Methodological test of efficiency of heterotrophic potential

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ABSTRACT. This study aimed at describing the kinetic aspects of glucose aerobic mineralization by different types of microorganisms selected by filtration membranes of different pore sizes: 0.22, 0.45, 2.2 and 7.4 μm, and also in glass wool. Water samples were collected from Óleo Lagoon (21º36´ S and 47º49´ W). About 30 mg of glucose was added into filtered water, and the samples were incubated in the dark at 21ºC under aerobic conditions. The dissolved oxygen consumption was determined periodically during 59 days (polarographic method; DOmeter YSI model 58).The data were fitted into a first-order kinetic model. The maximum values of consumed oxygen varied from 2.10 mg L^{-1} (pore size 0.22 μm treatment) to 32.83 mg L^{-1} (pore size 0.45 μm treatment). The glucose mineralization on the 0.22 μm treatment showed the lowest oxygen consumption coefficient (k_d = 0.03 day^{-1} and half time ($t_{1/2}$) = 23 days) and the highest value was observed on the 0.45 μm treatment and 2.2 μm treatment (k_d = 0.08 day^{-1} and $t_{1/2}$ = 9 days). Oxygen consumption was higher in the sample filtered into 0.45 μm, suggesting that bacterivory occurred in treatments with glass wool, 2.2 and 7.4 μm. The filtration into 0.22 μm pore size membranes favored chemical oxidation.

Key words: glucose mineralization, heterotrophic potential, long-term BOD experiments.

RESUMO. Teste metodológico da eficiência do potencial de heterotrofia. Este estudo teve por objetivo descrever os aspectos cinéticos da mineralização aeróbia da glicose pela ação de diferentes tipos de microrganismos selecionados por membranas de filtração com diferentes tamanhos de poros: 0,22, 0,45, 2,2 e 7,4 μm e em lã de vidro; para tanto, utilizaram-se amostras de água coletadas na lagoa do Óleo (21º36´ S e 47º49´ W). Aproximadamente 30 mg de glicose foram adicionadas à água filtrada e distribuídas em frascos escuros (1 L) que foram incubados no escuro a 21ºC sob condições aeróbias. As concentrações de oxigênio dissolvido das incubações foram determinadas periodicamente, durante 59 dias (método polarográfico; oxímetro YSI modelo 58). Os consumos acumulados de oxigênio foram ajustados a um modelo cinético de primeira ordem. Os valores máximos de oxigênio consumido variaram de 2,10 mg L^{-1} (no tratamento com membrana de 0,22 μm) a 32,83 mg L^{-1} (no tratamento com membrana de 0,45 μm). A mineralização da glicose, no tratamento com membrana de 0,22 μm, apresentou o menor coeficiente de consumo de oxigênio (k_d = 0,03 dia^{-1} e $t_{1/2}$ = 23 dias) e o maior valor foi observado para os tratamentos de 0,45 e 2,2 μm (k_d = 0,08 dia^{-1} e $t_{1/2}$ = 9 dias). O consumo de oxigênio foi maior nas amostras de água filtrada em 0,45 μm, sugerindo que nos tratamentos com lã de vidro, 2,2 e 7,4 μm ocorreram bacteriovorias. A filtração em membranas de poro de 0,22 μm favoreceu, provavelmente, as oxidações químicas.

Palavras-chave: mineralização da glicose, potencial de heterotrofia, experimentos de DBO de longo prazo.

Introduction

Detritus can be defined as any form of non-living organic matter found as particulate (POM) and dissolved organic matter (DOM) (Moore *et al.*, 2004). The detritus stabilizes both energy flux and transfer efficiencies across trophic levels (Azam, 1998). DOM consists of various fractions such as humic and non-humic compounds and low and high molecular weight components (Benner, 2002; Hansell, 2002) whose dynamics and characteristics influence a number of key processes, including the control of nutrient availability and element and energy cycling (Currie *et al.*, 1996; Williamson *et al.*, 1999). In aquatic ecosystems the dissolved organic matter is composed of two fractions: (i) the readily utilizable DOM or labile pool and (ii) the refractory DOM that are not easily utilized (Geller, 1986).

