BACTERIAL GROWTH AND DOC CONSUMPTION IN A TROPICAL COASTAL LAGOON

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ABSTRACT

The aims of this research were to determine the main limiting nutrient to bacterial growth in Imboassica lagoon, southeastern Brazil, to estimate the percentage of dissolved organic carbon (DOC) available for bacterial growth, and to determine the bacterial growth efficiency (BGE) of natural assemblages. Bacterial growth and DOC consumption were determined in batch culture experiments, in which water samples were supplemented with nitrogen and phosphorus together or separately, or incubated without nutrient additions. When added together, N and P stimulated higher bacterial growth rates and production, as well as higher DOC consumption. The BGEs and DOC consumption rates were strongly dependent on the method used to determine bacterial production. The BGE ranged from 11 to 72%. However, only a minor fraction of bulk DOC was consumed by the planktonic bacteria (from 0.7 to 3.4%). The results suggest that low availability of phosphorus and nitrogen coupled with excess organic carbon was the main factor responsible for the relatively low bacterial utilization of DOC in Imboassica lagoon.

Keywords: bacterioplankton, DOC consumption, BGE, coastal lagoons, phosphorus and nitrogen colimitation.

RESUMO

Crescimento bacteriano e consumo de COD em uma lagoa costeira tropical

Os objetivos desta pesquisa foram: determinar o principal nutriente limitante ao crescimento bacteriano na lagoa Imboassica, estimar a porcentagem de Carbono Orgânico Dissolvido (COD) disponível para o crescimento bacteriano e determinar a Eficiência de Crescimento Bacteriano (ECB) da comunidade bacteriana. O crescimento bacteriano e o consumo de COD foram avaliados em experimentos de culturas de diluição, nos quais nitrogênio e fósforo foram adicionados às amostras de água, juntos ou separadamente, e um controle foi preparado sem adições de nutrientes. Quando adicionados juntos, N e P estimularam um maior crescimento e produção bacterianos, assim como maiores taxas de consumo de COD. ECB e taxas de consumo de COD foram fortemente dependentes do método utilizado para determinar a produção bacteriana. ECB variou de 11% a 72%, porém apenas uma pequena fração do COD total foi consumida pelas bactérias planctônicas (de 0,7% a 3,4%). Sugere-se que as baixas disponibilidades de fósforo e nitrogênio são os principais fatores responsáveis pela baixa utilização de COD pelas bactérias na lagoa Imboassica.

Palavras-chave: bacterioplâncton, consumo de COD, eficiência do crescimento bacteriano, lagoas costeiras, colimitação por fósforo e nitrogênio.

INTRODUCTION

Dissolved organic carbon (DOC) is the main carbon stock in aquatic ecosystems. Globally, more organic matter occurs in dissolved form in seawater than in all land plants and marine organisms combined (Hedges & Keil, 1995). Low DOC concentrations are usually found in open ocean and in large lakes, while high DOC concentrations are observed in humic lakes and swamps. Several tropical lagoons of the Atlantic coast of Rio de Janeiro State have high DOC concentrations (Farjalla *et al*., 2001), comparable only to the DOC concentration of freshwater swamps (Bano *et al*., 1997). However, few studies related to DOC utilization in tropical coastal lagoons have been published.

For several decades DOC was considered to be biologically inert, and its loss from aquatic ecosystems was attributed to oxidation, sedimentation, and complexion with metals (Perdue, 1998). More recent observations have shown that bacteria can scavenge DOC with high efficiency and convert DOC into bacterial biomass, via bacterial production, or into CO_2, via respiration (Ducklow, 1994). Bacterial growth efficiency (BGE), *i.e*., the ratio of bacterial biomass produced to substrate consumed, is an important measurement used to understand DOC flux through aquatic trophic chains and DOC amounts released as CO_2 into the atmosphere (Søndergaard & Theil-Nielsen, 1997). Recent estimates have shown that BGE often falls within the range of 20 to 60% at natural DOC concentrations in freshwater, estuarine and marine ecosystems (del Giorgio *et al*., 1997; Søndergaard, 1997). Lower and higher BGE values were also observed in waters having low-quality DOC, and for bacterial degradation of aquatic macrophytes and phytoplanktonic debris (Cole & Pace, 1995; Søndergaard, 1997).

