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Distribution of Ammonium-Oxidizing Bacteria in Sediment with Relation to Water Quality at the Musi River, Indonesia

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ABSTRACT

The Musi River is located in the southern Sumatra, Indonesia. Most of activities, i.e. agricultural, industrial, and urban activities are considered as being major sources of chemicals and nutrients with their waste products effluent into the river. Nitrification, the microbial oxidation of ammonia to nitrite and nitrate, occurs in a wide variety of environments and naturally remove anthropogenic N pollution. The purpose of this research was to determine of distribution of ammonium-oxidizing bacteria (AOB) in sediment with relation to water quality at the Musi river area. This study was conducted on rainy and dry season 2016 at five sampling sites from the freshwater to seawater at high and low tide conditions, the sampling sites are station St1 (Gandus), station St2 (Palembang city), station St3 (Upang), station St4 (Sungsang), and station St5 (Sea). Sediment samples were collected from the surface layer by using an Ekman grab. Some water quality such as salinity, temperature, pH, and dissolved oxygen (DO) were directly analyzed in the field, while other water quality such as NH_4-N, NO_2-N, and NO_3-N were analyzed in the laboratory. The Density of AOB was determined by the most probable number of (MPN) method. The PCA was used to correlate variations of the AOB with physicochemical properties using software Xlstat. The results showed that the physicochemical properties had a range of salinity of 0 to 20 ppt, temperature of 29.21 to 31.82°C, pH of 4.88 to 7.93, DO of 3.44 to 11.33 mg/l, NH_4-N in sediment of 0.04 to 0.87 mg/l, NO_2-N in sediment of 0.01 to 1.77 mg/l, NO_3-N in sediment of 0.09 to 2.08 mg/l. The density of AOB ranged from 7.2 x 10^2 to 6.1 x 10^3 cells/g sediment. Principal component analyses showed that temperature, pH, DO, and concentrations of nutrient contributed to density of AOB.

1. Introduction

Musi river is a large river with its drainage area covering three provinces in Sumatra Island of Indonesia, namely South Sumatra, Lampung, and Bengkulu, and with multi uses of its resources. At the downstream of Musi river, around the Palembang city, industries are mayor activities with their waste products are discharged directly into Musi river. Those particular activities have negative ecological impact on the aquatic organisms including bacteria (Husnah *et al.* 2008).

Pollution of surface water with toxic chemicals and eutrophication of rivers and lakes with excess nutrients is a great environmental concern worldwide. Agricultural, industrial, and urban activities are considered as being major sources of chemicals and nutrients to aquatic ecosystems, while atmospheric deposition could be an important source of certain constituents such as mercury and nitrogen. The concentrations of toxic chemicals and biologically available nutrients in excess can lead to diverse problems such as toxic algal blooms, loss of oxygen, fish kills, loss of biodiversity, and loss of aquatic plant beds and coral reefs (Voutsa *et al.* 2001).

Nitrification, the microbial oxidation of ammonia (NH_3) to nitrate (NO_3-) via nitrite (NO_2-), plays a critical biogeochemical role in both individual ecosystems and the global nitrogen (N) cycle. Ammonia oxidation is the first, rate-limiting step in nitrification, the

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microbials mediated process in which ammonium is oxidized to nitrite and then to nitrate (Ward 2000). The abundance and the community structure of ammonia-oxidizing bacteria (AOB) can be affected by multiple global change indicators, such as atmospheric CO_2, nitrogen deposition, salinity, pH, dissolved oxygen, and temperature (Kim *et al.* 2008; Miranda *et al.* 2008; Erguder *et al.* 2009; Bernhard *et al.* 2010).

Despite the evident importance of nitrification, surprisingly little is known about the microorganisms that mediate this process in the natural environment. Furthermore, although numerous studies show the global variation of AOB, only a few have been focused on the freshwater and seawater ecosystems that were high turnover rates of land-use activities. Such study is also rarely conducted in the tropical aquatic ecosystem.

