

Potential cycling of organic matter in a eutrophic reservoir (Barra Bonita, SP - Brazil).

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ABSTRACT: Potential cycling of organic matter in a eutrophic reservoir (Barra Bonita – SP). **Water samples were collected in the Barra Bonita Reservoir - SP (22°32'34.5" S and 48°29'26.4" W) in two distinct periods (February/01 and July/01), filtered and enriched with glucose. After that, the samples were incubated at 20°C for 60 days, under aerobic and anaerobic conditions. During this period, aliquots were collected from the mineralization flasks to determine the organic carbon concentration (dissolved: DOC and particulate: POC). The global decay coefficient and the coefficients of the three routes admitted by the glucose mineralization model were determined by kinetics fitting. In February (rainy season), the mineralization coefficients were higher (c.a.10 times) than the mineralization coefficients of July (dry season). Considering the metabolism, in these two periods, the anaerobic one showed higher mineralization coefficients (1.6 times higher in February and 2.0 times higher in July), than the aerobic one. On the other hand, the carbon immobilization (POC formation) was higher in the aerobic metabolism, in the two periods.**

Key-words: Heterotrophic potential, decay kinetics, dissolved organic carbon (DOC), particulate organic carbon (POC).

RESUMO: Potencial de ciclagem de matéria orgânica em um reservatório eutrófico (Barra Bonita, SP -Brasil). **Amostras de água coletadas no reservatório de Barra Bonita - SP (22°32'34,5" S e 48°29'26,4" W) em dois períodos distintos (fevereiro/01 e julho/01) foram filtradas e enriquecidas com glicose. Em seguida foram incubadas a 20°C, durante sessenta dias, em condições aeróbias e anaeróbias. Durante esse período, alíquotas foram retiradas dos frascos de mineralização para se determinar as concentrações de carbono orgânico (dissolvido: COD e particulado: COP). Através de ajustes cinéticos foram determinados os coeficientes globais de decaimento e os coeficientes de mineralização das três rotas admitidas. Em fevereiro (período de maior precipitação pluviométrica) os coeficientes de mineralização foram superiores (@ 10 vezes) aos obtidos em julho (período de menor precipitação). Com relação ao metabolismo, nas duas oportunidades, o anaeróbio apresentou coeficientes superiores de mineralização (cerca de 1,6 em fevereiro e 2,0 em julho). Em contrapartida a imobilização de carbono em biomassa de bactérias (formação de COP) foi maior (@12 %) no metabolismo aeróbio, nos dois períodos.**

Palavras-chave: potencial de heterotrofia, cinética de decaimento, carbono orgânico dissolvido (COD), carbono orgânico particulado (COP).

Introduction

Microorganisms play a fundamental role in energy transference and cycling of the organic matter in aquatic ecosystems, since major part of carbon in these environments flows through the metabolism of bacteria and fungi (Pomeroy et al., 1988).

The largest fraction of organic matter in aquatic ecosystems occurs in the form of dissolved organic matter (DOM). A comparison between the organic fractions DOM and POM (particulate organic matter) gives relationships ranging from 6 to 10:1 (Wetzel, 1990). Only a small fraction (<5%) of DOM can be assimilated directly by bacteria (Ammerman et

al., 1984). The largest fraction of the dissolved organic compounds consists in substances with high molecular weight (Chróst, 1981; 1986) such as, proteins, polysaccharides, nucleic acid and humic substances that are not susceptible to microbial attack (Münster, 1984; 1985).

In the aquatic environment the concentration of dissolved oxygen is an important factor in the selection of microorganisms involved with the degradation processes. With respect to the characteristics of detritus processing it has been observed that aerobic degradation support a wide spectrum of compounds (Davis & Cornwell, 1991; Bianchini Jr., 1999a) generating stable final products (CO₂ and H₂O).

Considering carbohydrate uptake, the anaerobic decomposition can be accomplished by different metabolic routes: as glycolysis, Entner-Doudoroff pathway and phosphoketolase pathway (Stanier et al., 1976), that converge to a common product, the piruvate. This molecule formed during the catabolism of sugar is metabolized into specific metabolic pathways characteristic of each species, and gives different final products such as: lactate, ethanol, propionate, isopropanol, butanol, acetate and CO₂ (Lynch & Hobbie, 1988).

