The mineralization kinetics of Staurastrum iversenii Nygaard var. americanum.

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ABSTRACT: The mineralization kinetics of Staurastrum iversenii Nygaard var. americanum. The aim of this study was to describe and discuss kinetic aspects of the mineralization of ruptured Staurastrum iversenii var. americanum cells. This alga was isolated from the Barra Bonita Reservoir (SP; 22° 29' S and 48° 34' W), and cultivated in the laboratory. After 60 days, the cells were ruptured and used to set up decomposition chambers that were incubated under low and high dissolved oxygen concentrations and at four temperatures. The organic carbon concentrations (dissolved and consumed) were fitted to first order kinetic models. The coefficients varied between 0.015 and 0.075 day^{-1} for the decay of dissolved organic carbon and from 0.086 to 0.734 day^{-1} for the carbon mineralization. On average, the mineralization that developed under low dissolved oxygen concentration was more affected by temperature (Q_{10} = 2.51).
Key-words: decomposition, mineralization, organic carbon, Staurastrum iversenii var. americanum.

RESUMO: Cinética da mineralização de Staurastrum iversenii Nygaard var. americanum. Neste estudo visou-se estabelecer a cinética da mineralização de células rompidas de Staurastrum iversenii var. americanum e discutir os efeitos de alguns fatores abióticos sobre este processo. Esta alga foi isolada do reservatório de Barra Bonita (SP; 22° 29' S e 48° 34' W) e cultivada em laboratório. Após 60 dias, as células foram rompidas e utilizadas na montagem de câmaras de decomposição, que foram incubadas sob baixas e altas concentrações de oxigênio dissolvido e em diferentes temperaturas. As variações temporais das concentrações de carbono orgânico (dissolvido e consumido) foram ajustadas a modelos cinéticos de primeira ordem. Os coeficientes variaram de 0,015 até 0,075 dia^{-1} para o decaimento do carbono orgânico dissolvido e de 0,086 até 0,734 dia^{-1} para a mineralização do carbono. Em média, as mineralizações desenvolvidas sob baixas concentrações de oxigênio dissolvido foram mais sensíveis à temperatura (Q_{10} = 2,51).
Palavras-chave: decomposição, mineralização, carbono orgânico, Staurastrum iversenii var. americanum.

Introduction

The autochthonous organic matter is one of the main sources of detritus for aquatic ecosystems (Rooney & Kalff, 2000). The organic carbon detritus results from non-predatory loss of biomass coming from any trophic level (including excretion, secretion, etc.) or from the external input of organic carbon into the ecosystem (Wetzel, 1990). In aquatic environments, heterotrophic bacteria are the main consumers and re-mineralizing agents of the dissolved organic matter (DOM) and constitute a fundamental community for the operation of biochemical cycles (Raymond & Bauer, 2001). The interactions between the DOM and the bacteria constitute one of the main factors in the regulation of the dissolved

organic compounds concentrations in aquatic ecosystems. The availability of DOM for heterotrophic bacteria depends on its chemical composition and molecular mass, on the concentrations of nutrients of the detritus, as well on other environmental factors such as temperature (Mendelssohn et al., 1999). The decay of algae cells contributes to the enrichment of nutrients and organic matter in the water column. Besides the regeneration of nutrients, this process makes other substances available and drives the trophic transfer of carbon detritus through microbial loop (Mann, 1988).

Generally, the Division Chlorophyta is the one represented by the largest number of phytoplankton species, and the Order Zygnematales is the largest group of green algae with regard to the number of species. These algae are predominant in fresh water, rare species being found in salt water (Parra & Bicudo, 1995). Even a difference of 6ºC in temperature between winter and summer periods has influence on growth (Jati, 1998), as example of a factor that influences the growth of planktonic community in Barra Bonita Reservoir. However, underwater radiation has an influence due to the increase in suspended particulate material, mainly during the summer (Jati, 1998).

The genus Staurastrum is represented by five species in the Barra Bonita Reservoir, comprising 10% of the phytoplankton community (Jati, 1998). The importance of this genus in this environment is due to their high density and relative abundance, mainly in the winter period. In this context, this paper has attempted to establish the mineralizing kinetics of ruptured Staurastrum iversenii var. americanum cells and to discuss the effects of some abiotic factors upon this process and on the cycling of this resource in the reservoir.