2. Materials and Methods

2.1. Study Area

The study was conducted in Musi river area (Figure 1). Musi river is located in southern Sumatra, Indonesia. The river roughly flows from southwest to northeast, from Barisan mountains range that formed the backbone of Sumatra, in Kepahiang (Bengkulu province, Indonesia) to the Bangka Strait that formed the extension of South China Sea. It is about 750 kilometers and drains most of south Sumatra province, Indonesia. After flowing through Palembang, the provincial capital, it joins with the other rivers, including the Banyuasin river, to form a delta near the city of Sungsang. The river is navigable by large ships along Palembang, which is the site of major port facilities used primarily for exporting of petroleum, rubber, chemical fertilizer, and coal.

2.2. Sample Collection

Field samplings were done on rainy season in March 2016 with maximum rainfall of 316 mm and dry season in August 2016 with minimum rainfall of 89 mm (IAMCG 2016) at 5 sampling sites in Musi river area from the freshwater to seawater at high and low tide conditions. The description of these sampling sites and their respective land-use types are depicted in Table 1. Sediment samples were collected from the surface layer by using an Ekman grab (ABM, Indonesia) and then stored in a plastic bag. The samples were transported to the laboratory in a cool box. Each sample was further split into two equal parts, one for microbial analyses and another for chemical analyses.

2.3. Physicochemical Analyses

The salinity, pH, dissolved oxygen, and temperature were measured on sampling sites using a hand refractometer (ATAGO Co. Ltd, Tokyo, Japan), a pH meter (SM101, Milwaukee Instruments, Romania), a dissolved oxygen meter (HI 98193, Hanna Instruments Inc, USA), respectively. NH_4 measured by the phenol method spectrophotometrically at 640 nm, NO_2-N was determined by using sulfanilamide method that was measured spectrophotometrically at 543 nm, and NO_3-N was determined by using brucine method that was measured spectrophotometrically at 410 nm (APHA *et al.* 2005).

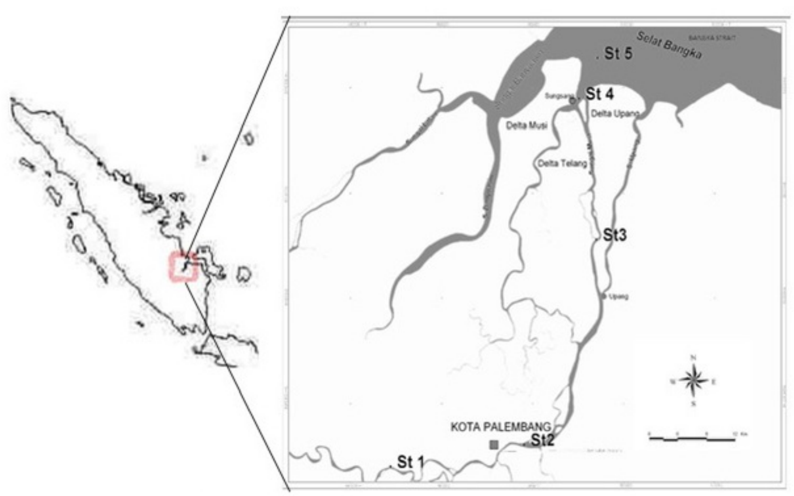

Figure 1. Map of the Musi river area and sampling sites

Figure 1. Map of the Musi river area and sampling sites

Land-use types	Sampling sites, number, and name	Position	Description of the land-use
Little activities of human	St1 - Gandus	03° 01' 52.0" (S) 104° 39' 55.0" (E)	Little activities of human, any little rivers effluent into the Musi river
Urban areas	St2 - Palembang city	02° 59' 07.3" (S) 104° 48' 20.0" (E)	Industrial area where industrial effluents drain into the river, wastes from the market place and sewage from nearby residential houses discharge into the river, construction to make Ampera bridge IV effluent into the river
Agricultural areas	St3 - Upang	02° 36' 51.2" (S) 104° 56' 48.2" (E)	Cultivation of mixed crops such as vegetables, rice, coconut, and maize are carried out 5-10 m from the river
Estuary	St4 - Sungsang	02° 22' 18.8" (S) 104° 54' 36.6" (E)	At this area, wastes from Sungsang village discharge into the river. An estuarine environment with a dense growth of mangroves
Sea	St5 - Sea	02° 15' 30.0" (S) 104° 56' 23.5" (E)	Fishing ground

