Salinity control of nitrification in saline shallow coastal lagoons

Controle da nitrificação pela salinidade em lagoas costeiras salinas

Santoro, A. L.[1,2] and Enrich-Prast , A.[2]

[1]Programa em Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro – UFRJ, Ilha do Fundão, CP 68020, CEP 21941-590, Rio de Janeiro, Brazil
e-mail:anasantoro@ufrj.br

[2] Laboratório de Biogeoquímica, Departamento de Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro – UFRJ, Ilha do Fundão, CP 68020, CEP 21941-590, Rio de Janeiro, Brazil
e-mail: aeprast@biologia.ufrj.br

Abstract: Aim: The present study was to show the importance of the salinity control of nitrification activity in sediments from saline shallow coastal lagoons; **Methods:** Potential nitrification rates were measured from nitrate production method and sediment chlorophyll-*a* concentration was used as an index of algae biomass. Potential nitrification rates, chlorophyll-*a* concentrations and water salinity were significantly different among the studied lagoons; **Results:** Potential nitrification rates varied from undetectable up to 120 nmol NO_3^- cm^{-3} h^{-1} and were negatively correlated with water salinity, showing a general exponential decay along a salinity range between 0 to 30‰; **Conclusions:** The lack of relationship between potential nitrification and chlorophyll-*a*, was attributed to the low density of algae at the studied sediments. For lagoons with reduced sediment algae colonization as the studied ones, the water salinity is probably a major regulating factor of sediment nitrification.

Keywords: nitrification, salinity, coastal lagoons, shallow lakes, tropics.

Resumo: Objetivos: O objetivo do presente estudo foi mostrar a importância do controle da salinidade sobre a atividade nitrificante no sedimento de lagoas costeiras rasas e salinas; **Métodos:** Foram mensuradas as taxas potenciais de nitrificação no sedimento utilizando o método de produção de nitrato ao longo de um curto período de tempo e a concentração de clorofila-*a* no sedimento foi utilizada como indicativo da biomassa algal; **Resultados:** As lagoas estudadas apresentaram diferenças significativas nas taxas potenciais de nitrificação, concentrações de clorofila-*a* no sedimento e salinidade da coluna d'água. As taxas de nitrificação variaram de valores não detectáveis a 120 nmol NO_3^- cm^{-3} h^{-1} e se correlacionaram negativamente com os valores de salinidade, apresentando um padrão de decaimento exponencial com a variação de salinidade entre 0 e 30 ‰; **Conclusões:** A ausência de correlação entre nitrificação potencial e clorofila-*a* pode ser atribuída à baixa densidade algal contemplada neste estudo. Para lagoas com reduzida colonização algal no sedimento, como as deste estudo, a salinidade da água é um importante fator de regulação do processo de nitrificação no sedimento.

Palavras-chave: nitrificação, salinidade, lagoas costeiras, lagos rasos, trópicos.

1. Introduction

Nitrification is defined as the oxidation of reduced nitrogen compounds and is an energy-yielding microbial process. Nitrification is a two step process, where ammonium (NH_4^+) is oxidized to nitrite (NO_2^-) and afterwards to nitrate (NO_3^-) under oxic conditions (Enrich-Prast et al., 2009). The availability of oxygen is a well-recognized controlling factor of nitrification in the sediment, as nitrifying bacteria are obligatory aerobes (Enrich-Prast, 2005).

Benthic microalgae colonize and change chemical conditions of the sediment-water interface, especially due to its O_2 production and potential for nutrient recovery (Wilkie et al., 2002; Muñoz and Guieysse, 2006). The activity of these organisms enhances oxygen penetration into the sediment, stimulating aerobic processes including nitrification (Risgaard-Petersen et al., 1994; Lorenzen et al., 1998; An and Joye, 2001; Travieso et al., 2006). However, part of the excreted photosynthetic products represent an additional carbon source to heterotrophic bacteria, increasing its activity in the sediment and can stimulate the competition between these organisms and nitrifying bacteria (Middelburg et al., 2000).

Benthic microalgae are commonly found in saline environments. In such systems a possible positive effect caused by benthic algae on nitrification can be buffered by a negative effect caused by salinity. Changes in water salinity can play an important role in regulating sediment nitrification (Risgaard-Petersen, 2004).