Materials and methods

Barra Bonita Reservoir was built in 1963, and is located in the central area of the state of São Paulo (22º 29' S and 48º 34' W), in the basin of Middle Tietê River. The morphometric characteristics and hydraulic regime of this reservoir were well described by Tundisi & Matsumura-Tundisi (1990).

Staurastrum iversenii Nygaard var. americanum selected for this study belongs to the Zygnematophyceae. This species is maintained in the phytoplankton culture collection of Universidade Federal de São Carlos. S. iversenii var. americanum axenic cultures were maintained in 8 liter glass bottles containing WC medium (Guillard & Lorenzen, 1972) under continuous aeration, with temperature ranging between 20 and 21ºC, illumination of 4 klux, at 12/12h light/dark photo-periods. From 60 day old cultures, the cells were separated from the medium by centrifugation (352 g; 30 min). For removal of adhered polysaccharides, the cells were washed with WC medium (without phosphate and nitrate), at a temperature of 50ºC (Paulsen & Vieira, 1994). The lysis of cell was induced with an ultra-sound device, with ferrule having amplitude of 30㎜ (MG brand, P-100 model) during 30 min. The rupture of cells was guaranteed by microscopic observations.

The mineralization experiment was conducted under low (LDO) and high dissolved oxygen (HDO) concentration at four temperatures: 17.7ºC (±1.2); 20.3ºC (±1.1); 22.4ºC (±1.3) and 27.0ºC (±1.3). For each temperature, 10 decomposition chambers were set up containing 400 ml of the Barra Bonita Reservoir water (previously filtered in glass wool) and the mixtures of ruptured cells (initial concentration of organic carbon: 52.02 mg/l). The LDO conditions (average concentration of dissolved oxygen: 1.50 ± 0.26 mg/l) of the incubations were obtained and maintained by bubbling nitrogen during 10 min at the beginning of the experiment and on sampling days. The HDO conditions (average concentration of dissolved oxygen: 6.93 ± 1.04 mg/l) were maintained by aeration (by bubbling clear air during c.a. 1 h) at the beginning of the experiment and when the solutions showed concentrations of dissolved oxygen below 2 mg/l. All of the chambers were covered with aluminum foil. On sampling day (0, 1, 3, 5, 10, 20, 30, 60, 90, and 120), for each temperature and oxygen availability, one decomposition chamber was deactivated and the concentrations of organic carbon determined by high temperature catalytic oxidation method using a TOC analyzer (Shimadzu - TOC-5000A). By successive carbon quantification and filtration of the samples

through ester cellulose membranes (Millipore - 0.22 mm pore size), the estimates of particulate (POC) and dissolved organic carbon (DOC) concentrations were obtained.

Considering the decomposition chambers as closed systems, in relation to the organic carbon (Bianchini Jr. et al., 2002), it was possible to estimate the amounts of mineralized carbon. The concentrations of consumed organic carbon were determined from the differences between the initial organic carbon values and the remainders in different forms (Equation 1).

$$COC = TOC - (POC + DOC) \qquad (1),$$

where: TOC = total amount of organic carbon added at the beginning of the experiment; POC = particulate organic carbon (detritus + bacterioplankton); DOC = dissolved organic carbon (x £ 0.22 mm) and COC = consumed organic carbon (mineralized).

To determine the decomposition reaction coefficients of the S. iversenii var. americanum ruptured cells, the temporal variations for DOC and COC were fitted to first order kinetic models (Jewell & McCarty, 1971; Fallon & Brock, 1979), Equations 2 and 3. The regressions were obtained by non-linear method (the Levenberg-Marquardt iterative algorithm) (Press et al., 1993). From the differences between the initial and final DOC concentrations, the conversion coefficients for these compounds were calculated. The COC conversion coefficients and their respective standard derivation were obtained from the regressions.

$$DOC_t = DOC_0 \times e^{-k_d t} \qquad (2),$$

where: DOC_t = DOC concentration in function of the time; DOC_0 = initial DOC concentration; e = natural logarithm base; k_d = decay coefficient (day^{-1}) and t = time (day).

$$COC_t = COC_{max} \times (1 - e^{-k_M t}) \qquad (3),$$

where: COC_t = amount of COC in function of the time; COC_{max} = maximum amount of COC and k_M = mineralization coefficient (day^{-1}).