In relation to the anaerobic decomposition, inorganic compounds can be used as final acceptors of electrons and generate different final products as NH₃ and H₂S. In that specific metabolism type, bacteria are generally strictly anaerobic. During fermentation, the final acceptors of electrons are organic substances and produce as final products CH₄ and CO₂.

Aspects related to the energy flow and cycling of matter through the bacterial metabolism have been studied, in detriment of the structural aspects of the microorganisms community as identification and succession of species (Thomaz, 1999). In the last decades modeling studies have been developed and their applications show the advantages of the mathematical models as a tool in the ecological studies (Jørgensen, 1994). The models are useful for observation of complex systems, to describe their properties and behavior variations within time, which includes seasonal changes and anthropogenic perturbation (Koch et al., 1998).

The ecological models are important tools in the management and restoration of ecosystems. Models supply information about the synergistic effects among biological, physical and chemical processes in the aquatic systems. Such studies can reveal the relationship among ecosystems, and show how a specific metabolic process (e. g. decomposition) can be affected by the environmental variations (Antonio & Bianchini Jr., in press). The models used to describe the decomposition process are usually based in exponential equation (Weider & Lang, 1982), from which the mass loss rates are established.

This study aimed at develop a model of the heterotrophic potential (using glucose as substratum) of a eutrophic aquatic system, in function of the availability of dissolved oxygen and limnological variables in two distinct periods (summer and winter).

Material and Methods

The Barra Bonita Reservoir is located in the basin of Middle Tietê river at an altitude of 430 m, in the central area of the São Paulo State, between the municipal districts of Barra Bonita and Iguaraçu (22° 29' to 22° 44'S and 48°10'W). The reservoir is sited in the most populous and developed areas of Brazil; it is a transition area between the tropical and subtropical climates, in which the annual climatic stations are not well defined. The seasonal changes are not very pronounced, being more evident difference verified in the summer (rainy season) and winter (dry season). The Barra Bonita Reservoir is a polymittic, eutrophic ecosystem, in which the seasonal cycles of the limnological events seem to be dominated by precipitation, wind, outflow and residence time varying from 1 to 6 months (Tundisi & Matsumura-Tundisi, 1990). During the rainy season, great input of nutrients occurs (Henry et al., 1985; Calijuri, 1988; 1999).

The water samples were collected from 3.0 km of the dam (22° 32' 34,5" S and 48° 29' 26,4" W), in the pelagic area, in two distinct periods: 17/February/2001 (high precipitation period/summer) and 09/July/2001 (low precipitation period/winter), using a

Van Dorn underwater sampler. The water sample was integrated; equivalent volumes of aliquots from surface, middle and bottom water were mixed and filtered in glass wool. Some physical and chemical variables (pH, temperature, dissolved oxygen, turbidity, conductivity electric, redox potential) were obtained with a multi-sensor Horiba U-23. The depth measure spacing was at 2-m intervals and was determined with depth sounding lines with platelike weights. The euphotic zone (Z_{eu}) was estimated through the depth of disappearance of the disk of Secchi (Z_{Ds}), multiplying the value of Z_{Ds} by 2.7 (Margalef, 1983).

In laboratory, the concentrations of total phosphorous, total organic nitrogen, ammonium, nitrate and nitrite of samples of water ($n = 2$), utilized in the mineralization experiments were measured using the analytical procedures described in APHA (1985), Golterman et al. (1978), Koroleff (1976), Mackereth & Talling (1978) and Golterman et al. (1978), respectively. Samples of water were previously filtered in glass fiber membrane (1.2 mm, Whatman) and enriched with glucose (solutions of 50 mg l⁻¹ = 20 mg l⁻¹ of DOC). The solutions were incubated in the darkness, under aerobic ($n=3$) and anaerobic ($n=3$) conditions, in a temperature of 19.9 ± 2.6°C, for 60 days. For each condition reference flasks ($n=2$) were also prepared with samples of filtered water of the reservoir (without glucose).