As salinity regulates sediment NH_4^+ adsorption capacity (Boatman and Murray, 1982) and also has a physiological impact on nitrifiers (Joye and Hollinbaugh, 1995; Rysgaard et al., 1999), nitrification is usually lower at high salinities. Besides that, the lower oxygen solubility at higher salinities decreases its penetration and availability within the sediment (Revsbech et al., 1980) reducing the thickness of the nitrification activity layer. However, some studies had also observed positive relationships between salinity and nitrification in estuary sediments.

Higher values of benthic nitrification were observed under intermediate salinity in estuarine sediment (Meyer et al., 2001; Magalhães et al., 2005). Culture experiments showed that some nitrifying species are able to adapt to changes in salinity and the salt tolerance generally reflects the original environment (Finstein and Bitzky, 1972). Helder and DeVries (1983) observed that an ammonium oxidizer could adapt to and grow at a salinity range from 0 to 35 ppt, but at higher salinities this growth occurred after a phase lag of up to 20 days. This long phase lag could be a disadvantage for nitrifying communities that live in very unstable environments like coastal shallow lakes, submitted to relatively rapid changes in water salinity as a response to rain, sea water inputs and evaporation intensity.

The aim of the present study was to show that changes in salinity can be an important regulating factor on nitrification activity in sediment colonized by benthic algae from coastal shallow lagoons.

2. Material and Methods

2.1. Study site

This research was performed at Pires, Preta, Catingosa and Visgueiro coastal lagoons, characterized by areas of 0.9, 2.2, 0.1 and 1.2 km^2, respectively. The lagoons are located in the National Park of Jurubatiba (22° 3' 19.3" S and 41° 40' 46.8" W), one of the most important coastal Brazilian conservation areas (Northeast of Rio de Janeiro state; Figure 1). This region shows annual mean temperature of 24 °C and a rainy period between November and April (Carmouze et al., 1991). All lakes are shallow (mean depth of 0.8 m) and parallel to the coast, classified as lakes formed in sand depressions that constitute a "Restinga" ecosystem (Martin and Dominguez, 1994). These ecosystems are influenced by terrestrial inputs of organic acid compounds and saline inputs from the sea groundwater (Suzuki et al., 1998). Lagoon water can vary from brackish to hipersaline within weeks (Enrich-Prast et al., 2004), showing high variation in the benthic algae colonization and salinity in a short period of time and inter-lakes.

2.2. Sampling and analyses

Sediment from all lagoons was sampled with Plexiglas tubes in 4 replicates in three different months (October/05, December/05 and January/06). The first 1 cm-sediment from each core was mixed and separated for later analyses of potential nitrification and chlorophyll-*a* concentration.

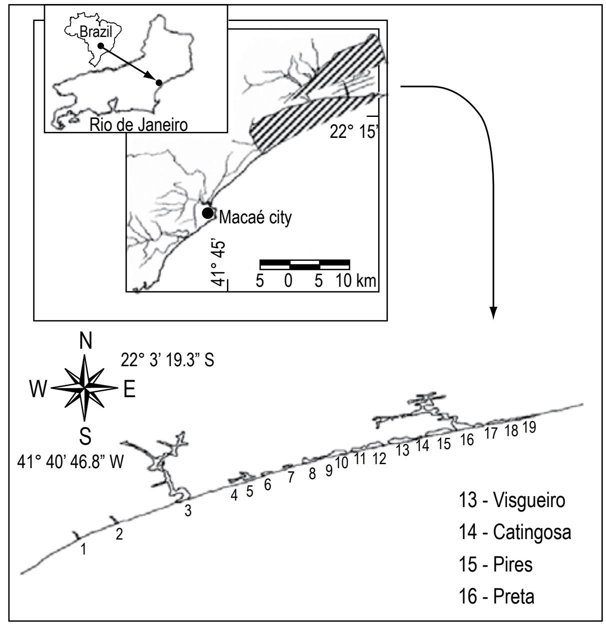

Figure 1. Illustration of the National Park of Jurubatiba lagoons, detailing the position of the four studied lagoons.

Limnological parameters of the water column were determined in situ: pH (Analion PM 608 pH meter), salinity and temperature (Thermosalinometer YSI-30) and ammonium concentrations in the water column were measured according to Bower and Holm-Hansen (1980).