2.4. The Density of Ammonium-Oxidizing Bacteria (AOB)

The density of AOB was determined by the most probable number (MPN) method in microplates 96 well 350 µl (IWAKI, Asahi Glass Co. Ltd. Japan). The microplates were filled with 90 µl sterile medium for ammonia-oxidizing bacteria as described by Cote and Ghena (1994). Composition of the medium was $(NH_4)_2SO_4$, 1.32 g/l; KH_2PO_4, 20 mg/l; $MgSO_47H_2O$, 0.1 g/l; $FeCl_26H_2O$, 0.014 g/l; $CaCl_22H_2O$, 0.18 g/l; $Na_2MO_42H_2O$, 100 mg/l; EDTA, 1 mg/l; phenol red, 0.002 g/l, and they dissolved in 70% artificial seawater. The medium was adjusted to pH 8 with Na_2CO_3. Ten microliters of the sediment sample were inoculated to the microplates in triplicates and serially diluted by tenfold serial. Incubation was carried out at 25°C for 20 days. Any well in the microplate that exhibited a color change from red to yellow due to acid production were further tested by adding 15 µl of a nitrite color reagent (sulfanilamide, 10 g/l; n-(1-naphtyl)-ethylenediamine 2 HCl, 0.50 g/l; concentrated HCl, 100 ml/l). Tubes that exhibited a red color after the addition of the reagent were scored positive for nitrite. The MPN values were calculated using the MPN Calculator Build 23 (Fegan *et al.* 2004).

2.5. Data Analysis

Principal component analysis (PCA) was used to correlate variations of the AOB with relation to water quality at Musi river, Indonesia in the rainy and dry seasons. PCA has been tested by using software Xlstat.

3. Results

The physicochemical properties of samples in all experiments in Musi river area did not fluctuate significantly. The salinity ranged from 0 to 20 ppt (Figure 2a), and the highest salinity was found at the St5 (Sea) in rainy and dry seasons at high and low tide, the lowest salinity was found from the St1 (Gandus) to the St4 (Sungsang) in rainy and dry seasons at high and low tide. The temperature ranged from 29.21 to 31.82°C (Figure 2b), the highest temperature was found at the St5 (Sea) in dry season at high tide, and the lowest temperature was found at the St5 (Sea) in rainy season at low tide. The pH varied between 4.88 to 7.93 (Figure 2c), the highest pH was found at the St3 (Upang) in dry season at high tide, and the lowest pH was found at the St5 (Sea) in rainy season at low tide. The DO of the present study was found between 3.34 to 11.33 mg/l (Figure 2d), the highest DO was found at the St5 (Sea) in dry season at high tide, and the lowest DO was found at the St3 (Upang) in dry season at low tide.

The concentrations of nutrients in the sediment samples during the both seasons (rainy and dry) in Musi River were very fluctuating. NH_4-N concentrations of the sediment samples ranged from 0.04 to 0.87 mg/l (Figure 2e). The highest NH_4-N concentration was found at the St3 (0.87 mg/l) in rainy season at low tide, while the lowest one was found at the St3 (0.04 mg/l) in dry season at high tide. NO_2-N concentrations between 0.01 to 1.77 mg/l (Figure 2f). The highest

NO_2-N concentration was found at the St2 (1.77 mg/l) in rainy season at low tide and the lowest one was found at the St4 (0.01 mg/l) in rainy season at low tide. NO_3-N concentrations that ranged between 0.09 to 2.08 mg/l (Figure 2g) was found in the high level at the St3 (2.08 mg/l) in rainy season at low tide, and its lowest level was found at the St5 (0.09 mg/l) in dry season at low tide.