Coastal lagoons are relatively shallow lakes occuring near the ocean, from which they are partially or entirely sealed off by sand bars built by tidal or wave action (Branco *et al*., 2000). Due to their proximity to the ocean, Brazilian coastal lagoons are often surrounded by cities and used as harbors, recreational areas, fisheries, or for sewage disposal and landfills (Esteves, 1998). In the present study, we estimated bacterial production, abundance, biomass, and BGE in the water column of a typical tropical coastal lagoon, the Imboassica, in Rio de Janeiro state. The objectives were 1) to determine the main limiting nutrient for bacterial growth; 2) to estimate available DOC to bacterial growth in the lagoon's waters; and 3) to determine BGE of its natural assemblages.

MATERIALS AND METHODS

Study area

Imboassica lagoon, which is located on a coastal plain (22° 50' S; 44° 42' W) in northern Rio de Janeiro State, Brazil (Fig. 1), was formed when the Imboassica River was dammed by a sand bar formed by wave action on the ocean shoreline (Esteves, 1998). A shallow lagoon, it has a maximum 2.1 m depth and an area of *ca*. 326 ha (Panosso & Esteves, 1999). Regional climate is warm and humid, presenting annual averages of 26.6 °C in temperature and 1,500 mm in precipitation. Approximately one third of the lagoon is shallow and presents dense aquatic plant growth (Fig. 1). This area is extensively colonized by aquatic macrophytes, *e.g.*, *Typha domingensis* and *Eleocharis mutata* (Palma-Silva, 1999).

Experimental design

In June 1998, water from the central portion of Imboassica lagoon was collected in 20 L plastic bottles previously rinsed with filtered water. They were immediately transported to the laboratory, and samples of initial DOC concentration, bacterial abundance, and bacterial biomass were collected and preserved for later estimations.

The complete experimental setup is described in previous works (Cimbleris & Kalff, 1998; Farjalla *et al*., 2002). In the laboratory, water was passed through a pore size 0.2 μm filter (SuporCap 100, Gelman Sciences) to remove bacteria. An inoculum was prepared by passing lake water through a GF/F filter (pore size 0.8 μm, Whatman) to remove large particulate matter from the water. To minimize organic and inorganic contamination from filters, we flushed the filters with approximately 1 L of lake water sample before filtrate collection. Bacteria were cultivated by combining 90% of 0.2 μm filtered lake water and 10% of inoculum. The filtered lake water and inoculum were poured into acid-rinsed (HCl 10%), heat-sterilized (120 °C, 1 atm autoclavation pressure) 200 mL

