

# Oxygen uptake during mineralization of photosynthesized carbon from phytoplankton of the Barra Bonita Reservoir: a mesocosm study

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(With 2 figures)

## Abstract

This study aimed to discuss and describe the oxygen consumption during aerobic mineralization of organic products (cells and excretion products) from five unialgal cultures: *Cryptomonas* sp., *Microcystis aeruginosa*, *Anabaena spiroides*, *Thalassiosira* sp. and *Aulacoseira granulata*. These species were isolated from Barra Bonita reservoir (22° 29' S and 48° 34' W) and cultivated in the laboratory. From each culture, two decomposition chambers were prepared; each chamber contained about 130 mg.L<sup>-1</sup> of carbon from water samples of the reservoir. The chambers were aerated and incubated in the dark at 20.0 °C. The concentration of dissolved oxygen, pH values and electrical conductivity of the solutions were determined during a period of 10 days. The results indicated increases in oxygen consumption for all the solutions studied and also for electrical conductivity. The pH values presented a decreasing tendency throughout the experiment. Oxygen consumption varied from 43 (*Aulacoseira granulata* chamber) to 345 mg O<sub>2</sub> g<sup>-1</sup> C (*Anabaena spiroides* chamber). Decrease in pH values was probably due to increase in CO<sub>2</sub> concentration from microbial respiration. Increase in electrical conductivity might be due to the liberation of ions during decomposition. The results demonstrate the potentiality of the studied genera in influencing oxygen availability followed by a die-off event. It also indicates the possibility of changing of the electrical conductivity and pH values in the water column due the aerobic algae mineralization.

**Keywords:** aerobic mineralization, algae, Barra Bonita Reservoir (Barra Bonita, SP, Brazil), oxygen uptake.

## Consumo de oxigênio da mineralização de carbono fotossintetizado de fitoplâncton do Reservatório de Barra Bonita: um estudo em mesocosmo

### Resumo

Este estudo teve por objetivo descrever e discutir aspectos do consumo de oxigênio decorrente da mineralização aeróbia de células e produtos de excreção provenientes de cinco culturas de algas: *Cryptomonas* sp., *Microcystis aeruginosa*, *Anabaena spiroides*, *Thalassiosira* sp. e *Aulacoseira granulata*. As algas foram isoladas do reservatório de Barra Bonita (22° 29' S e 48° 34' W) e cultivadas em laboratório. Para cada cultura, foram preparadas duas câmaras de mineralização; cada garrafa conteve, em base de carbono, cerca de 130 mg.L<sup>-1</sup> em amostras de água do reservatório. As câmaras foram aeradas e incubadas no escuro a 20 °C. Durante um período de 10 dias, foram determinadas as concentrações de oxigênio dissolvido, pH e condutividade elétrica das misturas. Os resultados indicaram incrementos nos consumos de oxigênio e de condutividade elétrica e decréscimos nos valores de pH. O consumo de oxigênio variou de 43 (experimento com *Aulacoseira granulata*) a 345 mg O<sub>2</sub> g<sup>-1</sup> C (experimento com *Anabaena spiroides*). Os aumentos dos valores de condutividade elétrica provavelmente decorreram da liberação de íons durante a decomposição. Para o pH, a diminuição dos valores foi provavelmente resultante do incremento das concentrações de CO<sub>2</sub> provenientes da respiração dos microrganismos. Os resultados sugeriram para a represa de Barra Bonita, a possibilidade de incremento das demandas de oxigênio em função da degradação dos gêneros

selecionados. Visto que esse reservatório é eutrófico, os resultados indicam também a possibilidade de alterações da condutividade elétrica da água e do pH devido à mineralização aeróbia de algas.

*Palavras-chave:* consumo de oxigênio, mineralização aeróbia, algas, reservatório de Barra Bonita (Barra Bonita, SP, Brasil).

## 1. Introduction

Phytoplankton is considered an important primary producer in many aquatic systems. Their photosynthetic products are one of the sources of organic material content in the water column of aquatic ecosystems. The degradation of photosynthetically fixed organic carbon in oceans, lakes and reservoirs is one of the most important transformations in the global carbon cycle (Chen and Wangersky, 1996; Daniels et al., 2006).