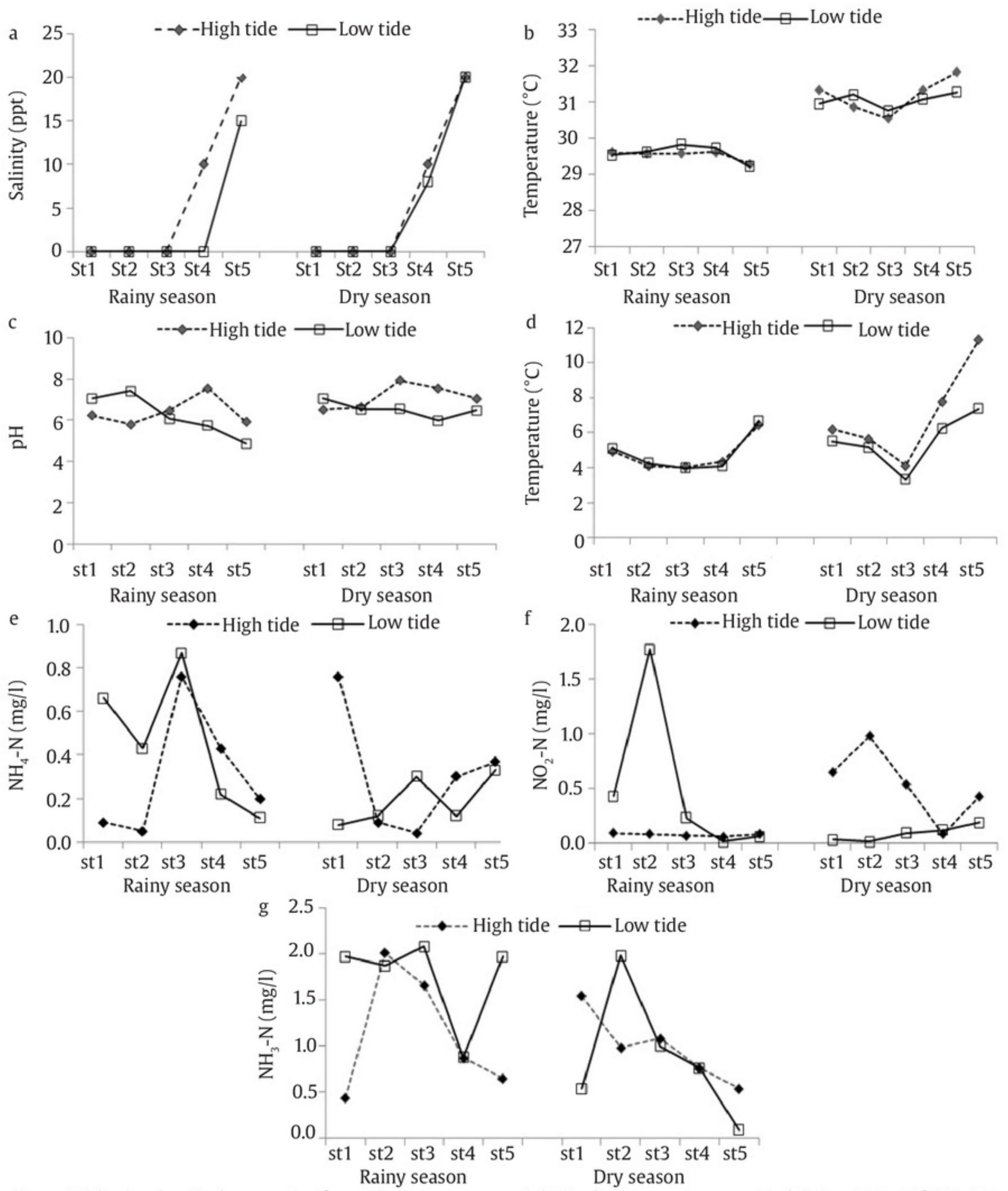

Figure 2. Physicochemical properties from Musi river area, a. Salinity, b. Temperature, c. pH, d. DO, e. NH_4-N, f. NO_2-N, g. NO_3-N

