HAYATI Journal of Biosciences March 2014
Vol. 21 No. 1, p 21-30
ISSN: 1978-3019

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.21.1.21

Independent Effects of Temperature, Salinity, Ammonium Concentration and pH on Nitrification Rate of the Ariake Seawater Above Mud Sediment

ALIM ISNANSETYO[1,2*], SAI GETSU[3], MASAHIRO SEGUCHI[1,3], MASUMI KORIYAMA[1,3]

[1]*Ariake Sea Research Project, Saga University, Honjo 1, Saga 840-8502, Japan*
[2]*Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Jalan Flora, Bulaksumur, Yogyakarta 55182, Indonesia*
[3]*Laboratory of Environment of Shallow Sea and Tidal Flat, Department of Agricultural Sciences, Faculty of Agriculture, Saga University, Honjo 1, Saga 840-8502, Japan*

Received June 11, 2013/Accepted January 27, 2014

The Ariake Sea located in the west parts of Kyushu Island is a semi-closed and macro-tidal shallow sea, and has the largest tidal flat in Japan. A large mud tidal flat with a productive ecosystem found along the western shoreline of the sea makes this area ideal as a major production site of nori (*Porphyra yezoensis*) in Japan. We determined the independent effect of temperature, salinity, ammonium concentration and pH on nitrification rates (NR) in the Ariake seawater above the mud sediment. The NR was determined by measuring accumulation of NO_2-N production after adding sodium chlorate, an inhibitor of NO_2-N to NO_3-N oxidation. NRs were relatively high at 20-35 ^oC (optimum at 29.5 ^oC), but the rates were very low at 5, 10, and 40 ^oC. NRs increased sharply when increasing the salinity from 13 to 20 ppt, but it decreased drastically at salinity levels more than 35 ppt (optimum at 19 ppt). The relationship between ammonium concentration and NR showed a typical kinetic curve of enzymatic reaction with the maximum NR (*V*max) of 0.029 μM $N.h^{-1}$ at 200 μM NH_4-N (the half saturation constant (Ks) = 35 μM NH_4-N). High NRs were determined at pH 7.5-8.0 (optimum pH 7.8). This is the first report on the independent effects of temperature, pH, salinity and NH_4-N concentration on the NR of seawater, specifically the Ariake seawater.

Keywords: nitrification, the Ariake sea, seawater, temperature, pH, ammonium, salinity

INTRODUCTION

Aerobic nitrification is a two-step process of microbial oxidation of ammonia (NH_3^-) to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-). The first oxidation step is mediated by ammonia-oxidizing bacteria (AOB), while the second one is mediated by nitrite-oxidizing bacteria. In the nitrogen cycle, nitrification links N mineralization to denitrification which produces N_2 gas. Therefore, nitrification is one of the prominent biochemical processes in the global nitrogen (N) cycle and in single ecosystems. Approximately, 30% of global fixed-N loss occurs in the sediments of estuaries and of the continental-shelf (Galloway *et al*. 2004). Coupled nitrification and denitrification in the estuary ecosystem also play an important role in removal processes of approximately 10 to 80% of anthropogenic N pollution (Seitzinger 1988). Nishio *et al*. (1983) estimated that 6-70% of the N_2 produced by the denitrification process originates from nitrogenous oxides (nitrate and nitrite) which are derived from nitrification.

*Corresponding author. Phone/Fax: +62-274-551218, E-mail: isnansetyo@yahoo.com

In the marine ecosystems, nitrification in seawater is less studied than that taking place in the sediment. The nitrification rate (NR) of sea water has been reported by McCarthy *et al*. (1984) and Horrigan *et al*. (1990) in Chesapeake Bay, Somville (1978, 1984) in Scheldt estuary, Enoksson (1986) in the Baltic sea, Owens (1986) in Tamar estuary, Berounsky and Nixon (1993) in Narragansett Bay, Bianchi *et al*. (1997, 1999b) in the Southern ocean and the NW Mediterranean Sea, Iriarte *et al*. (1998) in the estuary of the River Nervión, Capona *et al*. (1990) in Crane Neck, de Bie *et al*. (2002) in the Schelde estuary, O'Mullan and Ward (2005) in Monterey Bay, and Miranda *et al*. (2008) in Kochi backwaters. There is no report on the independent effect of environmental parameters on NR of seawater. Nitrification has been shown to be affected by environmental factors such as substrate concentration (Kim *et al*. 2008a; Miranda *et al*. 2008), dissolved oxygen (DO) (Kemp & Dodds 2002), temperature, pH, salinity (Jones & Hood 1980; Kim *et al*. 2008a; Miranda *et al*. 2008), organic carbon (C) availability and CN ratio (Strauss & Lamberti 2000; Strauss *et al*. 2002). In this paper, we report the experimental results of independent effect of environmental parameters on the nitrification of the Ariake seawater above mud sediment.