According to Straškraba and Tundisi (2000), in the Barra Bonita reservoir the nutrient inputs are due to anthropogenic processes related to agriculture, cattle breeding, industrial and domestic sewage. Some consequences are eutrophication with intensive growth of aquatic macrophytes and cyanobacteria blooms (*Microcystis* sp. in summer and *Anabaena* sp. during tropical winter). Decomposition of algae is considered to be an important process in the regeneration of organic and inorganic compounds in aquatic ecosystems (Brouwer, 1996). The extent to which the algal biomass will be decomposed and the factors that affect this process are of concern for the effect of algae decomposition on the oxygen budget of freshwater ecosystems like reservoirs and lakes. In eutrophic ecosystems the clarification of algal decay kinetics will provide information necessary to understand and avoid such serious consequences of algal decomposition that interfere in the global oxygen budget of the system.

The extent and rate of organic matter degradation throughout microbial communities metabolism are affected by environmental factors such as redox conditions (Otsuki and Hanya, 1972a, b; Pacobahyba et al., 2004), temperature (White et al., 1991), nutrient content of algal cells, decomposer density and the resistance of algae to microbial decomposition (Gunnison and Alexander, 1975). In this context, few data about algae decomposition are available, making discussion and comparison a difficult task. Considering that the phytoplankton biomass content in the Barra Bonita reservoir is increasing due to eutrophication (Barbosa et al., 1999), and the importance of phytoplankton in decomposition events, this study aimed at the description and discussion of oxygen uptake kinetics from decomposition experiments of five phytoplankton species from Barra Bonita Reservoir (São Paulo, Brazil).

## 2. Materials and Methods

### 2.1. Description of the area

Barra Bonita Reservoir (22° 29' S and 48° 34' W) is an artificial water system constructed in 1963, located

between Barra Bonita and Igarçu cities. Its morphometry is described by Straškraba and Tundisi (2000). The maximum depth is 25 m. Its hydrographic basin area (30% forests, 50% intensive agriculture, 20% pasture) comprises 324.84 km<sup>2</sup>. The reservoir has a volume of 33.6 x 10<sup>6</sup> m<sup>3</sup> and a theoretical retention time of 90 days. The anthropogenic pressure on Barra Bonita Reservoir affects the water quality of this system, characterized as eutrophic (Henry et al., 1985; Tundisi and Matsumura-Tundisi, 1990).

### 2.2. Species selection

The choice of the species for this study was based on their importance in the reservoir. The cyanobacteria *Microcystis aeruginosa* Kützing 1846 and *Anabaena spiroides* Klebahn 1895 and the diatom *Aulacoseira granulata* (Ehrenberg) Simonsen 1979 represent the dominant species related to biomass though *Thalassiosira* sp. and *Cryptomonas* sp. are not biomass predominant in the reservoir; they were also selected for their importance in nutrient cycling (Dellamano-Oliveira and Vieira, submitted).

### 2.3. Isolation and cultivation of algal cells

Methods for algal isolation as described in Vieira (1977) were applied for the isolation of the species, which are maintained in the freshwater phytoplankton culture collection at the Botany Department, Universidade Federal de São Carlos (WDCM 835). The culture media used were ASM-1 (Gorham et al., 1964) for the cyanobacteria species and WC (Guillard and Lorenzen, 1972) for other species. Algae cultures were grown in glass carboys of 4 L capacity, with 3 L of medium, using algae inoculum at the exponential phase. Culture conditions were constant temperature (20-22 °C), pH (6.8-7.0), irradiance (265 μmol.m<sup>-2</sup>/s) supplied by day-light fluorescent tubes of 40 W with a light-dark cycle of 12 : 12 hours and aeration by gently bubbling of filtered and moist air.

### 2.4. Experimental design

For each alga, two decomposition chambers were prepared with the unialgal cultures and glass wool filtered reservoir water for removal of gross material (initial concentration in carbon basis ca. 130 mg.L<sup>-1</sup>). The algae cultures were harvested at the beginning of the stationary phase. The chambers were incubated in the dark to avoid primary production, at 20.05 ± 0.99 °C. Aerobic conditions (dissolved oxygen near saturation) were maintained by 1 hour oxygenation during every sampling day. After the oxygenation, the DO was meas-

ured by an ODMeter (YSI model 58). At DO concentrations reaching 2.0 mg.L<sup>-1</sup>, the solutions were oxygenated again during the experiments, up to the saturation value correspondent to the temperature of incubation. The oxygen uptake was estimated periodically during 10 days. The pH and the electrical conductivity (EC) were determined potentiometrically (Digimed DMPH-2 and Digimed DM3). All the values of OD, pH and electrical conductivity were blank-corrected with measures using the reservoir water.

