakarta with the coordinates were in S.66°07.07” E 106.46’24.5” for Site 1, S.06°07.10.2” E 106°46’25.2” for Site 2, and S.06°06.45.8” E 106.45’05.5” for Site 3. Sediments were collected using Ekman grab and composited in 1 L container. Sampels were transported using coolbox filled with ice packs into the laboratory on the same day.

2.2. Media

Heterotrophic nitrification medium contained (per liter) 7.0 g K_2HPO_4 , 3.0 g KH_2PO_4 , 0.1 g $MgSO_4 \cdot 7H_2O$, 0.05 g $FeSO_4 \cdot 6H_2O$, and 10.0 g CH_3COONa . Addition of 20 g Agar was used to make the agar medium [4]. Glucose ($C_6H_{12}O_6$) and ammonium sulphate ($(NH_4)_2SO_4$) were added as the carbon and nitrogen source with the ammonia concentration expected to be 100 ppm. Preparation of 100 ppm of ammonia was done by adding 0.775 g ($(NH_4)_2SO_4$). Addition of glucose to prepare variation of C/N ratio varied with 2.05 g for 5:1, 4.1 g for 10:1, 6.15 g for 15:1, and 8.2 g for 20:1.

2.3. Assessment of ammonia oxidizing capability with C/N ratio variation

Working volume with 900 mL of heterotrophic nitrification medium and 100 mL of sediments were incubated inside an aerator-assisted 2 L gallon. Observation was made for 19 days with data sampling once every three days. During incubation, the samples were measured to determine the concentration of ammonia and nitrate using a spectrophotometer with $\lambda=640$ nm for ammonia and $\lambda=410$ nm for nitrate [6,7].

2.4. Identification of potential ammonia-oxidizing bacteria

Identification of bacterial isolates from the sediments was performed using VITEK 2 in Tangerang Regency General Hospital, Tangerang. Gram positive and negative identification cards were used during the process based on the biochemical characterization on each bacterial isolate.

2.5. Statistical analysis and graphical work

Statistical analysis and graphical work were conducted using SPSS Statistics version 22.0 and Microsoft Excel.

3. Results and Discussion

3.1. Changes of ammonia (NH_3-N) concentrations

Measurement of ammonia was performed using the phenate method according to SNI standard. The color blue of the compound indicates the ammonia concentration. The lighter the color indicates low concentration of ammonia whilst darker color indicates high concentration of ammonia. Regression equation acquired from measuring ammonia standard solution was $y = -0.0226x + 1.5531$ with the

coefficient of determination (R^2) is 0.99. The results of ammonia measurement during 19 days can be seen in figure 1.

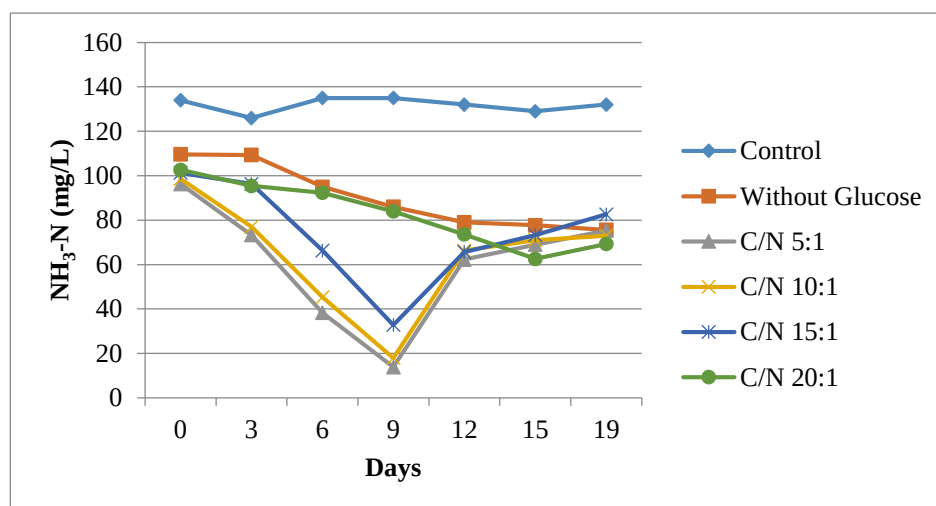


Figure 1. Ammonia concentration (mg/L) in 19 days.

Figure 1 shows no relatively significant decrease in the control group during incubation ($\alpha=0.05$) with 134 mg/L ammonia noted in the beginning and 132 mg/L in the end of incubation. Significant decline can be observed in the treated group of C/N ratio 5:1, 10:1, and 15:1. The concentration of ammonia fell to 13.83 mg/L, 18 mg/L, and 32.8 mg/L respectively at day 9. Ammonia oxidation level was relatively high in 5:1, 10:1, and 15:1 C/N ratio. The 5:1 and 10:1 group also shows more rapid oxidation with detected nitrate in both systems in figure 2. Oxidation of ammonia was relatively slow in the 20:1 C/N ratio and without glucose groups. Statistical analysis revealed a significant effect of C/N ratio variation on the concentration of ammonia.