In the aerobic condition, the solutions were maintained under concentrations of dissolved oxygen (DO) superior than 2.0 mg l⁻¹, by periodic bubbling with compressed air; the anaerobic incubations were maintained by periodic bubbling with nitrogen. The concentrations of DO were registered with DO meter (YSI - I model 58).

During the mineralization experiments, samples (c.a. 10 ml) of each flask in the following sampling days 0, 0.16, 0.33, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 15, 20, 30, 40, 50 and 60 days, were submitted to carbon analysis. The determinations of organic (TOC) and inorganic (TIC) carbon concentrations were performed with carbon analyzer (SHIMADZU TOC-5000A). After these determinations, the samples were filtered in ester cellulose membrane (0.22 mm; Millipore) for the separation of particulate and dissolved fractions (Wells et al., 2000) and again submitted to the determinations of carbon. The estimates of particulate organic carbon (POC) concentrations were calculated by differences between the concentrations of total organic carbon (TOC) and of dissolved organic carbon (DOC). In this study it was admitted that the concentrations of POC (> 0.22 mm) were equivalent to the microorganisms biomass.

According to Antonio & Bianchini Jr. (in press) the decomposition process of glucose can be described through three routes (Fig. 1). In the first, the formation of inorganic carbon (C_{in1}) was consequence of rapid processes (respiration and chemical oxidation). In the second, the formation of microorganisms (POC formation) and their mineralization (C_{in2}) were contemplated. The third route included the formation of refractory organic compounds (e.g. humic substances).

To determine the decomposition coefficients of DOC (C-glucose), the variations on the concentrations of DOC and of POC were fitted to a first order kinetic model (Bianchini Jr., 1999a), that includes parallel and serial irreversible reactions, represented by the Equations 1, 2, 3 and 4 (Levenspiel, 1986). For kinetic fittings of DOC and POC a non-linear regression, the iterative algorithm of Levenberg-Marquardt, (Press et al., 1993) was used.

C-glucose Global Decay

$$DOC = DOC_0 \times e^{-k_T t} \dots\dots\dots Eq.1$$

where:

- DOC₀ = initial concentration of dissolved organic carbon (mg l⁻¹);
- DOC = dissolved organic carbon concentration, equivalent to C-glucose (mg l⁻¹);
- k_T = (k₁ + k₂ + k₃) global coefficient from glucose conversion (day⁻¹);
- k₁ = oxidation coefficient (chemical or biological) (day⁻¹);
- k₂ = POC formation coefficient (day⁻¹);
- k₃ = refractory DOC formation coefficient (day⁻¹);
- e = natural log basis;
- t = time.

1st Pathway: Inorganic Carbon Formation (C_{in1}) from Rapid Processes (Biological and Chemical Oxidation)

$$C_{in1} = DOC_0 \times \frac{k_1}{k_T} [1 - e^{-k_T t}] \dots \dots \dots Eq. 2$$

where:

C_{in1} = inorganic carbon formation through 1st pathway (mg l⁻¹).

2nd Pathway: Inorganic Carbon Formation (C_{in2}) from Particulate Organic Carbon

$$C_{in2} = DOC_0 \times \frac{k_2}{k_T} \left[1 + \left(\frac{k_4}{k_T - k_4} \times e^{-k_T t} \right) + \left(\frac{k_T}{k_4 - k_T} \times e^{-k_4 t} \right) \right] \dots \dots \dots Eq.3$$

where:

C_{in2} = inorganic carbon formation through 2nd pathway (mg l⁻¹);

k_4 = POC mineralization coefficient (day⁻¹)

3rd Pathway: Formation of Refractory Organic Carbon

where:

DOC_r = dissolved refractory organic compounds formation (mg l⁻¹)

The DOC, POC and DOC_r conversions in percentile values were calculated considering 100% from the theoretical values of DOC_0 obtained from the kinetic fittings.