### 2.5. Oxygen uptake equations

The consumption of oxygen in the experiments was considered directly related with the oxidation of the organic resources present, and that this process can be represented by kinetic models (Characklis, 1990; Henze et al., 1997; Cunha-Santino and Bianchini Jr., 2002). The temporal variation of the evolved oxygen was fitted to a first-order kinetics model using a non-linear method, the Levenberg-Marquardt iterative algorithm, according to Press et al. (1993). From these considerations, it is possible to describe the variation in the DO decay (Equation 1) according to the following equations:

$$OC = OC_{max} (1 - e^{-k_D t}) \quad (1)$$

where: OC = accumulated value of consumed oxygen (mg.L<sup>-1</sup>); OC<sub>max</sub> = maximum amount of consumed oxygen (mg.L<sup>-1</sup>); k<sub>D</sub> = deoxygenation rate constant (per day); and t = time (day).

The half-time (t<sub>1/2</sub>) of deoxygenation derived from aerobic decomposition of algae was calculated according to the Equation 2.

$$t_{1/2} = \frac{\ln 0.5}{-k_D} \quad (2)$$

The effects of oxidation from the flasks with algae were corrected by subtraction of OC values from the control flasks. The OC, pH and EC data were statistically analyzed using analysis of variance (Kruskal-Wallis) followed by Dunn's test in order to verify for significant differences among treatments (p < 0.05).

## 3. Results

The kinetics of oxygen uptake during the aerobic mineralization of *Cryptomonas* sp., *Microcystis aeruginosa*, *Anabaena spiroides*, *Thalassiosira* sp. and *Aulacoseira granulata* are shown in Figure 1. From the kinetic fittings (Table 1), the mineralization of algae had the oxygen consumption (OC<sub>max</sub>) ranging from 43 mg.g<sup>-1</sup> C (*Aulacoseira granulata*) to 345 mg.g<sup>-1</sup> C (*Anabaena spiroides*). The values of the deoxygenation rate constant (k<sub>D</sub>) ranged from 0.0545 to 0.3785/day and their corresponding t<sub>1/2</sub> (half-time) varied between 2 to 13 days (Table 1). The highest k<sub>D</sub> was obtained with the mineralization of *Aulacoseira granulata* (0.3785/day), followed by *Microcystis aeruginosa*, *Cryptomonas* sp., *Thalassiosira* sp. and *Anabaena spiroides* (0.2099; 0.1911; 0.1575 and 0.0545/day, respectively). The de-

termination coefficients (r<sup>2</sup>) for the kinetic fitting varied from 0.91 to 0.99. Using the kinetics of oxygen consumption, the Kruskal-Wallis analysis resulted in significant differences among the values for *Cryptomonas* sp. and *Microcystis aeruginosa* (p < 0.01) and *Aulacoseira granulata* (p < 0.001); *Microcystis aeruginosa* results were statistically distinct of those from *Anabaena spiroides* (p < 0.001) and *Thalassiosira* sp. (p < 0.001).