The concentration of ammonia in C/N ratio of 5:1, 10:1, and 15:1 underwent an increase from day 9 towards the end of incubation. This phenomenon could be caused by the decomposition of nitrifying microbes allowing the nitrogen in the form of ammonia to be turned back into the system. Decomposed microbes that oozed ammonia out of the cells is known as ammonification which could cause the increase inside the system [5]. At a condition in which the decaying microbes are in high number, ammonification could occur with a faster rate than nitrification, ending in accumulation of ammonia [8].

Sufficient amount of carbon source will support reproduction of microbes thereby enough cells are produced to oxidize high concentration of ammonia. Moreover, the use of low ratio level could suppress operational cost on a large scale. The most probable cause of that was the most of the organic carbon source, in this case glucose, was utilized to build up cells before ammonia-oxidation occurred. Glucose was utilized by microbes for growth and could inhibit ammonia-oxidation in high concentration. The same outcome was reported in *Chryseobacterium* sp. R31 that showed optimal ammonia-oxidation in 10:1 C/N ratio while 20:1 C/N ratio resulted in low rate of nitrification [4]. Carbon source, such as glucose, is used by microbes to construct building block compounds as in polysaccharides, lipid, and proteins. On contrary, the absence of carbon source caused the slow rate of nitrification process[9,10].

3.2. Changes of nitrate ($\text{NO}_3\text{-N}$) concentrations

The measurement of nitrate standard solution's absorbance resulted a regression with a value of coefficient of determination (R^2) is 0.99 with the equation $y = 0.0137 + 0.0217x$. Nitrate concentration

was measured using a spectrophotometer at 409 nm. The results of such measurement can be seen in Figure 2.

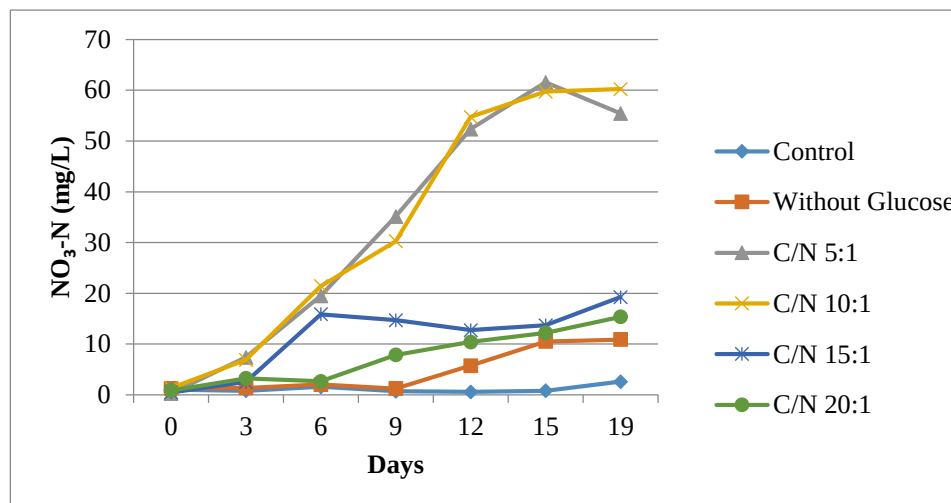


Figure 2. Nitrate concentration (mg/L) in 19 days

Figure 2 shows that nitrate concentration was relatively low in the beginning of incubation and keep increasing towards the end of incubation. Nitrate in high concentration can be observed the 5:1 and 10:1 groups. Kruskal-Wallis nonparametric test was performed to inquire difference in the nitrate concentration on non-normal data distribution. Results revealed that nitrate concentration in every group was not significantly different from each other. Variation of C/N ratio did not affect nitrate concentration during incubation.

The nitrate concentration in the reactors continued to rise towards the end of incubation suggest there was no denitrification process took place that could transform nitrate into nitrogen in the form of gas (N_2). Denitrification is a process where nitrate is reduced to nitrogen gas. The process occurs in a fast rate when oxygen is at the low level or in an anaerobic condition. Denitrification can also occur in an aerobic condition simultaneously with heterotrophic nitrification by microbes, however the observations are not easy to do. Nitrate formation takes place in an aerobic condition, meanwhile nitrate reduction goes on much slower rate in relatively high level of oxygen condition. Hence, further studies need to be done to observe the right level of dissolved oxygen for nitrification and denitrification to run optimal simultaneously [11,12].

3.3. Identification of potential nitrifying bacteria

Potential nitrifying bacteria performed using VITEK 2 resulted in three different species identified as *Acinetobacter ursingii*, *Pseudomonas aeruginosa*, and *Shewanella putrefaciens*. Several strains had been reported to have the ability to oxidize ammonia such as *Acinetobacter* strain SYF26, *Acinetobacter* sp.G107 and *Acinetobacter* sp. 81Y. *Acinetobacter* strain SYF26 had the ability to perform nitrification and denitrification simultaneously. Proposed pathway for that process was through nitrite formation where ammonia is oxidized into hydroxylamine to be further oxidized to nitrate [9,13].