Results and discussion

The concentrations of DOC from S. iversenii var. americanum detritus decreased faster during the first days of incubations (Fig. 1), suggesting that during this period a prevalence of labile fraction consumption. The high decrease in the DOC concentrations at the initial stages of decomposition can also be attributed to the cell rupture process that altered the quality of this resource, being more homogeneous and susceptible to microorganism hydrolysis. This fraction is probably constituted by protoplasmic content e.g. ammonium and phosphate (Pieczynska & Tarmanowska, 1996), fatty acids, chlorophyll, triacylglycerols (Afi et al., 1996) and hydrocarbons (Krog et al., 1986) released on the decomposing media. From 11 to 43% was found to be lost within 24 h; the leached from algae were rapidly utilized by bacteria and did not accumulate in the water column (Hansen et al., 1986). Krog et al. (1986) also showed high affinity of microorganisms to intermediate sized organic molecules (700 to 10.000 Daltons). Decomposition experiments showed that the fate of DOC presented at least three possible pathways: (i) conversion into in microbial tissues, (ii) formation of refractory compounds and (iii) conversion into inorganic compounds (Antonio & Bianchini Jr., 2003).

After this phase, the decay was less intense, probably due to (i) the remaining of 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 as consequence the decomposition became a slow process.

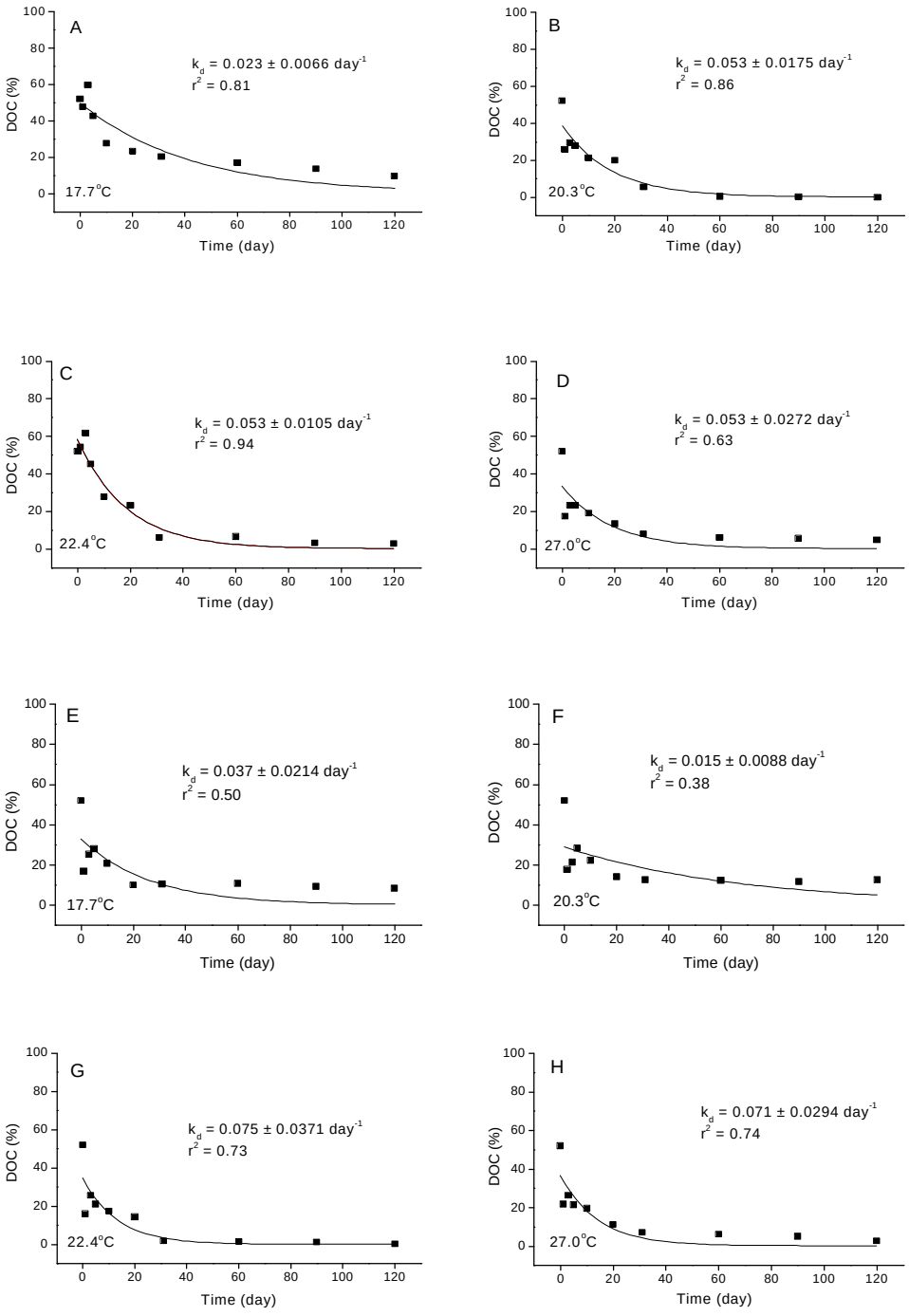

Figure 1: Kinetic fittings of DOC decay under high dissolved oxygen condition (A, B, C and D) and low dissolved oxygen condition (E, F, G and H).

The pattern of two stages of decay was also described by Otsuki & Hanya (1972), that demonstrated during Scenedesmus sp decomposition that algal cell content may be divided in two constituents (labile and refractory) according to resistance to bacterial action. Jewell & McCarty (1971) also observed a refractory fraction that remained undecomposed for periods up to 700 days. This pattern was also verified for the decomposition of Chlorella salina at 30 °C, in which the first fraction (labile) decayed in a few hours, and the second fraction could take years to decompose (Chan, 1985).

During the decomposition of 14 phytoplankton species, Gunnison & Alexander (1975) observed that Straurastrum sp. was moderately resistant to microbial hydrolysis; an explanation for this resistance was that the excretion of (extracellular, intracellular or attaches at cell wall) toxins constituted an inhibitory to potential lytic of microorganisms. The authors also observed that the walls of this genus did not appear to be attacked, even after 40 days of incubations. Concerning to these observations during S. iversenii var. americanum cells decomposition, the decay of labile and refractory fraction will be processed within the sediment.