The density of AOB has been detected between 7.2 x 10^2 to 6.1 x 10^3 cells/g sediment during the rainy season (Figure 3). The highest density of AOB was observed was found at the St2 (6.1 x 10^3 cells/g sediment) at high tide. The lowest density of these bacteria was found at the St1 (7.2 x 10^2 cells/g sediment) at low tide. In dry season, the density of AOB between 9.4 x 10^2 to 6.1 x 10^3 cells/g sediment. The highest density of AOB was found at the St1 (6.1 x 10^3 cells/g sediment) at high tide. The lowest one was at the St5 (9.4 x 10^2 cells/g sediment) at high and low tide.

The PCA on correlation matrix of the physicochemical and density of AOB in Musi river area in rainy season indicated that the cumulative eigen values was 74.55% and squared minimum was 0.5 from F1 and F2 axis (Figure 4). The variability at the F1 axis (47.41%) and F2 axis (27.14%) showed that there were two groups. The first group consisted of the St4 (Sungsang) related to high pH at high tide was found 7.55, it could be indicated by wastes from Sungsang village discharge into this area and contributed to density of AOB. The second group, the St5 (Sea) related to the highest salinity (high and low tide) was 20 and 15 ppt, the highest DO in high and low tide was found 6.45 and 6.66 mg/l, it did not contribute significantly to density of AOB.

At dry season, the PCA of the physicochemical and AOB of Musi river area indicated that the cumulative eigen values was 73.11% and squared minimum was 0.5 from F1 and F2 axis (Figure 5). The variability at

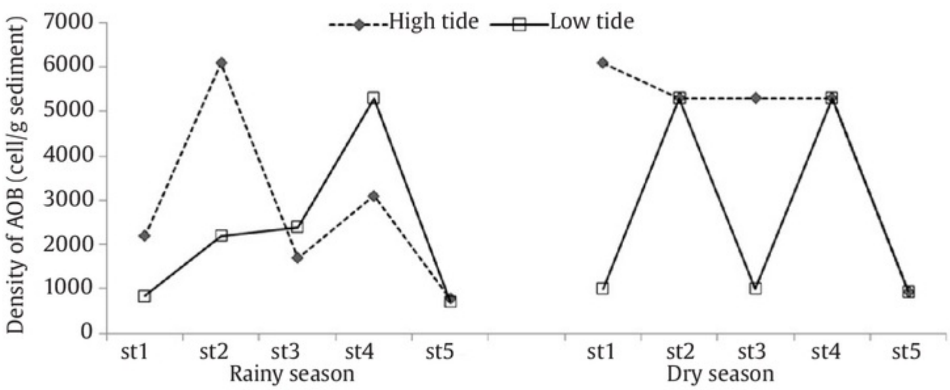

Figure 3. The Density of AOB in Musi river area

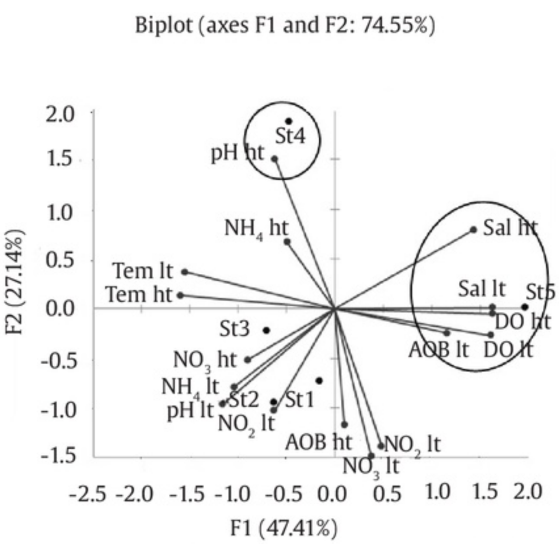

Figure 4. PCA analyses of the physicochemical and AOB properties from Musi river area in rainy season