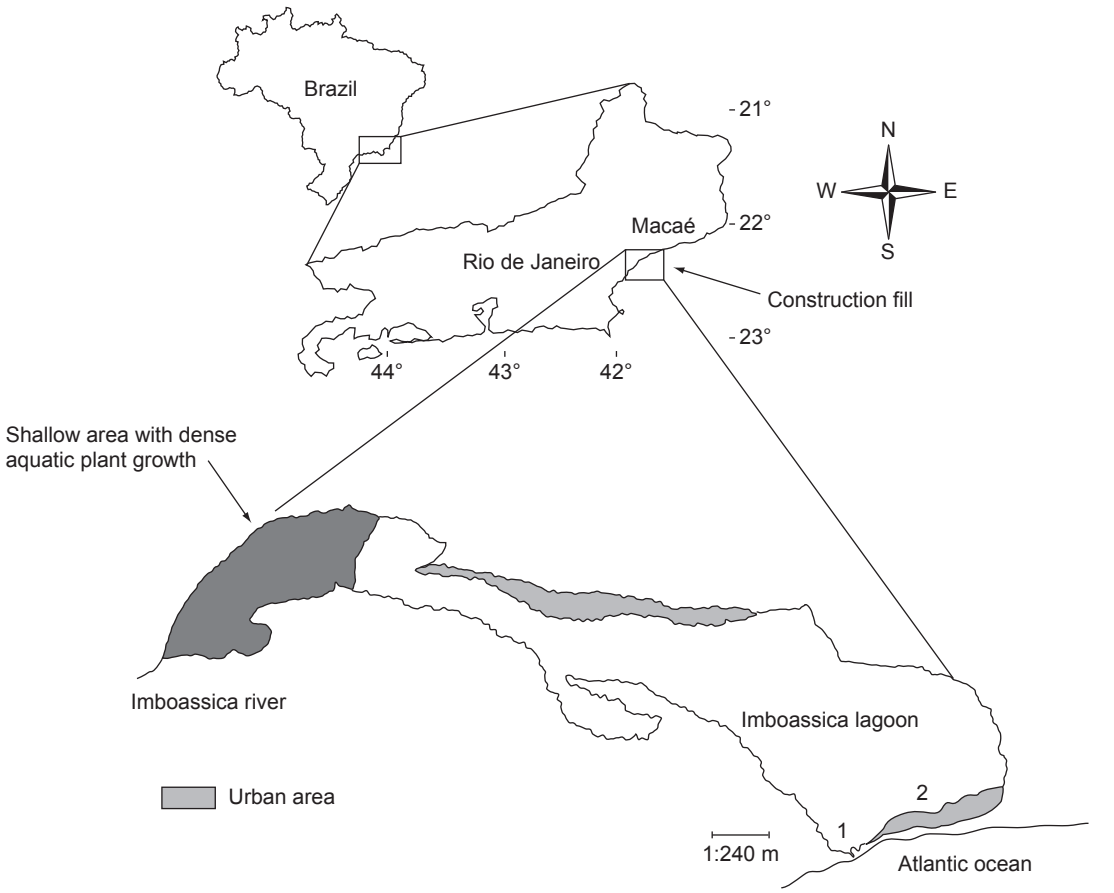

Fig. 1 — Map indicating location of Imboassica lagoon, Macaé, Rio de Janeiro, Brazil.

glass flasks. These cultures were used to determine bacterial abundance, biomass, and production. The filtered lake water and inoculum were also put in 60 mL Winkler glass flasks, leaving no headspace, to measure bacterial respiration. The complete process generally took 6-8 h.

Four sets of experiments, each replicated four times, were performed: 1) control treatment: culture incubated without nutrient additions; 2) N treatment: culture incubated with addition of NH_4NO_3 (50 µM-N); 3) P treatment: culture incubated with addition of KH_2PO_4 (5 µM-P); and 4) NP treatment: culture incubated with addition of NH_4NO_3 and KH_2PO_4 together (50 µM-N and 5 µM-P). The cultures were incubated in the dark at room temperature; bacterial growth was followed by measurements after 24, 48, and 72 h of bacterial production, abundance, biomass, and respiration.

We measured bacterial production in water cultures by two methodologies, the first of which is based on the incorporation of ^{3}H-leucine by bacteria (Smith & Azam, 1992), and the other based on bacterial biomass increase over time in the cultures. In the latter case, bacterial biomass was obtained through the conversion of bacterial biovolume by two different factors: 308 fg C μm^{-3} (Fry, 1990) and 105 fg C μm^{-3} (Theil-Nielsen & Søndergaard, 1998).

Bacterial respiration was estimated by the Winkler method based on dissolved oxygen consumption, which was converted to respired bacterial carbon using a respiratory quotient of 1.0.

Bacterial growth efficiency (BGE) was calculated during the growth phase, as BP/(BP + BR), where BP is the bacterial production rate and BR, the bacterial respiration rate (Søndergaard & Theil-Nielsen, 1997). The DOC bioavailability was calculated as follows: $DOC_L = (DOC_B + DOC_R) /DOC_T$, where DOC_L is labile DOC for bacteria, DOC_B is dissolved organic carbon converted into bacterial biomass, DOC_R is dissolved organic carbon respired by bacteria, and DOC_T is initial DOC bulk.The DOC bioavailability was estimated during log phase (Carlson & Ducklow, 1996).