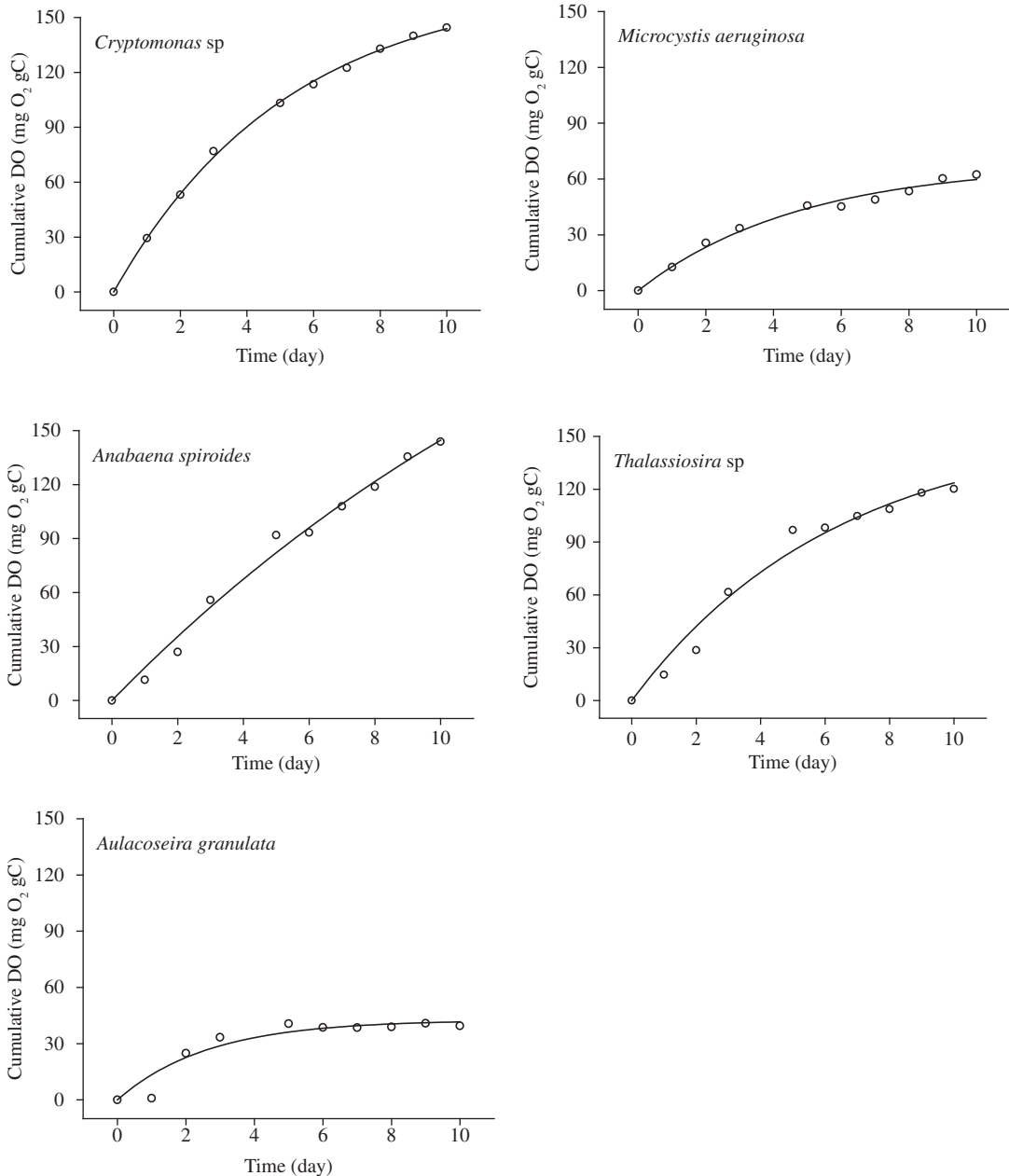
The pH values of incubations varied from 6.81 in the decomposing media of *Aulacoseira granulata* to 7.41 for incubation with *Cryptomonas* sp. (Figure 2). pH values tended to decrease, except for *Thalassiosira* sp., which presented a tiny decaying variation. The statistical analyses pointed to differences in the temporal variation of pH of the *Aulacoseira granulata* chambers in relation to *Cryptomonas* sp. (p < 0.001), *Microcystis aeruginosa* (p < 0.05) and *Thalassiosira* sp. (p < 0.01) experiments. *Cryptomonas* sp. experiments also differed in pH (p < 0.05) from the *Anabaena spiroides* experiments.

The minimum and maximum values for electrical conductivity (EC) among the incubations were 48.3 and 59.1 μS.cm<sup>-1</sup> (Figure 2). The incubations with *Microcystis aeruginosa* and *Anabaena spiroides* showed increases in EC during degradation, while with *Aulacoseira granulata*, *Cryptomonas* sp. and *Thalassiosira* sp., an attenuated EC decrease was shown. Differences in the temporal variation of CE among the incubations with *Microcystis aeruginosa* and *Aulacoseira granulata* (p < 0.05), *Cryptomonas* sp. (p < 0.001) and *Thalassiosira* sp. (p < 0.001) were revealed by statistical analyses. Values of CE for *Anabaena spiroides* incubations differed to the CE of *Cryptomonas* sp. (p < 0.01) and *Thalassiosira* sp. (p < 0.001) incubations.