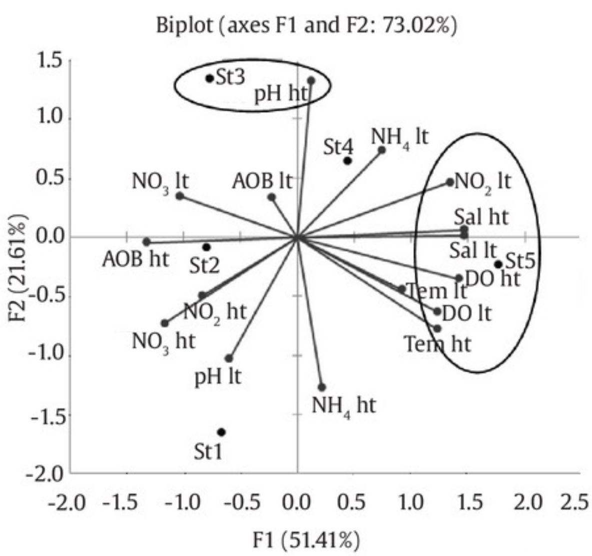

Figure 5. PCA analyses of the physicochemical and AOB properties from Musi river area in dry season

the F1 axis (51.35%) and F2 axis (21.76%) showed that there were two groups. The first group was consisted of the St3 (Upang) related to the highest pH was 7.93 at high tide and contributed to density of AOB. The second group was the St5 (Sea) related to the highest NO_2-N was 0.19 mg/l at low tide, the highest salinity was 20 ppt at high and low tide, the highest DO was found 11.33 mg/l at high tide and 7.37 mg/l at low tide, and the highest temperature was 31.82°C at high tide, and these physicochemical conditions did not contribute significantly to density of AOB.

The relationship between the physicochemical properties and the density of the AOB, in which the physicochemical properties may increase or decrease the AOB density. In the rainy season, salinity ranged from 15 to 20 ppt, DO >5 mg/l, and pH <5 caused a decrease in the density of AOB (St1 at low tide condition, St5 in high and low tide condition). Likewise, in the dry season, salinity >15 ppt and DO >7 mg/l decreased AOB density (St5 in high and low tide condition) (Table 2).

4. Discussion

Musi river is an important river in Palembang city, Indonesia and serves as hydro-biologically place where freshwater from upstream is continuously mixing with seawater from the South China Sea. Combinations of diverse fluctuating parameters are responsible for occurrences and distribution of different microorganisms in the river environment. Besides a lot of industry, fishing boats, vessels or trawlers and container ships operating along the river are hampering the status of water, sediment, and microorganism.

The water samples in this study showed a typical characteristic range of estuarine salinity (0 to 20 ppt). Somville (1984) reported similar findings that nitrification of estuarine water are high at salinity between 0 to 20 ppt. Bianchi *et al.* (1994) described that ammonium oxidation increases by increasing salinity up to 8.5 psu in the Rhone River plume. The water temperature in the Musi river area was

Table 2. The relationship between the physicochemical properties and the density of the AOB