Analytical methods

Bacterial abundance was measured using a Beckton Dickinson FACSort flow-cytometer, according to methodology proposed by del Giorgio *et al*. (1996). Syto 13 stain (50 µM, Molecular Probes) and Fluoresbrite™ Carboxy YG Microspheres (Ø = 1.58 µm, *ca*. 3×10^5 mL^{-1}, Polysciences) were added to 1 mL subsamples. The cytometer was controlled with CellQuest 1.2 software. Bacterial cells and microspheres were separated in a log-log scattergram of green fluorescence intensity (FL1) and side scattering (SSC). Samples were run for one minute or until 10,000 cells were counted. Bacterial abundance in the samples was calculated using as an internal standard microspheres, the number of which in standard stock solution was analyzed by epifluorescence microscopy. Bacterial biomass was estimated by measuring bacterial biovolume in an epifluorescence microscope connected to a video camera with an image analysis system. Bacteria samples were previously stained with DAPI, according to Porter and Feig (1980). Images were captured with Image Grabber-24 software (Neotech) and processed with IPLab Spectrum 3.1a software (Signal Analytics). At least 150 bacteria on 3 images were measured in each sample; cell volume was calculated using a formula proposed by Fry (1990). The previously mentioned conversion factors of 308 fg C μm^{-3} and 105 fg C μm^{-3} were used to convert bacterial biovolume to bacterial biomass.

Bacterial secondary production in these cultures was obtained through the increase in bacterial biomass (see method above) or from the incorporation of ^{3}H-leucine (Smith & Azam, 1992). For ^{3}H-leucine incorporation, 1.3 mL of the culture was incubated in Eppendorf® 1.5 mL tubes containing 20 nM of ^{3}H-leucine (5-fold diluted solution, 159 Ci $mmol^{-1}$, Amersham), at room temperature in the dark for 45 min. A control was prepared for each culture by adding 90 µl of TCA (100%) to an identical tube. After incubation, 90 µl of TCA (100%) was also added to each of the replicates to stop the reaction. Each tube was washed sequentially with 5% TCA and 80% ethanol. Five hundred µl of scintillation cocktail (Aquasol 2, Dupont) was added to each tube and radioactivity measured using a Beckman LS-5600 Liquid Scintillation System. Bacterial production was calculated assuming an intracellular leucine dilution factor equal to two; and a cellular carbon-to-protein ratio equal to 0.86 (Wetzel & Linkens, 1991).

A Shimadzu Carbon Analyzer TOC-5000 was used to measure DOC concentrations, with DOC ascertained by subtracting from the carbon total the DIC, which was measured by an infrared sensor through acidifying inorganic forms of carbon to CO_2. At least three DOC and DIC measurements were made for each sample, resulting in a coefficient of variation (CV) of less than 2%. Dissolved nitrogen concentration was obtained through digestion at 320 °C and distillation of the NH_4^+ formed (Mackereth *et al*., 1978). Dissolved phosphorus was measured by autoclaving and formation of ammonium molybdate (Golterman *et al.*, 1978). Chlorophyll *a* was extracted with 90% ethanol, measured by absorbance at 665 nm and corrected for turbidity at 750 nm (Nusch & Palme, 1975). Absorbance at 430 nm was used to estimate Imboassica water-sample color.

Statistics

Differences among control, N, P, and NP treatments were tested by one-way ANOVA, followed by a Tuckey post-hoc test, according to Zar (1996). A probability level of $\alpha = 0.05$ was used throughout to determine statistical significance.

RESULTS

Water pH (8.16) and salinity (6.0 ‰) of Imboassica lagoon were relatively high, showing the influence of the neighboring ocean. We also observed a high DOC concentration (1.06 mM C) and, consequently, high C:N:P molar ratio

(2078:12:1). On the other hand, the lagoon had a low transparency (0.6 m) that, however, was not due to its water color (Abs 0.008 at 430 nm), but probably because of the moderately high chlorophyll *a* values (8.11 μgL^{-1}).

Bacterial abundance in Imboassica lagoon was around 3.4 x 10^9 bacteria L^{-1}, representing a 2.4 µM C bacterial biomass. Initial bacterial production was 0.032 µM C h^{-1}. Calculation showed that approximately 3 days would be adequate for the bacterial population to double its biomass in the lagoon.

Control, N, P, and NP cultures demonstrated a classic sigmoidal growth curve (Fig. 2). Cultures were initiated with approximately 3.5 x 10^8 cells L^{-1} and reached the stationary phase after 2-3 days. After 72 h, the NP treatment reached the highest bacterial abundance of 4.2 x 10^9 cells L^{-1}, and also showed the only bacterial growth ratio significantly higher (ANOVA, $p < 0.05$) than those obtained in the other three treatments. The second highest bacterial abundance (2.7 x 10^9 cells L^{-1}) occurred in the P treatment, which approximated the final bacterial abundance observed for both control and N treatments (2.1 x 10^9 cells L^{-1}). No significant increase was observed in bacterial biovolume during the logarithmic and stationary phases in any of the treatments (ANOVA, $p < 0.05$, Fig. 3).