## 4. Discussion

Several studies have demonstrated a sudden die-off of phytoplankton blooms in aquatic ecosystems (Barica, 1974; Lee and Lee, 1995). As a primary consequence of this event, fast oxygen depletion occurs and ammonia increases in concentration. The total amount of OC<sub>max</sub>, obtained in the dark, is usually employed as a measure of total heterotrophic activity in samples of lake water and sediments, and it is therefore reasonable to use OC<sub>max</sub> to follow the process of a microbial reaction in aerobic environments (Characklis, 1990). The long-term BOD tests are the practical procedure that expresses this variable (Cunha-Santino and Bianchini Jr., 2003a). The extent of temporal evolution of the oxygen consumption describes the metabolic activity of the microorganisms involved in the mineralization process and the recalcitrance of the structural compounds of phytoplanktonic cells.

Considering the high determination coefficients (r<sup>2</sup>: from 0.91 to 0.99) obtained on the fittings of the kinetic model (Figure 1) to the experimental results (Table 1), the proposed model (Equation 1) represents the kinetics of oxygen uptake. The oxygen uptake observed during algae decay presented similarities with that observed in the mineralization process of aquatic macrophytes



**Figure 1.** Oxygen consumption during the aerobic mineralization of *Cryptomonas* sp, *Microcystis aeruginosa*, *Anabaena spiroides*, *Thalassiosira* sp and *Aulacoseira granulata*.

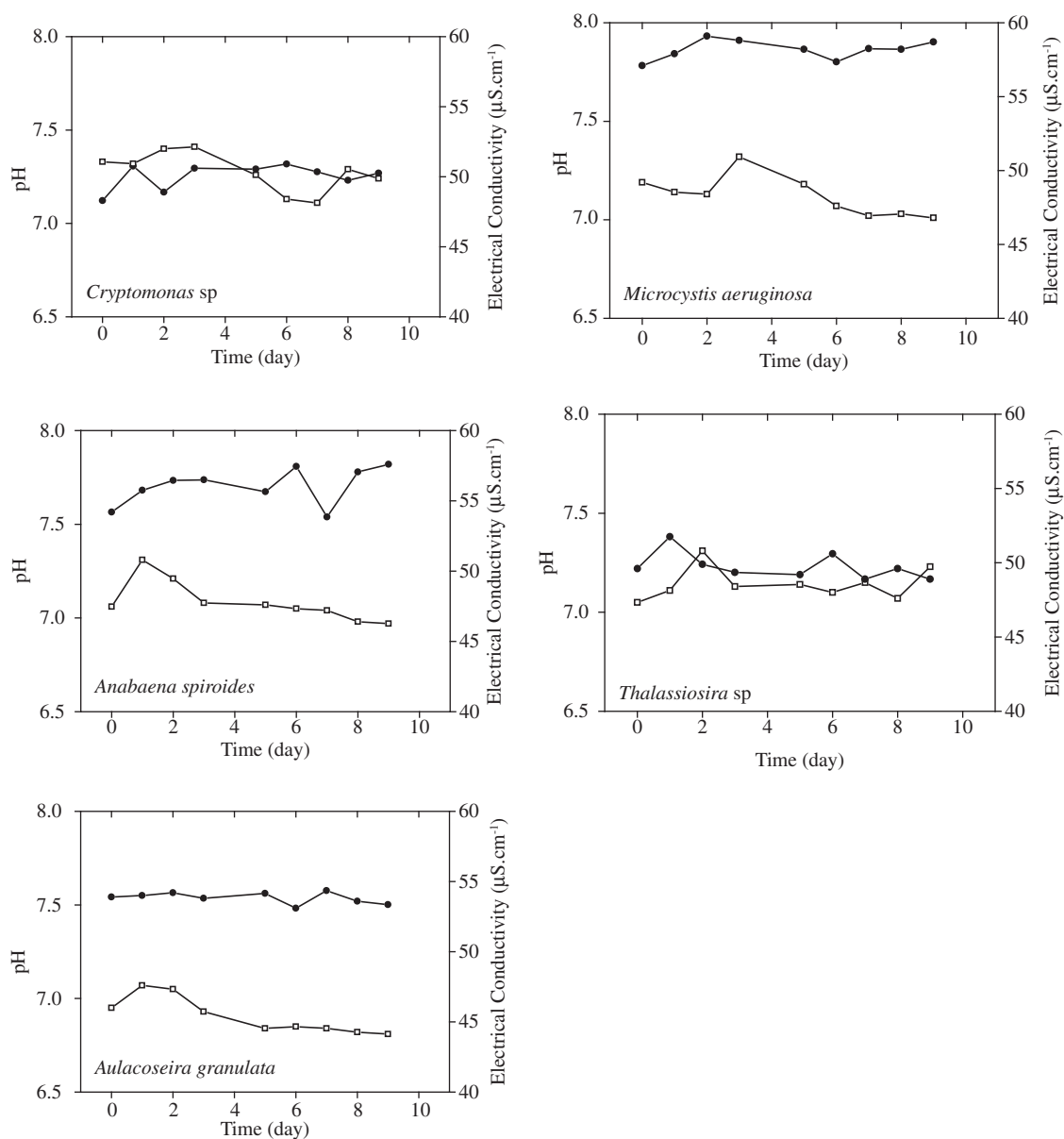
(Bianchini Jr. et al., 2006). The mineralization process tended to have an accentuated consumption of the dissolved oxygen in the beginning of the process, followed by a period tending to stabilization of oxygen consumption. After this phase, the decay was less intense, probably due to i) the remaining refractory compound derived from the cellular walls, which are composed of cellulose impregnated with pectin (Brook, 1981) that gives to the algae detritus more resistance to bacterial attacks (refractory fraction) and ii) the utilization of the nutrients in the first stage, limiting the growth of microorganisms and