Low DOC content (zero and 0.19%) was observed at the end of experiments for HDO (at 20.3°C) and LDO conditions (22.4°C), respectively. For HDO condition, the lowest value of DOC decay coefficient (k_d) was 0.023 ± 0.006 day^{-1} (at 17.7°C); the highest value (0.075 ± 0.038 day^{-1}) occurred at 22.4 °C, under LDO condition (Fig. 1). The half-time ($t_{1/2}$) of these coefficients were 30 and 9 days, respectively. A low value for the aerobic DOC decay coefficient (0.0156 day^{-1}) of other Chlorophyceae (Scenedesmus sp) was also registered (Otsuki & Hanya, 1972). Studying the anaerobic degradation of algae at 20°C, Foree & McCarty (1970) obtained mean values of k_d = 0.022 day^{-1} (SD = 0.007 day^{-1}; n = 16). For the mineralization of cultures of young algae, under aerobic conditions, Jewell & McCarty (1971) estimated coefficients with lower values (ranging from 0.01 to 0.06 day^{-1}); for old cultures theses values varied between 0.01 to 0.03 day^{-1}. On average, in the experiment with S. iversenii var. americanum cells the k_d values (0.047 day^{-1}; $t_{1/2}$ = 15 days) were higher than those obtained by Jewell & McCarty (1971) for old cultures decay and than that registered by Foree & McCarty (1970) for anaerobic degradation of algae. Overall, the differences in these coefficients could be a result from: (i) variations on chemical structure of the cell walls and protoplasmatic contents (e.g. quantity and quality of lipids, carbohydrates and proteins) of algae species, (ii) the ability of microorganisms to compete for nutrients and carbon sources, (iii) the microbial ability to adherence on algae cell walls and (iv) the effects of allelopathic compounds, released by algae cells or by the formation of intermediate metabolites.

Regardless of the temperature and the availability of oxygen, the mineralization was fast on the first days, and after the 20th day a stabilization of the processes was noticed. The highest k_M (0.735 day^{-1}; $t_{1/2}$ = 1 day) was estimated for the LDO condition at 27.0°C, while the lowest (0.086 day^{-1}; $t_{1/2}$ = 8 days) was detected for the HDO condition at 20.3°C (Fig. 2). On average, the k_M obtained for LDO condition was 3 times higher than for HDO condition. These differences probably referred the ability and adaptability of the microorganisms to the incubations conditions (e.g. dissolved oxygen and nutrients availability).