Bacterial secondary production was measured in all treatments by ^{3}H-leucine incorporation

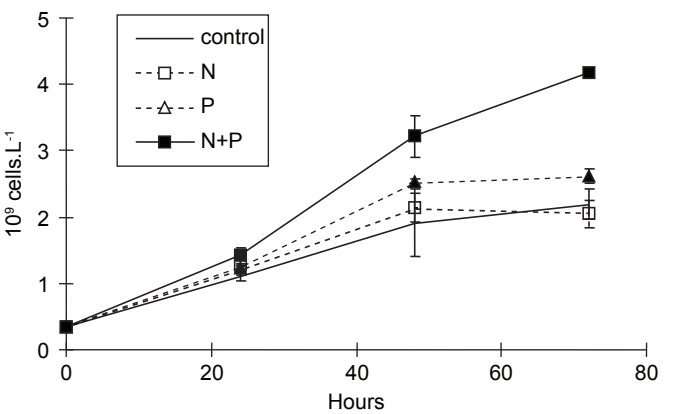

Fig. 2 — Bacterial growth curves in Imboassica water cultures. Bars = SD, n = 4.

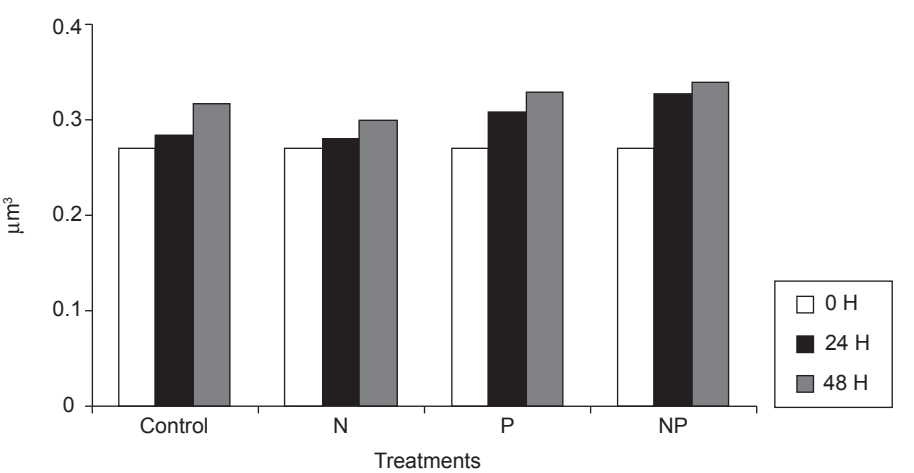

Fig. 3 — Bacterial biovolume changes during bacterial growth in Imboassica cultures.

(Fig. 4) and by bacterial biomass increase, using the bacterial biovolume-biomass conversion factors of 308 fg C μm^{-3} (Fig. 4) and 105 fg C μm^{-3}. We observed an increase in the bacterial production rate in all treatments measured by changes in bacterial biomass during the logarithmic phase (from 0 to 48 h; Fig. 4a). On the other hand, a decrease in all treatments in bacterial production rates occurred from the middle (24 h) to the end of logarithmic phase (48 h) as measured by incorporation of ^{3}H-leucine. These decreases occurred independently of the increase in bacterial abundance (Fig. 4b).

While the lower bacterial production rates were found using the ^{3}H-leucine method, the higher were found using the bacterial biomass method, obviously with the higher conversion factor, *i.e.*, 308 fg C μm^{-3} (Table 1). Bacterial production rates ranged from 0.027 to 0.535 $\mu M\ C\ h^{-1}$, with higher rates in the NP treatments and the lower rates in the control and N treatments (Table 1).