turning the decomposition a slow process. Considering that the algal detritus is a heterogeneous source of organic matter presenting labile and refractory compounds, the oxygen uptake was probably related to the labile fractions. Hence differences in oxygen consumption were related to the chemical composition of the plant material, presenting a positive relation with higher concentrations of nitrogen and lower content of cellulose (Almazan and Boyd, 1978).

Experiments of the aerobic decomposition of cells of *Staurastrum iversenii* under different temperatures

**Table 1.** Parameterization of the kinetic model during *Cryptomonas* sp., *Microcystis aeruginosa*, *Anabaena spiroides*, *Thalassiosira* sp and *Aulacoseira granulata* mineralization process:  $OC_{max}$  = oxygen consumption;  $k_D$  = DO consumption coefficient;  $t_{1/2}$ : DO consumption half-time;  $r^2$  = determination coefficient and error = error referred to the kinetics fittings.

Specie/Genera	$OC_{max}$ ( $mg.g\ C^{-1}$ )	Error	$K_D$ (per day)	Error	$t_{1/2}$	$r^2$
<i>Cryptomonas</i> sp.	169	3	0.1911	0.0075	4	0.99
<i>Microcystis aeruginosa</i>	68	4	0.2099	0.0291	3	0.98
<i>Anabaena spiroides</i>	345	112	0.0545	0.0220	13	0.99
<i>Thalassiosira</i> sp.	156	20	0.1575	0.0370	4	0.98
<i>Aulacoseira granulata</i>	43	4	0.3785	0.1086	2	0.91



**Figure 2.** Temporal variation of pH and electrical conductivity values during the aerobic mineralization of the algae incubations: pH (□) and electrical conductivity (●).

presented differences in the values of  $OC_{max}$  (Table 2). The decay verified in the incubation with glucose and water from Barra Bonita reservoir produced an  $OC_{max}$  of 496  $mg.g^{-1} C$  (Panhota and Bianchini Jr., 2003). In comparison with glucose, the algae species used in the present experiment showed a low  $OC$ , indicating a greater refractability of the cell materials. Considering as 26% the average of phytoplankton carbon content (Platt and Irwin, 1973), an experiment of oxygen consumption with five species of algae (Almazan and Boyd, 1978) showed  $OC_{max}$  varying from 106  $mg.g^{-1} C$  (*Euglena proxima*) to 633  $mg.g^{-1} C$  (*Anabaena circinalis*; Table 2).

Aerobic decomposition differs in the Barra Bonita Reservoir according to the type of algae predominating. This aquatic system presented its dissolved oxygen budget most affected by *Aulacoseira granulata*, followed by *Microcystis aeruginosa*, *Thalassiosira* sp., *Cryptomonas* sp. and *Anabaena spiroides*. Depending on the species, biomass and composition of a phytoplankton bloom, the oxygen demand during its die-off will regulate the oxygen availability in the water column. Species presenting higher content of recalcitrant material will contribute with benthic oxygen demand once that debris tends to accumulate on the sediment surface.