According to the compilation made by Bianchini Jr. (1999), mineralization coefficients varying between 0.027 to 0.094 day^{-1} were registered for exuded Ankistrodesmus densus. During the aerobic mineralization of COD and carbohydrates leachated from a mixed sample of phytoplankton, the k_M varied from 0.81 to 0.016 day^{-1} and from 1.24 to 0.047 day^{-1}, respectively. According to the experiments on glucose decay (Antonio & Bianchini Jr., 2003), higher decomposition coefficients were registered for both the aerobic (k_M = 0.309 day^{-1}) and the anaerobic condition (k_M = 0.144 day^{-1}), demonstrating that the decomposition of this monosaccharide was faster than observed for S. iversenii var. americanum cells in HDO conditions.

Carbon consumption (mineralized) during LDO condition was faster, since the k values were higher than those estimated for HDO condition. It is evident that dissolved oxygen did not favor the decay of S. iversenii var. americanum cells. However, the mean

conversion yields for COC was higher in the HDO condition (88.6%). These results suggest that the decay under high oxygen availability condition favored the formation of CO_2, in detriment to intermediary compounds (e.g. re-synthesized products). On the other hand, the LDO condition was faster and generated more remaining compounds (19.5%).

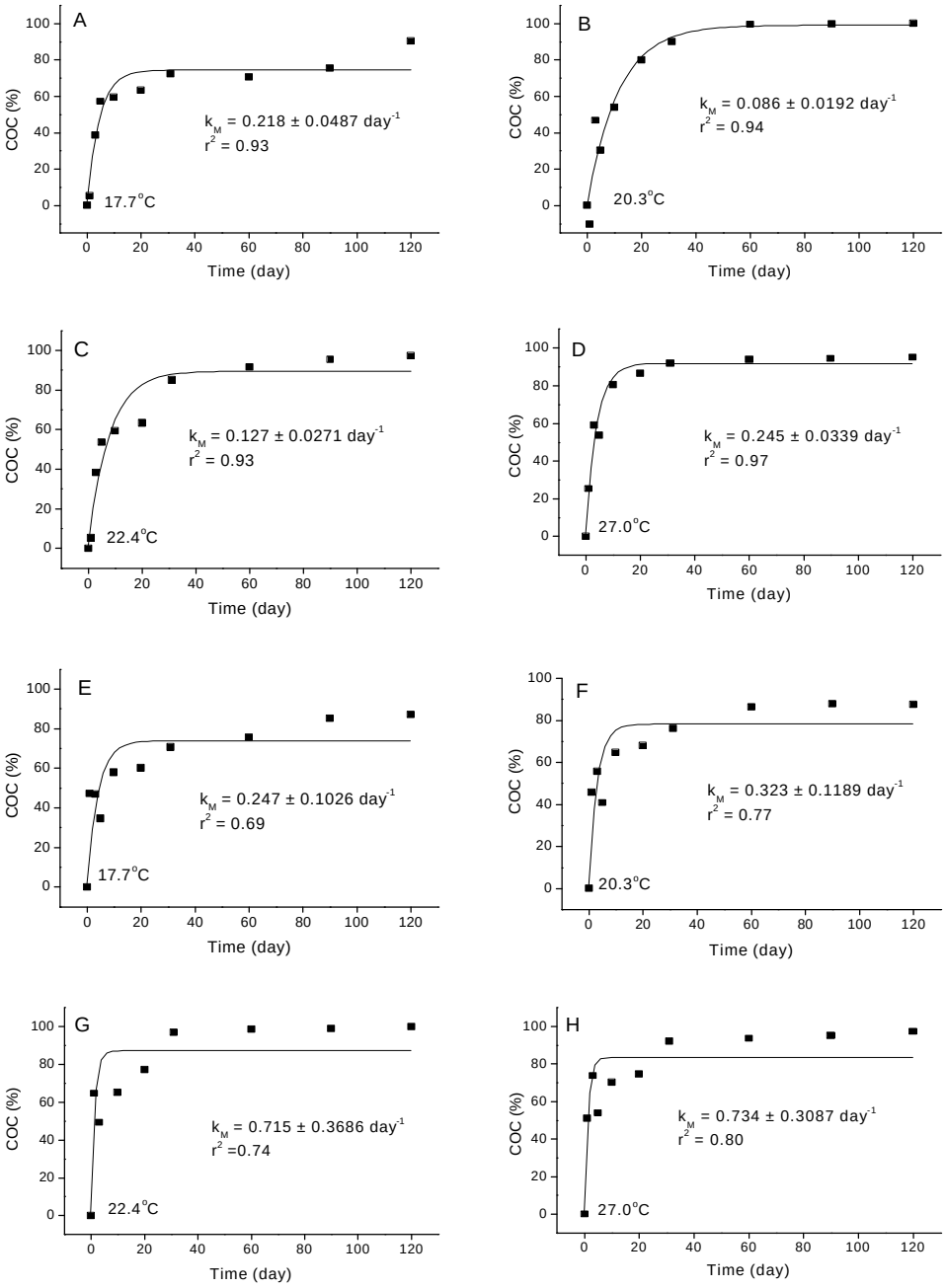

Figure 2: Kinetic fittings of the global consumption of carbon under high dissolved oxygen condition (A, B, C and D) and low dissolved oxygen condition (E, F, G and H).

DOC conversion by microorganisms, under both conditions, ranged from 80 to 99% (Fig. 3B and D). COC formation varied between 73 and 99% (Figures 4B and D). Antonio & Bianchini Jr. (2003) calculated that the yield of glucose respiration was 19.4%; this value was lower than the yield of DOC conversion and COC formation obtained in the present study. The COC formation during degradation of Synechococcus sp, Dunaliella sp and Cylindrotheca fusiformis were respectively: 60, 40 and 58% (Biddanda, 1988). The COC yields for Microcystis sp incubations with low-alkalinity waters were 68% and for acid waters 40%; these results for Chlamydomonas reinhardi were 60 and 20% (Schoenberg et al., 1990).