The Ariake Sea located in the west part of Kyushu island, Japan, is a semi-closed shallow sea with macro-tidal and several well-mixed estuaries. This sea covers 1,700 km^2 of a long inner bay with 96 km of the bay axis and 18 km of the average width. The Ariake Sea is characterized by a macro tidal range of 3-6 m, which is the largest tidal range in Japan (Kato & Seguchi 2001; Hiramatsu *et al*. 2005). A vast tidal flat area has developed in the sea, and constitutes almost 40% of the total tidal flat area in Japan. The sediments that are transported by several rivers to the bay reach around 440,000 ton per annum. Coarse sediment settles in the eastern part of the bay, but the fine sediment makes up the Ariake clay formation and mud tidal flat along the western shoreline of this area (Kato & Seguchi 2001). This tidal flat is the main area for the production of sea laver (*Porphyra yezoensis*) production in Japan, which contributes 40% of the total Japanese sea laver production (Yanagi & Abe 2005). The most important nutrient for production of *P. yezoensis* is nitrogen; either NO_3^- or NH_4^+, but NO_3^- is a better source of N in terms of growth (Hafting 1999). The uptake of these two inorganic nitrogen fractions in *Porphyra* species including *P. yezoensis* occur at similar rates (Kraemer *et al*. 2004). Moreover, the uptake is affected by the frequency and duration of cycles of immersion and exposure (Kim *et al*. 2008b), which occur naturally in the high tidal range area as found in the Ariake sea.

Although nitrogen is a crucial nutrient contributing to high productivity of the Ariake sea, there has been little research on the nitrogen cycle in this area. Moreover, water nitrification in this area has also not been studied. A report on the independent effects of environmental parameters on NR of water from the marine system, especially in the Ariake sea has not been conducted hitherto. This paper provides the experimental results of independent effects of temperature, NH_4-N concentration, pH and salinity on nitrification of the Ariake seawater.

MATERIALS AND METHODS

Water Sample. Water samples used in this study were collected in Higashi Yoka in the intertidal zone at high tide. Higashi Yoka, around 8 km south of Saga City, is located in the interior part of the Ariake sea and it has muddy sediment. This is the predominant sediment in the interior parts of the Ariake sea tidal flat (Figure 1). Samples were

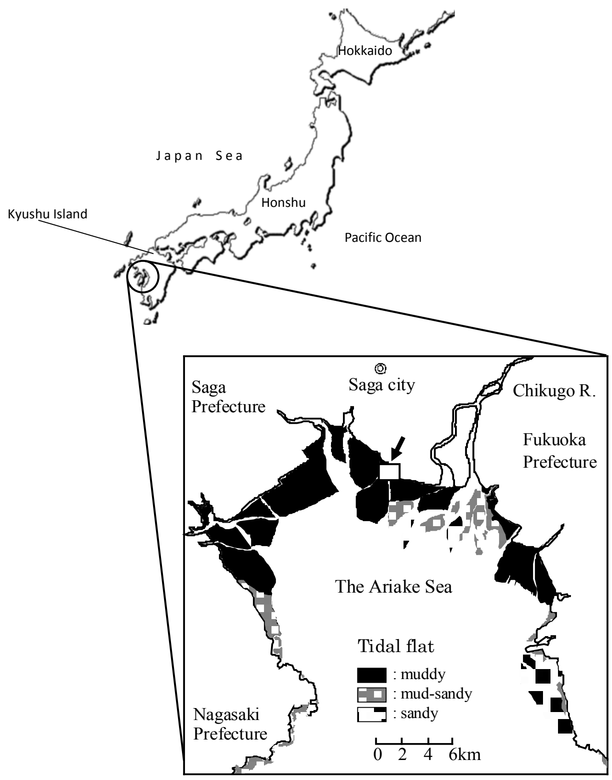

Figure 1. Map of the interior parts of the Ariake sea and distribution of sediments in its tidal flat [32]. An arrow indicates the sampling point.

collected in polyethylene bottles and the samples were transported to the laboratory in a cool box.

Water Geochemistry. Water samples were filtered through 0.45 µm-pore-size cellulose ester filter (Advantec, Toyo Roshi Kaisha, Tokyo, Japan), and frozen immediately until analysis. NH_4-N, NO_2-N, NO_2+NO_3-N, PO_4, total nitrogen (TN) and total phosphate (TP) were analyzed by an automated water analyzer (Water auto-analyzer, swAAT, BLTEC, Tokyo, Japan). NH_4-N concentration was determined by means of the alkali phenol-hypochlorite reaction detected photometrically at 630 nm. NO_2-N concentration was analyzed by diazotizing samples with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form a highly colored azo dye which was detected photometrically at 550 nm. NO_3-N was measured using the same method as for NO_2-N after NO_3 was reduced by the cadmium reduction process. PO_4 was determined by the ascorbic acid method at 800 nm. TN and TP concentrations were measured by peroxodisulfate oxidation (Ebina *et al*. 1983).

Density of Ammonium-Oxidizing Bacteria (AOB) in Water. Density of ammonium-oxidizing bacteria was determined by the most probable number (MPN) method in 1.5 ml sterile microtubes. The microtubes were filled with 900 µL sterile medium for ammonia-