Pseudomonas aeruginosa was identified as one of the microbes in the reactors that had contribution on the nitrification process. The species is ubiquitous that could be found in most types of habitats such as soil, water, and including in animals or humans. It is known as one of the bacteria that could perform nitrification and denitrification simultaneously. Reduction of nitrate in *P. aeruginosa* can happen in two pathways which are through assimilative or dissimilative pathway. In the dissimilative

pathway, nitrate act as the final electron acceptor. Besides nitrification and denitrification, *P. aeruginosa* also has *nifH* gene that could help in nitrogen fixation[9, 16].

The motile *Shewanella putrefaciens* is a common bacteria isolated from water, soil, and crude oil. *S. putrefaciens* is known to reduce nitrate in an aerobic condition known as co-respiration. Co-respiration can be beneficial in a condition with fluctuating oxygen level. Nitrate reductase and nitrite reductase were known to help in the process that was said to be better performed than *E. coli* LE 392 [16,17].

4. Conclusion

In this study, the addition C/N ratio variations had significant effect on ammonia-oxidation whilst no significant differences between treated groups were noted in nitrate concentration. The ammonia concentration could be well-reduced to certain levels in relatively 9 days by low ratios, 5:1 and 10:1, of C/N contents before ammonification would inhibit the process by prolonged incubation. Potential nitrifying bacteria isolated from the systems need to be further studied and observed in performing beneficial simultaneous nitrification-denitrification process.

5. References

- [1] Jenie B S L 1993 *Penanganan Limbah Industri Pangan (Treatment of Food Industry Wastes)*(Yogyakarta: Kanisius) p 184
- [2] Chao Wang, Wanying Zhai, Wei Yin and Baoqing Shan 2015 The limiting role of oxygen penetration in sediment nitrification *Environ.Sci.Pollut. Res. Int.* **22** 10910-18
- [3] Jung H S, Hirai M and Shoda M 2005 Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* no.4 *J. Biosci.Bioeng.* **100** 184-91
- [4] Kundu P, Pramanik A, Dasgupta A, Mukherjee S and Mukherjee J 2014 Simultaneous heterotrophic nitrification and aerobic denitrification by *Chryseobacterium* sp. R31 isolated from abattoir wastewater *Biomed Res. Int.* **2014** 1-12
- [5] Pincus D H 2006 Microbial identification using the Biomerieux VITEK 2 system *Encyclopedia of rapid microbiological methods*(vol 2) ed M J Miller (Illinois: PDA/DHI)p 1-32
- [6] Ambarsari H, Pristi N A, and Sitomurni A I 2017 Potensi mikroalga *Spirulina platensis* untuk pengolahan air limbah domestik Center for Environmental Technology, Assessment and Application of Technology (Serpong: Assessment and Application of Technology) p 1-12
- [7] Anderson J M and Ingram J S I 1993 *Tropical Soil Biology and Fertility: A Handbook of Methods* (Oxford, CAB International) p 221
- [8] Kadlec R H and Wallace S 1995 *Treatment Wetlands* 2nd edition (USA: CRC Press) p 1016
- [9] Kathiravan, V and Krishani K K 2013 *Pseudomonas aeruginosa* and *Achromobacter* sp.: nitrifying aerobic denitrifiers have a plasmid encoding for denitrifying functional genes *World J. Microbiol. Biotechnol.* **14** 1187-98
- [10] Hauser B 1996 *Practical Manual of Wastewater Chemistry* (USA: CRC Press) p 135
- [11] Hogg S 2005 *Essential Microbiology* (UK: John Wiley & Sons) p 468
- [12] Vymazal J and Kropfelova L 2008 *Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow* (Springer science & business media) p 566
- [13] Yamanaka T 2008 *Chemolitho-Autotrophic Bacteria: Biochemistry and Environmental Biology* (Japan: Springer) p 156
- [14] Moura I, Moura J J G, Pauleta S R, and Maia L B 2016 *Metalloenzyme Denitrification: Applications and Environmental Impacts* (UK: Royal Society of Chemistry) p 452
- [15] Palleroni N J 2015 *Pseudomonas In Bergey's Manual of Systematics of Archaea and Bacteria Online* (USA: John Wiley & Sons, Inc.) p 105
- [16] Bitton G 2005 *Wastewater Microbiology* 3rd edition (USA: John Wiley & Sons, Inc.) p 746
- [17] Krause B and Nealson K H 1997 Physiology and Enzymology Involved in Denitrification by *Shewanella putrefaciens* *Appl.Environ.Microbiol.* **63** 2613-18

Acknowledgements

This study was supported by Agency for Assessment and Application of Technology (BPPT) and INSINAS 2017—2018 Ministry of Research, Technology and Higher Education. Research Grant on behalf of Dr. Hanies Ambarsari, B.Sc, M.Appl.Sc. The publication funded by the PITTA 2018. Grant on behalf of Prof. Dr. Wibowo Mangunwardoyo.