It is possible to verify that the highest values for carbon mineralization were obtained under HDO conditions (Fig. 4B and D). However, the percentages registered for the LDO process were slightly lower. Based on these results, it is noticed that the temperature directly influenced the reaction coefficients (k_M and k_d) as the carbon conversion rate. These results suggest that the optimum temperature range for carbon decomposition (in the Barra Bonita Reservoir) is between 20 and 24°C.

When comparing the DOC decay coefficients and those of COC formation (mineralization), it is noticed that the dissolved fraction decay processes were slower than those for the mineralization of the entire resource, since the DOC decay coefficients were lower (for both the LDO and HDO conditions). These results suggest that the DOC was slowly being transformed into refracting substances, while the POC fractions (particulate detritus and microorganisms biomass) were relatively less refractory and supported the respiratory processes. This event was also registered by Mills & Alexander (1974) during Asterococcus superbus decomposition. Generally, based on the determination coefficients, it can be seen that the selected models are satisfactory representations of COD and COC variations. The model used to represent the kinetics of mineralization was more appropriate (r^2: between 0.69 and 0.97) than the one employed to describe the COD decay rates (r^2: between 0.38 and 0.86).

The mineralization coefficients, a function of temperature, were used to determine the Q_{10} values (Fig. 3 and 4). From these parameters it was possible to observe that the decay of ruptured S. iversenii var. americanum cells was more affected by temperature under LDO conditions (2.43 and 2.60), than under HDO conditions (1.59 and 1.64).