Bacterial respiration varied from 0.119 to 0.212 µM C in control and NP treatments, respectively. The DOC consumed throughtout the logarithmic growth phase ranged from 0.7 to 3.4%, depending on the bacterial production method used (Table 1). Consumption was higher in the NP treatment, followed by P, N, and control treatments. The BGE ranged from 11 to 72% and, as previously mentioned, was strongly influenced by the bacterial production method (Table 1). Higher BGE values were usually found for control and NP cultures, while lower ones generally resulted from the N treatment (Table 1). During incubations temperature was maintained at around 24 °C.

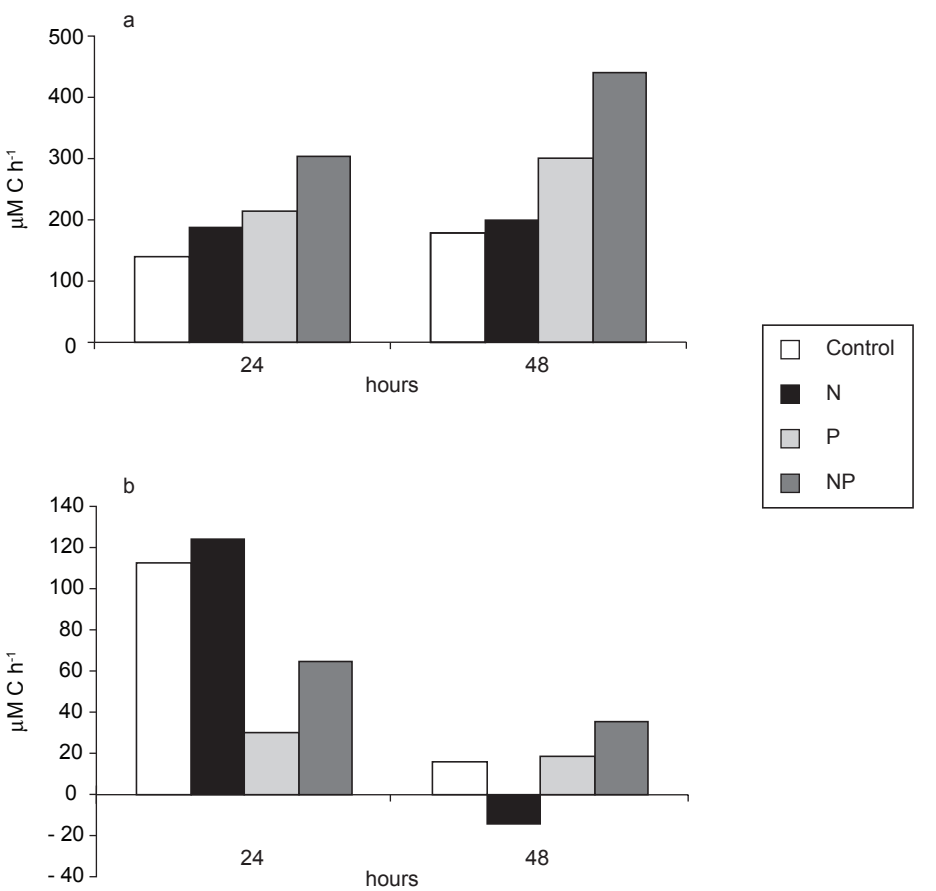

Fig. 4 — Percentage of change in bacterial production rate in relation to incubation initiation: a) bacterial production measured by bacterial biomass increase (conversion factor of 308 fg C μm^{-3}); and b) bacterial production measured by ^{3}H-leucine incorporation.

TABLE 1
Bacterial production rate, bacterial respiration rate, DOC consumed, and bacterial growth efficiency in Imboassica water cultures.

Treatments	BP rate (μM C h^{-1})			BR rate (μM C h^{-1})	DOC consumed (%)			BGE (%)		
	A	B	C		A	B	C	A	B	C
Control	0.037	0.107	0.275	0.119	0.7	1.0	1.8	24	47	70
N	0.027	0.115	0.294	0.212	1.1	1.5	2.3	11	35	59
P	0.037	0.154	0.394	0.190	1.0	1.6	2.6	17	45	67
NP	0.043	0.209	0.535	0.212	1.2	1.9	3.4	17	50	72

A) Bacterial production rate (BP), DOC consumed, and BGE estimated from ^{3}H- leucine incorporation at the end of logarithmic phase; B) Bacterial production rate (BP), DOC consumed, and BGE estimated from bacterial biomass increase during the logarithmic phase. Biomass (Theil-Nielsen & Søndergaard, 1998) = (abundance x mean cell volume) x 105 fg C μm^{-3}; and C) Bacterial production rate (BP), DOC consumed, and BGE estimated from bacterial biomass increase during the logarithmic phase. Biomass (Fry 1990) = (abundance x mean cell volume) x 308 fg C μm^{-3}.