Potential nitrification rates were measured from nitrate production during a 3-4 hours incubation period. The possibility of growth of nitrifying bacteria was excluded due to the short-term incubation in our measurements (Kaplan, 1983). The incubation was performed using 50 mL tubes on a rooter at 100 rpm, adding 1 mL of 20 mM NH_4Cl and 1 mL of 4 mM KH_2PO_4 to avoid nutrient limitation (Belser, 1979). A slurry was formed with 30 mL of lagoon water and 2 mL of the previously homogenized sediment from each lake. At each incubation time (1 hour), 6 mL of this slurry was centrifuged at 5000 rpm for later NO_3^- determination with cadmium reduction analysis using a flow injection analysis system (ASIA/Ismatec). Potential Nitrification Rates (PNR) were calculated from the slope (α) of the linear regression of nitrate concentrations accumulated during incubation time. This rate was obtained according to Equation 1:

$$\mathrm{PNR}\left(\mathrm{nmol\ NO_3^{-}cm^{-3}\ h^{-1}}\right) = \alpha\left(\mathrm{nmol\ NO_3^{-}\ L^{-1}h^{-1}}\right)\frac{\mathrm{water\ volume\ (L)}}{\mathrm{sediment\ volume}\left(\mathrm{cm^{-3}}\right)} \quad (1)$$

Chlorophyll-*a* concentrations were used as an index of algae biomass and were obtained from spectrophotometry after pigment extraction using 90% acetone according to Dalsgaard et al., (2000).

2.3. Statistical analysis

Data (n = 3 or 4) were not normally distributed (Kolmogorov-Smirnov, $p < 0.05$) and variances were heterogeneous (Bartlett, $p < 0.05$). Even transformed data did not fulfill parametric assumptions (Zar, 1996). Therefore non-parametric Kruskall-Wallis test (significance $p < 0.05$) followed by Dunn's multiple comparison (significance $p < 0.05$) was used in order to identify differences among lagoons in chlorophyll-*a* concentration, potential nitrification rates and water salinity. Non-parametric Spearman correlations and linear regression model (significance $p < 0.05$) were also used among nitrification rates and sediment chlorophyll-*a* concentrations and salinity and temperature values.

3. Results

Production of NO_3^- was linear during incubations in all sediment slurries ($r^2 = 0.75 – 0.99$), indicating that nitrification activity began when incubation started. The linear nitrate production also indicated no significant growth of nitrifying bacteria during the incubation time.

Potential nitrification rates were significantly different among some lagoons (Kruskall-Wallis; $p < 0.05$; Figure 2a), ranging from undetectable up to 120 nmol NO_3^- cm^{-3} h^{-1}. The highest and lowest nitrification rates measured in this study were observed in Pires and Visgueiro lagoons, respectively. Sediment chlorophyll-*a* concentrations were also significantly different among some of the lagoons (Kruskall-Wallis; $p < 0.05$; Figure 2b), but not significantly different between Pires and Visgueiro (Dunn's test; $p < 0.05$). Those two lagoons showed higher sediment chlorophyll-*a* concentration, but with a restricted variation, as the highest concentrations did not exceed 10.0 mg chl-*a* m^{-2} (Figure 2b).

Water salinity also showed some significantly differences among lagoons (Kruskall-Wallis; $p < 0.05$; Figure 3). Preta Lagoon showed the lower median and range (median = 4.0; range (max – min) = 3.9) for salinity, while Visgueiro Lake presented the higher ones (median = 26.20; range = 11.7). Water temperature and pH were very similar among all lagoons with values around 28 °C and pH 7, respectively, during the studied period. Water ammonium concentration ranged between 3 and 7 µM and was also very similar among the lagoons. Potential nitrification rates estimated for those lagoons were not significantly correlated (Spearman; $r^2 = 0.005$, $p > 0.05$) with sediment chlorophyll-*a* concentrations, pH or temperature, but the correlation was significant (Spearman $r = -0.78$; $p < 0.05$) with water salinity.

A general exponential decay of all sediment nitrification data along the salinity range was observed ($R^2 = 0.5345$; Figure 4). Considering each environment independently we observed a significant negative linear regression for all lagoons. The higher and lower negative linear regression slope and r^2 were found in Preta and Visgueiro lagoons respectively (Table 1). Despite the significant differences in sediment nitrification and chlorophyll-*a* among some lagoons, there was no clear relationship between both parameters.