Station	Tides	Salinity	Temperature	pH	DO	NH_4-N	NO_2-N	NO_3-N	AOB (X 10^2 cells/g)
				.... Rainy season					
St1	ht	+	+	+	+	+	+	+	22
	lt	+	+	+	-	+	+	+	8.4
St2	ht	+	+	+	+	+	+	+	61
	lt	+	+	+	+	+	+	+	22
St3	ht	+	+	+	+	+	+	+	17
	lt	+	+	+	+	+	+	+	24
St4	ht	+	+	+	+	+	+	+	31
	lt	+	+	+	+	+	+	+	53
St5	ht	-	+	-	-	+	+	+	7.8
	lt	-	+	-	-	+	+	+	7.2
				.... Dry season					
St1	ht	+	+	+	+	+	+	+	61
	lt	+	+	+	+	+	+	+	10
St2	ht	+	+	+	+	+	+	+	53
	lt	+	+	+	+	+	+	+	53
St3	ht	+	+	+	+	+	+	+	53
	lt	+	+	+	+	+	+	+	10
St4	ht	+	+	+	+	+	+	+	53
	lt	+	+	+	+	+	+	+	53
St5	ht	-	+	+	-	+	+	+	9.4
	lt	-	+	+	-	+	+	+	9.4

ht: high tide condition, lt: low tide condition, +: relationship that increase the AOB density, -: relationship that decrease the AOB density high at a temperature in the dry season (30.54 to 31.82°C) and was low at a temperature in the rainy season (29.21 to 29.82°C). The ammonia oxidation rate increased significantly with the increase in the temperature from 10 to 30°C (Kim *et al.* 2008).

The pH is one of the most important factors in nitrification both in freshwater and marine systems (Strauss *et al.* 2002; Miranda *et al.* 2008). The optimum pH for nitrification varies depending on nature of the system. The pH ranges from 4.88 to 7.93 in the Musi river area. Strauss *et al.* (2002) determined that the maximum nitrification rate occurs at pH 7.5 over a range pH of 5.9 to 8.7 in the freshwater sediment.

In this study, DO in the range of 3.34 to 11.33 mg/l significantly affected to a density of AOB in the Musi river area. Philips *et al.* (2002) hypothesized that, nitrogen losses in a water column overlaying an oxygen-limited sediment containing ammonium (DO <0.1 $mgO_2\ L^{-1}$), especially together with nitrite, could be partially attributed to OLAND (oxygen-limited autotrophic nitrification and denitrification). Near the water surface, higher amounts of ammonia oxidizers could be detected. Together with the observation of gas bubbles, and the N2 production, these findings support the proposed theory of exerting OLAND in order to survive, and to escape from, oxygen deficiency. Overall these results show that the majority of AOB can be found in and is very likely adapted to various concentrations and availability of NH_4-N, NO_2-N, and NO_3-N. Hatzenpichler *et al.* (2008) reported that ammonium as the primary energy source might promote the activity of ammonia oxidizers, and previous studies also reported that high concentrations of ammonium partially inhibited the activity of ammonia-oxidizing prokaryotes. NH_4-N concentrations in Musi river area was the highest in the St3 (Upang) in a rainy season and the lowest in the St3 (Upang) in a dry season, this area used for agricultural purposes, probably due to an application of fertilizers in farms that eventually end up in the river system due to runoff.

The density of AOB in sediment samples during rainy and dry season estimated by MPN method ranged from 7.2 x 10^2 to 6.1 x 10^3 cells/g sediment. These densities are lower than the results reported by Isnansetyo *et al.* (2011) that the AOB density in mud sediment of the Ariake Sea tidal flat, which ranges from 7.17 x 10^3 to 1.64 x 10^5 cells/g sediment. However, the density AOB estimated by MPN method were relatively lower than molecular methods. Dollhopf *et al.* (2005), reported that by using PCR primers targeting the amoA gene, which encodes the active site of ammonia monooxygenase, a key enzyme in AOB, in salt marsh sediment, which ranges from 5.6 x 10^4 to 1.3 x 10^6/g of wet sediment. Therefore, the use of molecular techniques is necessary to enumerate AOB in the Musi river area in the future.