DISCUSSION

Due to its location, Imboassica lagoon is subject to two severe types of anthropogenic impacts, the first of which occurs with the opening of the sand bar separating the lagoon from the ocean. This not only alleviates pollution by draining lagoon waters, but also allows access by ocean fish in incoming waters. The second impact is the domestic effluent release directly into the lagoon. Whereas the high salinity and, consequently, high pH observed in this lagoon is related to the last sand bar opening in March 1998, its cultural eutrophication - caused by sewage disposal - stimulates phytoplankton growth and, consequently, the relatively high chlorophyll *a* values (8.11 μg Chl *a* L^{-1}) in its water column (see Melo, 2001). In contrast, thirteen other relatively unpolluted tropical coastal lagoons in Rio de Janeiro State have an average of 3.65 μg Chl *a* L^{-1} (Farjalla *et al*., 2001).

The observed initial bacterial abundance (3.4 x 10^9 cells L^{-1}), biomass (2.4 μM C), and secondary productivity (0.032 μM C h^{-1}) are within the range of most tropical freshwater ecosystems (Torréton *et al*., 1994; Benner *et al*., 1995; Lindell & Edling, 1996; Anesio *et al*., 1997; Thomaz & Esteves, 1997; Bouvy *et al*., 1998). Direct disposal of sewage into Imboassica lagoon, although stimulating phytoplankton growth (Melo, 2001), has not resulted in higher bacterial abundance, biomass, and secondary productivity than that found in other, less impacted Brazilian tropical coastal lagoons (Farjalla *et al*., 2001; Farjalla *et al*., 2002). According to Bratbak and Thingstad (1985), phytoplankton competes with bacteria for phosphorus supplies. In clear-water ecosystems with high light availability, phytoplankton is more capable than bacteria of scavenging phosphorus. Moreover, bacterial-algal competition seems to be less favorable to bacteria as lake entrophy increases (Vadstein & Olsen, 1989; Currie, 1990). Therefore, in Imboassica lagoon, that sewage disposal did not result in marked increases in bacterial biomass might be due to the competition between phytoplankton and bacteria for the phosphorus supply. Nitrogen may also be a limiting factor for bacterial growth. Experiments in a eutrophic reservoir showed an increase in bacterial growth and production when surface water was supplemented with ammonium (Wang *et al*., 1992). Furthermore, bacterial abundance in a meso-eutrophic lake was only significantly correlated to Chl *a* following ammonium nitrate additions, implying that both bacteria and phytoplankton were mutually influenced by the N supply (Le *et al*., 1994).

Bacterial populations in aquatic ecosystems can be regulated by several factors, divisible into bottom-up factors (*e.g.*, nutrient concentrations and temperature), which are generally considered the main regulators of bacterial community in aquatic ecosystems, or top-down factors, *e.g.*, grazing by zooplankton and viral lysis (Pace & Cole, 1994; Pace & Cole, 1996). Microbial growth requires a balanced supply of organic carbon and nutrients (Neidhardt *et al*., 1990). In Imboassica lagoon,

bacteria are limited by availability of phosphorus and nitrogen, since those nutrients when added together significantly stimulated bacterial growth in cultures (Fig. 2). The high C:N:P ratio of 2078:12:1, compared to the actual content of carbon, nitrogen, and phosphorus in bacteria (50:10:1, Fagerbakke *et al.*, 1996), further corroborates bacterial growth as being colimited by phosphorus and nitrogen in Imboassica lagoon.

That chlorophyll *a* concentrations, in relation to those of less impacted coastal lagoons, are relatively high in Imboassica lagoon suggests that in it phytoplankton is an important DOC source. Other important sources are the large aquatic macrophytes beds surrounding the lagoon and the raw sewage input. The DOC from phytoplankton exudates and that leached from macrophytes, both considered biologically labile, are readily available to bacteria (Baines & Pace, 1991; Carlson & Ducklow, 1996; Mann & Wetzel, 1996).