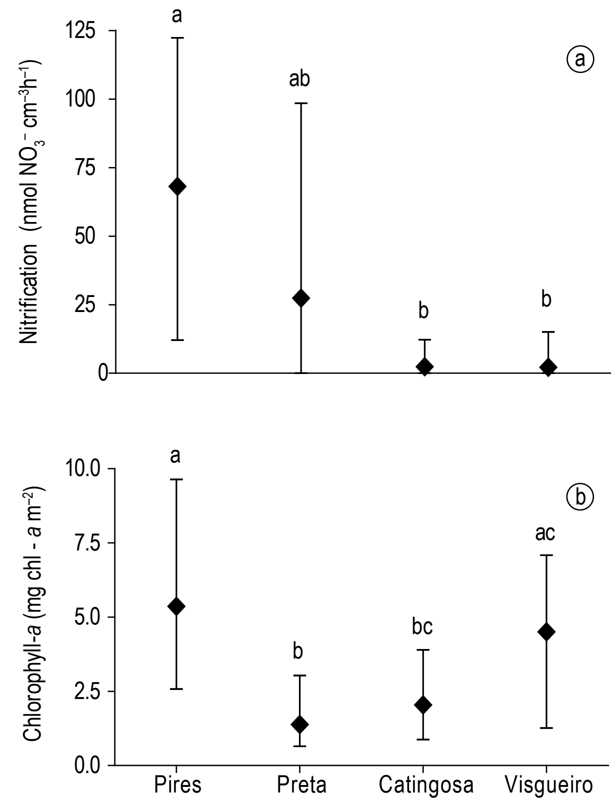

Figure 2. a) Sediment potential nitrification rates and b) chlorophyll-*a* concentrations at the studied lagoons. Symbols represent median and range. Different letters indicate significative differences (Kruskall-Wallis, $p < 0.05$).

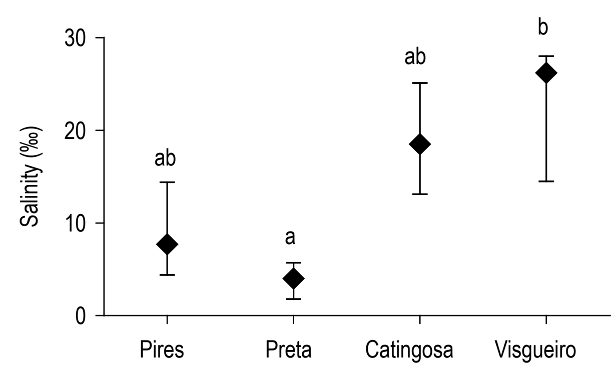

Figure 3. Water salinity at the studied lagoons at the moment of sediment sampling. Symbols represent median and range. Different letters indicate significative differences (Kruskall-Wallis, $p < 0.05$).

Table 1. Linear regression model between sediment nitrification and salinity for each studied lagoon.

	Pires	Preta	Catingosa	Visgueiro
Linear regression slope	–6.797 ± 1.339	–16.29 ± 2.795	–0.7163 ± 0.1594	–0.4990 ± 0.1807
r^2	0,7411	0,7905	0,6917	0,4587
P value	0,0007*	0,0003*	0,0015*	0,0221*
N value	11	11	11	11

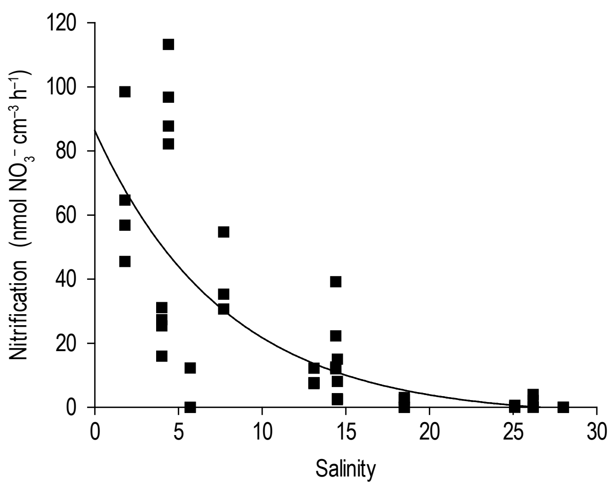

Figure 4. Exponential decay model between sediment nitrification and salinity at the studied lagoons. Equation: y = 89.29 * exp (–0.1285 * x) + (–2.929); R^2 = 0.5345; n = 44.

4. Discussion

Sediment potential nitrification rates varied up to two orders of magnitude between the studied lagoons.