Conclusion

The Musi river used for any purpose may affect land-use activities like industry, fishing boats, vessels or trawlers and container ships, cultivation of mixed crops, wastes etc. With the relative physiochemical parameters against the density of AOB both in the rainy and dry season, it was observed that positively correlated density of AOB with temperature, pH, DO and concentrations of nutrient. This study concluded that maximum density of AOB from St2 (Palembang city) and St1 (Gandus) in Musi river area can be explained by the industrial activity, wastes, and agricultural areas.

Acknowledgements

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References

[APHA] *et al.* 2005. Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association (APHA), Washington, DC, USA.

Bernhard AE *et al.* 2010. Abundance of ammonia-oxidizing archaea and bacteria along an estuarine salinity gradient in relation to potential nitrification rates. *Appl Environ Microbiol* 76:1285–1289. doi: 10.1128/AEM.02018-09

Bianchi M *et al.* 1994. Bacterial nitrification and denitrification rates in the Rhone River plume (northwestern Mediterranean Sea). *Mar Ecol Prog Ser* 103:197–202. doi: 10.3354/meps103197

Cote R J, Ghena RL. 1994. Nutrition and media. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (Eds). *Methods for General and Molecular Bacteriology*. Am Soc Microbiol pp. 155-277.

Dollhopf SL *et al.* 2005. Quantification of ammonia-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. *AEM* 71:240–246. doi: 10.1128/AEM.71.1.240

Erguder TH *et al.* 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiol Rev* 33:855–869. doi: 10.1111/j.1574-6976.2009.00179.x

Fegan N *et al.* 2004. Enumeration of escherichia coli O157 in cattle faeces using most probable number technique and automated immunomagnetic separation. *Lett Appl Microbiol* 38:56–59. doi: 10.1046/j.1472-765X.2003.01439.x

Hatzenpichler R *et al.* 2008. A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proc Natl Acad Sci USA* 105:2134–2139. doi: 10.1073/pnas.0708857105

Husnah *et al.* 2008. Fish community structure in relation to water quality of the downstream of Musi river, South Sumatera, Indonesia. *Ind Fish Res J* 14:51–65.

IAMCG. 2016. Forecast of dry season 2016. Indonesian Agency for Meteorological, Climatology and Geophysics (BMKG), Palembang.

Isnansetyo A *et al.* 2011. Nitrification potential of mud sediment of the ariake sea tidal flat and the individual effect of temperature, pH, salinity and ammonium concentration on its nitrification rate. *Research Journal of Environmental and Earth Sciences* 3:587–599.

Isnansetyo A *et al.* 2014. Independent effects of temperature, salinity, ammonium concentration and pH on nitrification rate of the Ariake seawater above mud sediment. *HAYATI J Biosci* 21:21-30. DOI: 10.4308/hjb.21.1.21

Kim JH *et al.* 2008. Comparison study of the effects of temperature and free ammonia concentration on nitrification and nitrite accumulation. *Process Biochem* 43:154–160. doi: 10.1016/j.procbio.2007.11.005

Miranda J *et al.* 2008. Nitrification in kochi backwaters. *Estuar Coast Shelf Sci* 78:291–300. doi: 10.1016/j.ecss.2007.12.004

Philips S *et al.* 2002. Oxygen-limited autotrophic nitrification/denitrification by ammonia oxidisers enables upward motion towards more favourable conditions. *Appl Microbiol Biotechnol* 59:557–566. doi: 10.1007/s00253-002-1059-8

Somville M. 1984. Use of nitrifying activity measurements for describing the effect of salinity on nitrification in the Scheldt estuary. *Appl Environ Microbiol* 47:424–426.

Strauss EA *et al.* 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. *Can J Fish Aquat Sci* 59:554–563. doi: 10.1139/f02-032

Voutsa D *et al.* 2001. A study of surface water quality in Macedonia, Greece speciation of nitrogen and phosphorus. *Water Air Soil Pollut* 129:13–32.

Ward BB. 2000. Nitrification and the marine nitrogen cycle. In: Microbial ecology of the oceans, Kirchman D. Wiley-Liss, New York. pp. 427–453.

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