The DOC assimilated by bacteria may be converted to respiratory CO_2 or be transformed into bacterial biomass. We observed not only bacterial abundance and biovolume increases in our cultures (Figs. 2 and 3), but also an increase in respiration following nutrient additions (Table 1). Therefore, BGE did not necessarily increase in nutrient-enriched water because respiratory losses also increased. It has actually been shown that bacteria can dissociate catabolic and anabolic processes, *i.e.*, there is no obligatory coupling between respiration and growth and, consequently, BGE is theoretically highly variable (Middelboe & Søndergaard, 1993).

We used two methods in estimating bacterial production in Imboassica cultures. The first method, which results in instantaneous bacterial production measurement, is based on bacterial incorporation of labeled leucine in 0, 24, and 48 h of bacterial growth; the other method is based on bacterial biomass increase throughout the growth phase in the cultures (from 0 to 48 h). In the first case, decrease in bacterial production from 24 to 48 h (Fig. 4b) should be related to a decrease in bacterial metabolism between 24 and 48 h and is independent of bacterial abundance increase. Bacterial production based on bacterial biomass increase is an integrated analysis of the entire bacterial growth in the cultures. However, it is strongly dependent on the conversion factor used (Table 1, columns b and c).

When considering bacterial biomass increase, Imboassica cultures showed high BGE values (Table 1, column c) compared to cultures in other lake water (see Søndergaard & Theil-Nielsen, 1997) and coastal systems (Toolan, 2001). Several studies in the literature have focused on determining bacterial biovolume-biomass conversion factors, based on direct measurement of bacterial carbon content, on natural bacteria growth in natural conditions, and on bacteria growth in cultures (Watson *et al*., 1977; Bratbak, 1985; Bjørnsen, 1986; Norland *et al*., 1987; Fry, 1990; Carlson & Ducklow, 1996; Theil-Nielsen & Søndergaard, 1998). These studies have yielded a wide spectrum of conversion factors varying from 105 to 560 fg C μm^{-3}. We used conversion factors suggested by Fry (1990) and Theil-Nielsen and Søndergaard (1998). The Fry factor (308 fg C μm^{-3}) has already been widely used in previous studies, while the Theil-Nielsen and Søndergaard factor (105 fg C μm^{-3}) is particularly suited to our circumstances, as it was based on bacterial growth in freshwater and marine cultures. According to Norland *et al*. (1987), the expected carbon content of a bacterial cell with a 0.27 μm^{-3} biovolume, which was usually observed in our batch cultures (Fig. 3), would imply a conversion factor of lower than 200 fg C μm^{-3}. In fact, the 105 fg C μm^{-3} conversion factor, as well as the ^{3}H-leucine incorporation method, produced BGE values within a range observed in recent estimates (Toolan, 2001). Furthermore, our respiration rates were similar to those reported in the literature.

Bacteria in Imboassica cultures consumed only a minor DOC fraction, regardless of the bacterial production method used, and the high DOC concentration suggests a DOC accumulation in Imboassica lagoon (Table 1). Zweifel *et al*. (1995) observed a net DOC accumulation in the Bothnian Sea coastal areas, where bacterial community growth was limited by the phosphorus supply. In addition, DOC may accumulate if bacterial growth rates remain low due to competition with algae for inorganic nutrients (Thingstad *et al*., 1997). Given nutritional environments largely determine substrate utilization by bacteria, as demonstrated by physiological studies which exhibit an order of magnitude energy-cost savings in biosynthesis

of cellular components, notably protein and nucleotides, when bacteria grow in a rich medium (Neidhardt *et al*., 1990). Furthermore, plant decomposition rates correlate positively to nitrogen and phosphorus concentrations (Enríquez *et al*., 1993). Likewise, DOC biodegradability increases upon N (Kirchman *et al*., 1991) and P (Pomeroy *et al*., 1995) additions in oceanic waters, and by supplementation by both in estuarine waters (Coffin *et al*., 1993). Bacteria doubled DOC scavenging in Imboassica cultures after simultaneously adding phosphorus and nitrogen. Our results indicate that low availability of phosphorus and nitrogen combined with excess organic carbon, is the main factor accounting for relatively low DOC utilization in Imboassica lagoon.

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