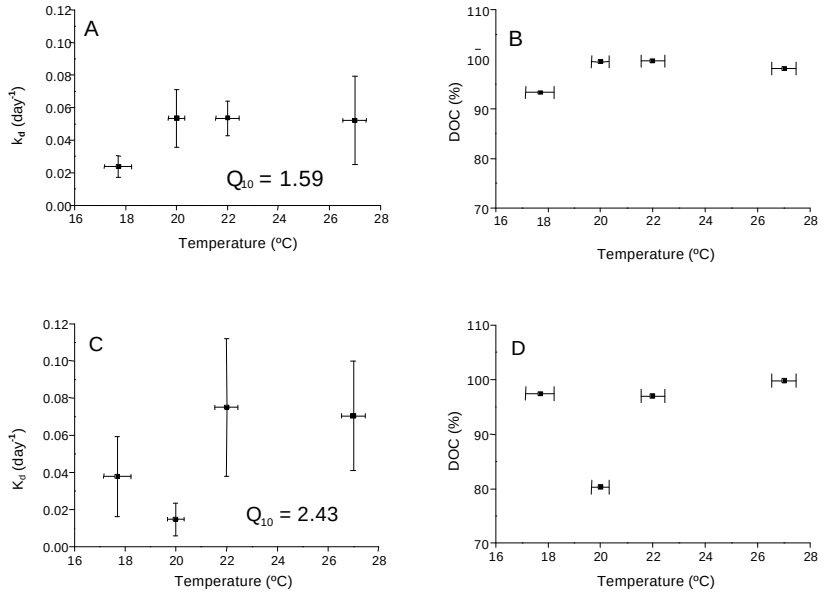

Figure 3: Decay coefficients of DOC (A and C) yield of dissolved organic carbon (B and D) in function of temperature and experimental conditions (high dissolved oxygen condition: A and B; low dissolved oxygen condition: C and D).

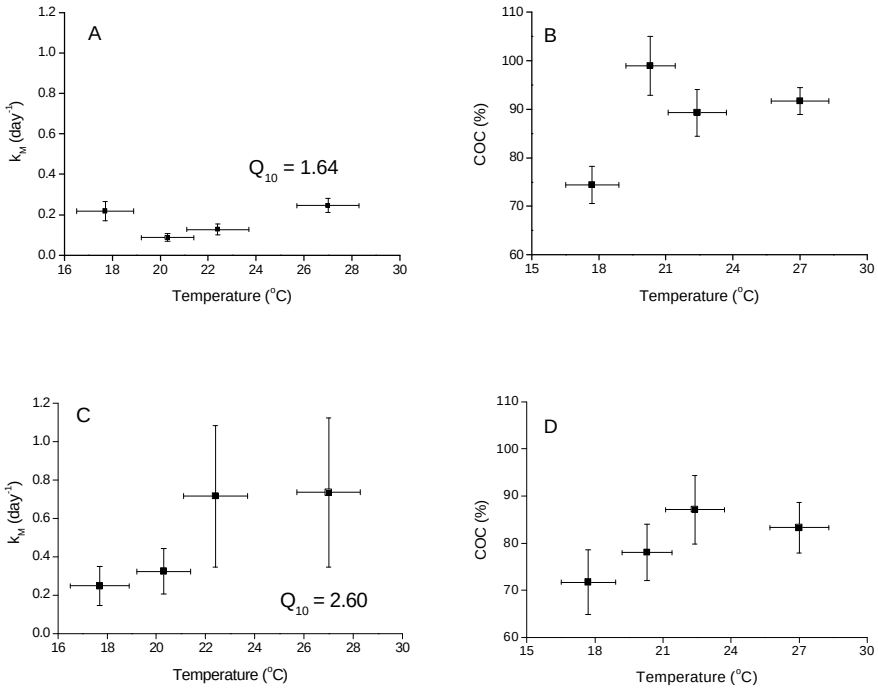

Figure 4: Decay coefficients and mineralization percentage of DOC in function of temperature and experimental conditions (high dissolved oxygen condition: A and B; low dissolved oxygen condition: C and D).

The Q_{10} values did not depend on the oxygen availability, suggesting the participation of a similar bacterial community in the two processes (DOC decay and COC formation). These results indicate that given a 10°C increase in temperature, the DOC cycling coefficient will be 1.53 times higher for the LDO condition. For the mineralization of organic carbon, the community adapted to low oxygen availability also showed to be more sensitive (58%). Such results further suggest that the facultative aerobic organisms of the Barra Bonita Reservoir are more sensitive to the effects of temperature than aerobic organisms; consequently they are more efficient in mineralization process (Fig. 4C and D).

According to methodological procedures adopted, we concluded that: i) the decomposition process of the ruptured of S. iversenii var. americanum cells was relatively faster than the one registered for other species of algae. This fact may be related to the mechanical cell rupture procedure that favored the release of the labile fraction and the bacterial contact surface on the refractory fractions; ii) the mineralization process under HDO conditions was slower than LDO condition, but tended to be more efficient in resource conversion; iii) the Q_{10} values were higher in the LDO condition, suggesting that in the Barra Bonita Reservoir the community of alternate anaerobic and/or aerobic organisms is more sensitive to temperature variations than the strictly aerobic community.

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