The absence of positive or negative correlation between benthic algae biomass and nitrification was attributed to the low density of algae that was two or three orders of magnitude lower than usually found (Risgaard-Petersen, 2003). A positive relationship between benthic algae biomass and nitrification,as described in the literature (Lorenzen et al., 1998; Risgaard et al., 1999; An and Joye, 2001; Travieso et al., 2006), was not observed in this study. Sediment ammonium concentrations and pH values were not determined in this study and could also have been important regulator factors of nitrification. However, the direct effect of sediment ammonium or pH on nitrification would demand the use of microeletrodes to evaluate ammonium, O_2 and pH profiles. These equipments were not available in our laboratory while this study was conducted.

The strong relationship between nitrification rates and water salinity indicates that salinity shall be a main regulating factor of sediment nitrification, especially in lagoons with low chlorophyll-*a* concentrations in the sediment. Rysgaard et al. (1999) observed that nitrification decreased as salinity increased along a saline gradient in Danish estuaries. The most pronounced reduction of sediment NH_4^+ absorption capacity was observed when the salinity raised from 0 to 10‰. That is comparable to the salinity variation found in Preta Lagoon, where it was observed the most accentuated slope of the linear regression model between salinity and sediment nitrification .

The salinity influence on nitrification can be more drastic in environments with low salinity because nitrifiers need more time to recover. The results obtained by Helder and DeVries (1983) showed that adaptation of nitrifiers from saline to fresh water was particularly slow. The slope relating nitrification and salinity obtained for Pires and Preta lagoons (where the salinity median was below 10‰) were much higher than the slopes obtained for Catingosa and Visgueiro lagoons. These results suggest that a salinity increase in a fresh or almost fresh water environment can be much more critical for nitrification than the same increase in a saline environment.

The results obtained in our study indicated that nitrification might show large variability, and that the salinity is probably a major regulating factor on nitrification in coastal environments, especially when salt concentrations are relatively low.

References

AN, SM. and JOYE, SB. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnol. Oceanogr.*, 2001, vol. 46, no. 1, p. 62-74.

BELSER, LW. Population ecology of nitrifying bacteria. *Annu. Rev. Microbiol.*, 1979, vol. 33, p. 309-333.

BOATMAN, CD. and MURRAY, JW. Modeling exchangeable NH_4^+ adsorption in marine sediments: process and controls of adsorption. *Limnol. Oceanogr.*, 1982, vol. 27, no. 1, p. 99-110.

BOWER, CE. and HOLM-HANSEN, T. A salicylate-hypochlorite method for determining ammonia in seawater. *Can. J. Fish. Aquat. sci.,* 1980, vol. 37, no. 5, p. 794-798.

CARMOUZE, JP., KNOPPERS, B. and VASCONCELOS, P. Metabolism of a subtropical Brazilian lagoon. *Biogeochemistry*, 1991, vol. 14, no. 2, p. 129-148.

DALSGAARD, T. (Ed.), NIELSEN, LP., BROTAS, V., VIAROLI, P., UNDERWOOD, G., NEDWELL, DB., SUNDBACK, K., RYSGAARD, S., MILES, A., BARTOLI, M., DONG, L., THORNTON, DCO., OTTOSEN, LDM., CASTALDELLI, G. and RISGAARD-PETERSEN, N. *Protocol handbook for NICE – Nitrogen Cycling in Estuaries*:

a project under the EU research programme: Marine Science and Technology (MAST III). Silkeborg: National Environmental Research Institute, 2000. p. 62.

ENRICH-PRAST, A., BOZELLI, RL., ESTEVES, FA. and MEIRELLES-PEREIRA, F. Lagoas costeiras da restinga de Jurubatiba: descrição de suas variáveis limnológicas. In ROCHA, CFD., ESTEVES, FA. and SCARANO, FR. (Eds.). *Pesquisas de longa duração na restinga de Jurubatiba*: Ecologia, história natural e conservação. São Carlos: RiMa Editora, 2004. p. 376.

ENRICH-PRAST, A. Caminhos do nitrogênio em ecossistemas aquáticos continentais. In ROLAND, F., CESAR, D. and MARINHO, M. (Orgs.). *Lições de Limnologia*. São Carlos: RiMa Editora, 2005. p. 209-227.

ENRICH-PRAST, A., BASTVIKEN, D. and CRILL, P. Chemosynthesis. In GENE E. LIKENS. (Org.). *Encyclopedia of Inland Waters*. Oxford: Elsevier, 2009. vol. 1, p. 211-225.