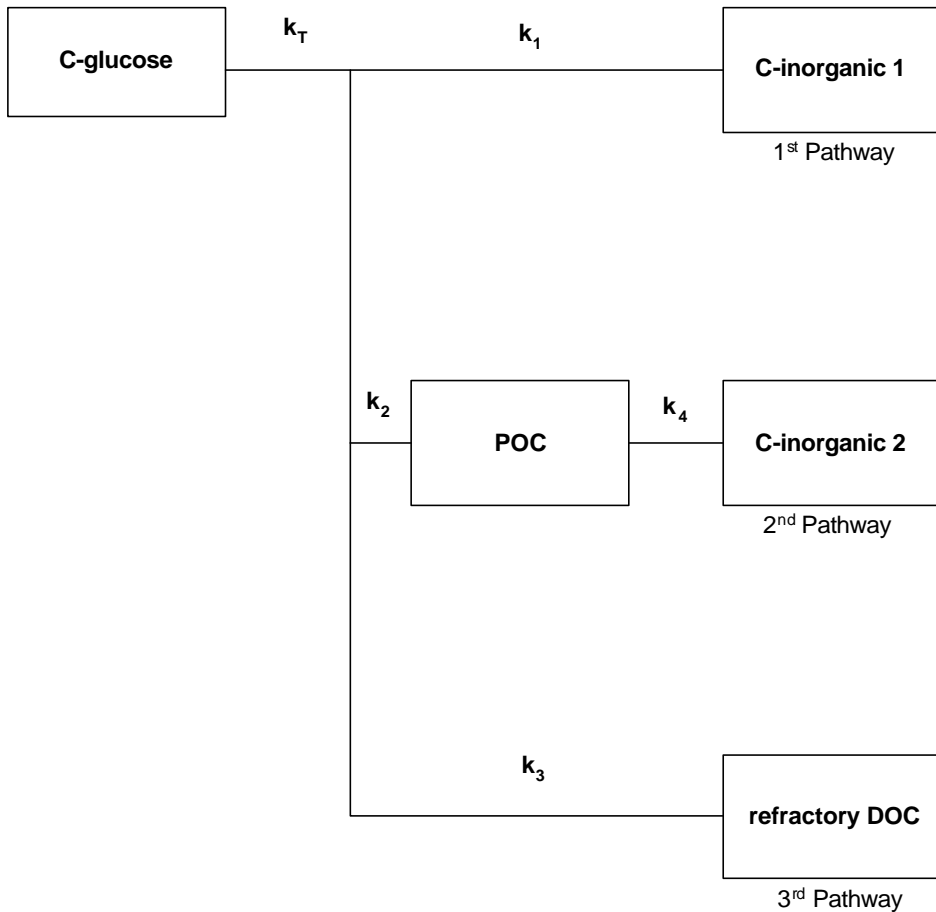


Figure 1: Kinetic model proposed to the glucose decomposition. k_T : global coefficient of glucose decay ($k_T = k_1 + k_2 + k_3$); k_1 = oxidation coefficient (chemical or biological); k_2 = POC formation coefficient; k_3 = refractory DOC formation coefficient; k_4 = POC mineralization coefficient.

Results and discussion

Values of depth (maximum and from the euphotic zone) and physical-chemical variables (dissolved oxygen, temperature, pH, electric conductivity, redox potential) of the sampling site (rainy and dry periods) are shown in Tab. I. Carbon and nutrients (total phosphorous, total organic nitrogen, ammonia, nitrate and nitrite) concentrations of the water samples used in the mineralization experiments are also presented in Tab. I.

Table I: Physical and chemical variables measured in the sampling station in the Barra Bonita Reservoir - SP in February (summer) and July (winter).