In our experiments, the values of the deoxygenation coefficients ( $k_d$ : 0.055 to 0.379/day) for the aerobic mineralization of algae presented great variations among the species studied. The oxygen uptake during the decomposition of *Aulacoseira granulata* was ca. 7 times faster than for *Anabaena spiroides* decomposition. According to Hartemink and O'Sullivan (2001), slow decomposition is mainly caused by high C/N ratio. More organic cell surface favors the biofilm development, with a faster decomposition in these cases than on inorganic cell surface. Other organic substrates showed the following  $k_d$  values: 0.39/day (tannic acid, Cunha-Santino et al., 2002); 0.11/day (leaves), 0.52/day (branches), 0.36/day (barks) and 0.11/day (litter;

Antonio et al., 1999); 0.016/day (glucose), 0.025/day (sucrose), 0.050/day (starch) and 0.048/day (lysine; Cunha-Santino and Bianchini Jr., 2003b).

Considering the microbial loop of the aquatic systems, the effect of the microbial decay on the aerobic regulation of the dissolved organic matter concentrations is directly related with the  $k_D$ . Mineralization processes presenting higher  $k_D$  (i.e. short half-time) have a great potential for biodegradability. Due to the higher  $k_D$ , these fractions do not tend to accumulate in the ecosystem. In opposition, the fractions with lower  $k_D$  probably remain unaffected for longer periods, and consequently, are present at the highest concentrations, tending to accumulate in sediments. Hence, the organic matter generated at Barra Bonita reservoir and its  $k_D$  acts directly on the quality and quantity of the carbon cycle in this system. Another fact related to the potentiality of the biodegradability is the indigenous microbiota of the reservoir water. The adaptability of these organisms to the immobilization and mineralization will also affect the concentrations of the carbon compounds in the aquatic environment.

Owing to the algae chemical composition and their specific mineralization rates, the effects on acidification and release of ionic species in the water column are distinct among *Aulacoseira granulata*, *Microcystis aeruginosa*, *Thalassiosira* sp., *Cryptomonas* sp. and *Anabaena spiroides* biomass decomposition. It is possible to infer that some species release more H protons than others. In addition, the decomposition releases  $CO_2$  as end product, thus producing carbonic acid in aqueous solutions. The acidification can cause a change in the microbial community. The acidification tendency occurred probably by the low quantities of humic substances formed due to the algae mineralization. In addition, buffer capacity of humic substances is mainly formed by polyphenols (Stevenson, 1982); the lack of these compounds in phytoplankton cells does not promote an effi-

**Table 2.** Maximum oxygen consumption ( $OC_{max}$ ) reported in the literature for algae.

Species	$OC_{max}$ ( $mg.g^{-1} C^{-1}$ )	Citation
<i>Staurastrum iversenni</i> – 18 °C	124	Pacobahyba (2002)
<i>Staurastrum iversenni</i> – 21 °C	118	Pacobahyba (2002)
<i>Staurastrum iversenni</i> – 25 °C	121	Pacobahyba (2002)
<i>Staurastrum iversenni</i> – 27 °C	198	Pacobahyba (2002)
<i>Euglena proxima</i>	106	Almazan and Boyd (1978)
<i>Anabaena circinalis</i>	633	Almazan and Boyd (1978)
<i>Spirogyra</i> sp.	201	Almazan and Boyd (1978)
<i>Pithophora kewensis</i>	89	Almazan and Boyd (1978)
<i>Chara braunii</i>	116	Almazan and Boyd (1978)
<i>Cryptomonas</i> sp.	169	This study
<i>Microcystis aeruginosa</i>	68	This study
<i>Anabaena spiroides</i>	345	This study
<i>Thalassiosira</i> sp.	156	This study
<i>Aulacoseira granulata</i>	43	This study

cient buffer system. The regular liberation of ions, represented by the EC values, can support phytoplankton and macrophyte growth, increasing the primary production in eutrophic systems through the regeneration of nitrogen and phosphorous (Andersen and Jensen, 1992).

This study demonstrates how the selected algae (the main species of local phytoplankton regarding to biomass) can affect, in a short time, the oxygen availability in the water column in Barra Bonita Reservoir, owing to the aerobic decay during a die-off event. It also indicates the possibility of the increase in electrical conductivity and pH values in the water column due mainly to the aerobic algae mineralization.

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