FENCHEL, T., KING, GM. and BLACKBURN, H. *Bacterial biogeochemistry:* the ecophysiology of mineral cycling. 2 ed. San Diego: Academic Press, 1998. p. 307.

FINSTEIN, MS. and BITZKY, MR. Relationships of autotrophic ammonium-oxidizing bacteria to marine salts. *Water. Res.*, 1972, vol. 6, no. 1, p. 31-40.

HELDER, W. and DEVRIES, RTP. Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard Estuary). *Neth. J. Sea. Res.*, 1983, vol. 17, no. 1, p. 1-18.

JOYE, SB. and HALLINBAUGH, JT. Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science*, 1995, vol. 270, no. 5236, p. 623-625.

KAPLAN, WA. Nitrification. In CARPENTER, EJ. and CAPONE, DJ. (Eds.). *Nitrogen in the marine environment*. New York: Academic Press, 1983. p. 139-190.

LORENZEN, J., LARSEN, LH., KJAER, T. and REVSBECH, NP. Biosensor determination of the microscale distribution of nitrate, nitrate assimilation, nitrification, and denitrification in a diatom-inhabited freshwater sediment. *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 9, p. 3264-3269.

MAGALHÃES, CM., JOYE, SB., MOREIRA, RM., WIEBE, WJ. and BORDALO, AA. Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. *Water. Res.*, 2005, vol. 39, no. 9, p. 1783-1794.

MARTIN, L. and DOMINGUES, JML. Geological history as coastal lagoons. In KJERVE, B. (Ed.). *Coastal lagoon processes*. Netherlands: Elsevier Oceanography Series, 1994. vol. 60, p. 41-68.

MEYER, RL., KJAER, T. and REVSBECH, NP. Use of NO_x^- microsensors to estimate the activity of sediment nitrification and NO_x^- consumption along an estuarine salinity, nitrate, and light gradient. *Aquat. Microb. Ecol.*, 2001, vol. 26, no. 2, p. 181-193.

MUÑOZ, R. and GUIEYSSE, B. Algal–bacterial processes for the treatment of hazardous contaminants: a review. *Water. Res.*, 2006, vol. 40, no. 15, p. 2799-2815.

REVSBECH, NP., SORENSEN, J., BLACKBURN, TH. and LOMHOLT, JP. Distribution of oxygen in marine-sediments measured with microelectrodes. *Limnol. Oceanogr.*, 1980, vol. 25, no. 3, p. 403-411.

RISGAARD-PETERSEN, N., RYSGAARD, S., NIELSEN, LP. and REVSBECH, NP. Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnol oceanogr.*, 1994, vol. 39, no. 3, p. 573-579.

RISGAARD-PETERSEN, N., NICOLAISEN, MH., REVSBECH, NP. and LOMSTEIN, BA. Competition between ammonia-oxidizing bacteria and benthic microalgae. *Appl. Environ. Microbiol.*, 2004, vol. 70, no. 9, p. 5528-5537.

RISGAARD-PETERSEN, N. Coupled nitrification–denitrification in autotrophic and heterotrophic estuarine sediments: on the influence of benthic microalgae. *Limnol. Oceanogr.,* 2003, vol. 48, no. 1, p. 93-105.

RYSGAARD, S., THASTUM, P., DALSGAARD, T., CHRISTENSEN, PB. and SLOTH, NP. Effects of salinity on NH_4^+ absorbtion capacity, nitrification and denitrification in Danish estuarine sediments. *Estuaries*, 1999, vol. 22, no. 1, p. 21-30.

SUZUKI, MS., OVALLE, ARC. and PEREIRA, EA. Effects of sand bar openings on some limnological variables in a hypertrophic tropical coastal lagoon of Brazil. *Hydrobiologia*, 1998, vol. 368, p. 111-122.

TRAVIESO, L., BENÍTEZ, F., SÁNCHEZ, E., BORJA, R. and COLMENAREJO, MF. Production of biomass (algae–bacteria) by using a mixture of settled swine and sewage as substrate. *J. Environ. Sci. Health. A.*, 2006, vol. 41, no. 3, p. 415–419.

WILKIE, AC. and MULBRY, WW. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresource Technol.*, 2002, vol. 84, no. 1, p. 81-91.

ZAR, JH. *Biostatistical analysis*. 3 ed. Upper Saddle River: Prentice Hall International Editions, 1996. 662 p.

Received: 18 May 2009
Accpeted: 19 June 2009