Variables	February (summer)				July (winter)			
	max	min	average	SD	max	min	average	SD
Z(m)	-	-	19	-	-	-	16	-
Z _{eu} (m)	-	-	3,85	-	-	-	5,62	-
DO (mg.l ⁻¹)*	3,2	0,4	1,16	1,11	9,1	6,8	7,96	0,84
Temperatura (°C)*	30,5	27,3	27,8	0,96	21,9	19,9	20,3	0,64
Conductivity (µS.Cm ⁻¹)*	210	210	210	0	280	280	280	0
pH*	6,7	6,3	6,48	0,13	7,1	6,3	6,94	0,25
Turbidity (NTU)*	24	9	11,4	4,34	23	2	7,24	7,32
Redox Pontential (mV)*	103	-222	58,3	102,6	84	63	76,11	8,31
Organic Carbon (mg.l ⁻¹)**	-	-	8,15	1,32	-	-	6,71	1,25
Total P (mg.l ⁻¹)**	-	-	215	3	-	-	121	5
Total Organic N (µg.l ⁻¹)**	-	-	406	21	-	-	277	245
NH ₄ ⁺ (mg.l ⁻¹)**	-	-	199	13	-	-	124	3
NO ₃ ⁻ (mg.l ⁻¹)**	-	-	3236	36	-	-	5395	455
NO ₂ ⁻ (mg.l ⁻¹)**	-	-	491	7	-	-	55	0

(* measured at interval of 2 m; (**) mixed samples (n=2); SD: standard deviation.

The values of limnological variables (Tab. I) showed in the sampling periods were relatively different, especially related with DO concentrations, temperature and nutrients. The concentration of DO in summer (rainy season) was, on average, 1.16 ± 1.11 mg l⁻¹. During winter (dry season) the mean concentration of DO was 7.96 ± 0.84 mg l⁻¹. The temperature of these periods corresponds to 27.8°C and 20.3°C, respectively. The differences in the temperature probably interfered on the metabolic activities of microbial assemblage of this ecosystem, e. g. the mineralization rates (Cunha, 1999). As observed in experiments of heterotrophic potential in an oxbow lake of Mogi-Guaçu river, during decomposition the anaerobic process was more influenced by temperature variation than aerobic (Antonio, 1996).

On average, the turbidity was more elevated in the rainy (11.40 ± 4.34 NTU) than dry period (7.24 NTU \pm 7.32). It was probably related with the increasing of suspended particles input derived from storm water and rivers, and also with the bloom of *Microcystis aeruginosa* registered during the sampling in that period (February), which decreased the euphotic zone (Tab. 1). The values of electric conductivity were relatively elevated in the two samplings, being 210 mS cm⁻¹ in February and 280 mS cm⁻¹ in July. The ions have an important influence on the global productivity of aquatic ecosystems, giving support to the primary production processes (Esteves, 1988). In such high concentrations it is not probable that ions act as limiting factor in the decomposition processes. Most of the bacteria lives in a wide range of pH, reaching the maximum grow rate at pH ranging between 6.0 and 9.0 (Stanier, 1976). The pH variation in the two periods was relatively low, thus the hydrogen ion concentration could not strongly influence the progress of the glucose mineralization process.

Regarding the nutrients (N and P) concentrations, the highest value was found in the rainy period (summer); the only exception was observed for nitrate, which presented higher concentrations in the dry period. Once in this period the aerobic conditions prevailed, it is possible that the nitrification processes predominated (Esteves, 1988). Santos (1996) also found in that reservoir the higher concentrations of the nitrogenous species during summer period, except for nitrate.

Studies carried out by Calijuri & Santos (2001) reported that the concentrations of total phosphorous in the summer were 2 times that in the winter, corroborating the results observed in the present study (Tab. I). This fact is related to the decomposition of the suspended organic material and to desorption of phosphate from suspended inorganic material, which increase due to the leachate from adjacent soil from the drainage basin (Calijuri & Santos, 2001; Calijuri & Tundisi, 1990). The nitrogenous forms (total organic nitrogen, ammonia, and nitrite) were found in concentrations more elevated in the rainy period, probably due to the entrance of nutrients originating from agricultural activities, excretion of nitrogenous compounds by Cyanophyceae (McCarthy, 1980) and intensification of the decomposition processes. The higher concentrations of ammonium and nitrite were related to the ammonification process and to the low efficiency of the nitrification process, due to the low concentrations of DO.

Another important factor that decreases the input of nutrients in the dry period is that great part of the water from Tietê river is deviated to the Billings Reservoir (São Paulo). In the rainy season this water management strategy is ceased and consequently the contribution of the river to the input of nutrients increases.

The kinetic fittings of DOC (C-glucose) decay and the formation and mineralization of POC, under aerobic and anaerobic conditions in sampling periods (rainy and dry periods), are presented in Fig. 2 and 3, respectively.

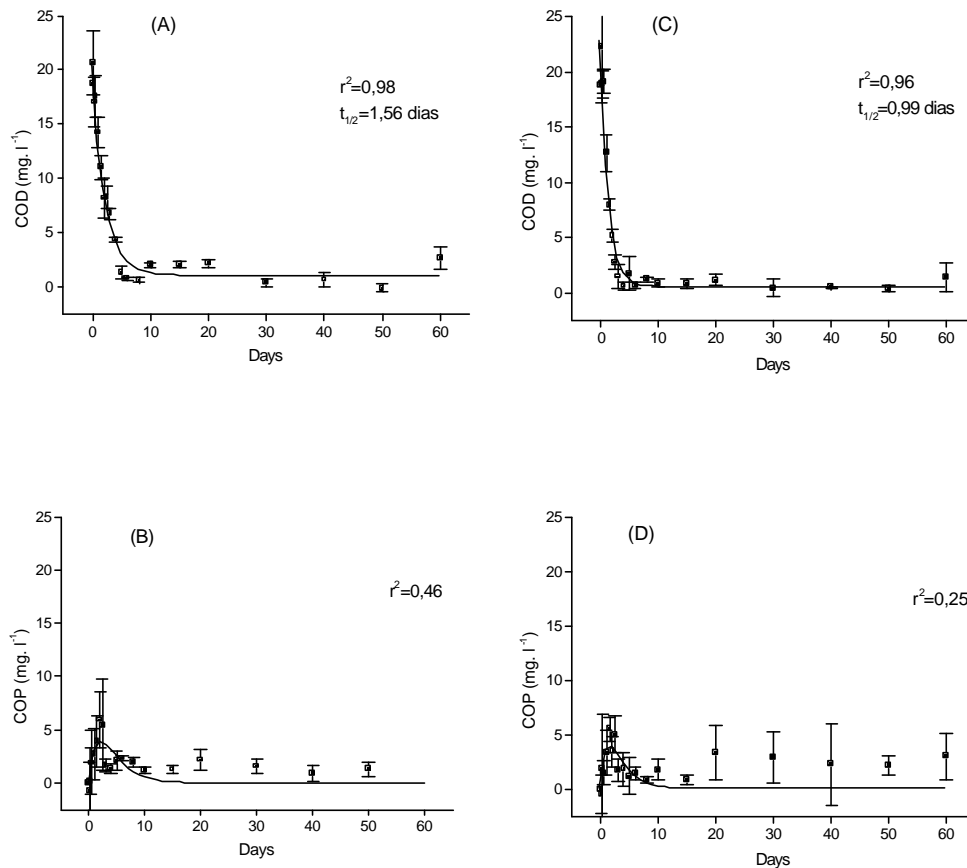


Figure 2: Kinetic fittings of DOC and POC from incubation of February/2001: aerobic processes (A and B) and anaerobic processes (C and D).

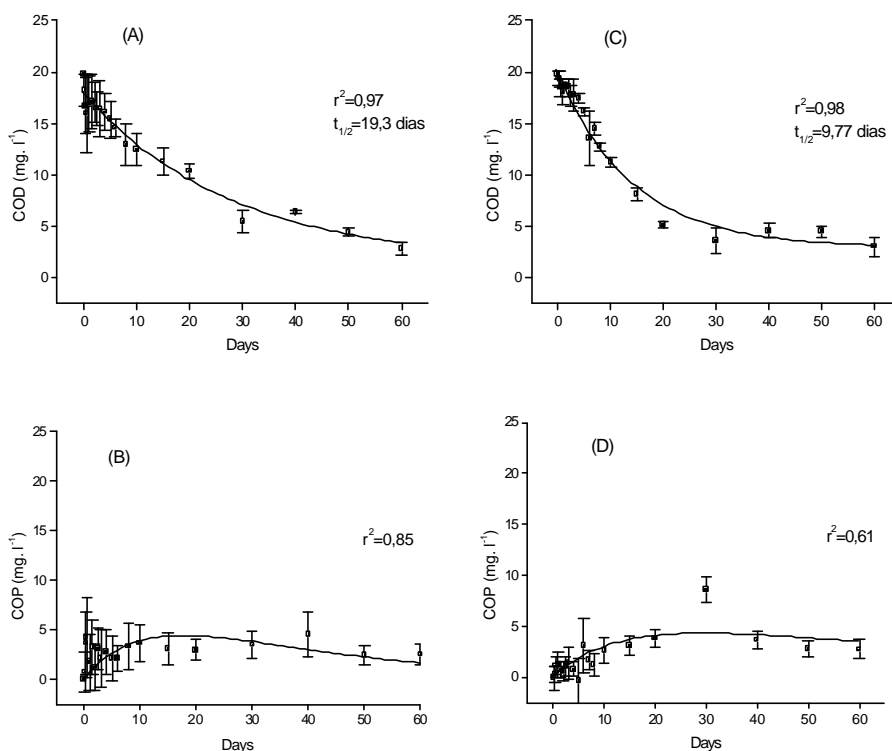


Figure 3: Kinetic fittings of DOC and POC from incubation of July/2001: aerobic processes (A and B) and anaerobic processes (C and D).

Tab.II presents the values of the parameterization of the Equations 1, 2, 3 and 4: the percentile values of initial DOC, POC, DOCr, from the inorganic carbon during the rapid processes (respiration and chemical oxidation) (C_{in1}); the coefficients of global decay of DOC (C-glucose) (k_1), formation of the inorganic carbon due to rapid processes (respiration and chemical oxidation) (k_2), formation of POC (k_3), formation of DOCr (k_4) and mineralization of POC (k_5).

Table II: Parameters from the model determined from the kinetic fittings of DOC and POC. DOC_0 = initial dissolved organic carbon; POC = particulate organic carbon; DOCr = refractory dissolved organic carbon; C_{in1} = inorganic carbon formation through 1st pathway; k_1 = ($k_1 + k_2 + k_3$) global coefficient from glucose conversion; k_1 = oxidation coefficient (chemical or biological); k_2 = POC formation coefficient; k_3 = refractory DOC formation coefficient; k_4 = POC mineralization coefficient.

	Summer (February/2001)				Winter (July/2001)			
	Aerobic		Anaerobic		Aerobic		Anaerobic	
	Parameter	SD	Parameter	SD	Parameter	SD	Parameter	SD
DOC ₀ (%)	100	3,43	100	4,60	100	1,85	100	18,48
POC (%)	50,40	15,04	39,37	13,35	43,24	9,47	30,57	5,91
DOCr(%)	4,97	2,02	2,15	2,18	8,09	8,37	14,07	3,43
C _{in1} (%)	44,63	15,55	58,48	14,28	48,67	12,73	55,36	7,00
K ₁ (day ⁻¹)	0,4486	0,0437	0,7030	0,0850	0,0360	0,0071	0,0709	0,008
K ₂ (day ⁻¹)	0,3454	-	0,5999	-	0,0175	-	0,0392	-
K ₃ (day ⁻¹)	0,0444	-	0,0879	-	0,0155	-	0,0217	-
K ₄ (day ⁻¹)	0,0078	-	0,0150	-	0,0029	-	0,0100	-
K ₅ (day ⁻¹)	0,4406	0,1936	0,4761	0,2417	0,0814	0,0323	0,0125	0,0085

SD: standard deviation.

In the rainy period (summer) the yields of the mineralization pathways for the aerobic metabolism were 44.63% for the C_{in1} , 50.40% for POC and 4.97% for DOCr. For the anaerobic metabolism these values were 58.48% for the C_{in1} , 39.37% for POC and 2.15% for DOCr. In the dry period (winter) the yields of the mineralization pathways for the aerobic metabolism were 48.73% for the C_{in1} , 43.08% for POC and 8.03% for DOCr. For the anaerobic metabolism these values were 55.36% for the C_{in1} , 30.57% for POC and 14.07% for DOCr.

Based in the kinetic data, the samples of water of the reservoir collected in the two periods presented differences regarding the heterotrophic capacity and the yields of the metabolic pathways of the mineralization of the glucose by the bacterioplankton.

According to the parameterization, the global coefficients (k_d) of glucose decomposition was 12.5 times higher for the aerobic processes and 10 times for the anaerobic in the rainy sampling in comparison to the coefficients obtained in the dry period. In rainy season, the time of half-life for aerobic glucose decomposition was 1.56 days, for the anaerobic condition this value was 0.99 day. In dry season these values were 19.3 days and 9.77 days, respectively. Those differences are probably related with climatic and limnological factors of the sampling periods, that selected qualitative and quantitatively the bacterioplanktonic communities. The rainy period sampling was coincident with a bloom of *M. aeruginosa*. Studies on the phytoplankton community found the dominance of this species in the Barra Bonita Reservoir during summer period (Santos, 1996). That event, together with the largest input of nutrients and organic matter and also the high temperatures, could be viewed as an intensification of the decomposition processes, what could be confirmed by the low concentration of DO in the water column in that sampling (on average 1.16 mg l⁻¹). The high development of *M. aeruginosa* could result in an increase of the excretion products and cellular lyses of the Cyanophyceae (Hansen et al., 1986), that gives support to the bacterioplankton and consequently this fact could increase the bacterial population (Nalewajko et al., 1980).

Studies in Barra Bonita Reservoir demonstrated that the main limiting factor for the growth of phytoplankton is the phosphorous (Henry et al., 1985). As observed in the present work the concentration of total phosphorous in February was of 215 µg l⁻¹ and 121 µg l⁻¹ in July. The high concentration of phosphorous associated with high temperature of the water column could be viewed as the main cause for the high development of the *M. aeruginosa* population in that period, and consequently, associated with the largest heterotrophic activity of the bacterioplankton. It is well known that the relationship between bacteria and phytoplankton is the most important regulation system of the organic and inorganic carbon in the aquatic ecosystems (Kato & Sakamoto, 1981).

The global glucose decomposition coefficients during the anaerobic processes were higher (1.6 times in February and 1.9 times in July) than during aerobic processes. Despite that, frequently, aerobic processes are considered faster than the anaerobic ones (Wetzel, 1983; Antonio, 1992), some studies showed that the anaerobic processes can be faster (Antonio, 1996; Bianchini Jr. et al., 1997). Those authors suggest that in this reservoir, the bacteria community (aerobic and facultative anaerobic) is well adapted to the anaerobic conditions, once these communities depend much more on symbiotic relationships than aerobic populations (Zhender, 1988).

Regarding the conversion of the glucose into POC (immobilization), the aerobic bacteria showed great efficiency (50.4% in the summer and 43.2% in the winter) than the facultative anaerobic or anaerobic (39.4% in the summer and 30.6% in the winter). The larger yield observed for the aerobic bacteria was already observed (Bianchini Jr., 1999b). The high efficiency of the aerobic bacteria in the immobilization of the carbon could be related to the largest energetic efficiency during the aerobic breakdown of the glucose, generating larger amount of energy for the anabolic processes.

The metabolism of the Barra Bonita Reservoir depends on the hydrologic cycle, the turbulence and to the external factors, e. g. pulse of suspended material. It must be considered that the aquatic system expresses the tension received by the adjacent

ecosystems (terrestrial) on the drainage basin, modifying its operation and tending to minimize the consequences of such tension (Calijuri, 1990). In this study it could be observed that one of the response to the largest inputs of nutrients and materials during the rainy period was the acceleration of the decomposition processes. Therefore the mathematical model adopted in the determination of the heterotrophic potential performed well, showing the accentuated difference between the heterotrophic potential of the two sampling periods.

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