Although the definition of labile DOM is somewhat complex, the labile pool is generally thought to consist mainly of sugars, amino acids, peptides and other simple compounds (Moran and Hodson, 1990), and accounts for less than 20% of the total DOM. The refractory pool, which is composed mostly of higher molecular weight humic and fulvic acids, is more abundant, but its turnover is slower and has been considered relatively less important as a substrate for bacterial growth (Bertilsson and Tranvik, 2000) than the labile pool. Nevertheless, some components of the refractory pool may be altered photochemically to produce more bioavailable compounds (Moran *et al.*, 2000; Santos, 2005).

The term DOM is generally applied to organic material that passes through a membrane filter with a pore size of 0.45 μm (Wotton, 1994). This material represents a direct source of food for the biota, especially to the microorganisms; some of which can themselves pass through membrane filters with pore sizes of 0.45 μm (Vähätalo and Søndergaard, 2002). DOM consuming by bacterioplankton forms a link between detritus and planktonic food webs (Wetzel, 1983).

Microbial productivity linkages to DOM cycling are of great importance in lacustrine systems (Bertilsson and Tranvik, 1998). Most primary production is not consumed by herbivores, but rather returned to the environment as detritus to play critical roles in organizing and sustaining ecosystems (Crawley, 1997). Microorganisms of the aquatic environment play an important role in the mineralization process and regeneration of nutrients, as well as in the creation of the basic food resources (Sorokin and Kadota, 1972). Microorganisms are not only responsible for nutrient cycling but, through the microbial loop, they represent an exceptional trophic link between detritus and the classical food chain. Therefore, nutrients, carbon and energy are efficiently transferred from a lower level of the food chain to its higher levels (Biddanda, 1985; Pomeroy and Wiebe, 1988), and it is also a major source of food for some detritivores (Mann, 1972). In this context, in this study we verified the efficiency of microbiota on glucose cycling, describing the kinetic aspects of glucose aerobic mineralization by different types of microorganisms selected by membranes of different pore sizes, as well as the interactions among these organisms.

Material and methods

Sampling site description: Óleo Lagoon (21º36´S and 47º49´W) is one of the many oxbow lagoons in the Mogi-Guaçu River floodplain, located within the Jataí Ecological Station (21º33´ to 21º37´S and 47º45´ to 47º51´W; Luiz Antonio, São Paulo, Brazil). Its morphometry is described by Cunha-Santino (2003) and Petracco (2006). The maximum depth is 5.1 m ($Z_{average}$ = 2.5 m). The flooded area comprises 19,470 m^2. It is an acidic (pH 5.49 ± 0.65) lagoon with low concentrations of dissolved organic carbon (3.05 ± 0.98 mg L^{-1}) and dissolved oxygen (3.57 ± 2.18 mg L^{-1}). The annual water temperature varies from 18 ± 2ºC (winter) to 30 ± 1ºC (summer). The analysis of chemical and biological variables of the lagoon suggests that this system is oligotrophic (Wisniewski *et al.*, 2000).

Experimental set-up: About 30 mg of glucose were incubated in duplicate at 21 ± 0.6ºC, in BOD ottles (1 L) containing filtered lagoon water in membranes of different pore sizes: 0.22 μm (Millipore, cellulose ester); 0.45 μm (Millipore, cellulose ester), 2.20 μm (Schleicher and Schull; Blue ribbon 389) and 7.40 μm (Schleicher and Schull; Black ribbon 389) or in glass wool. The dissolved oxygen (DO) concentrations were measured periodically during 59 days through a DOmeter (YSI, model 58). The bottles were oxygenated during 1 hour to keep DO near saturation when the concentrations of DO decreased to *ca.* 2.0 mg L^{-1}. In order to neutralize the quantity effects of organic matter present at Óleo Lagoon, the average values of oxygen concentration of control flasks (n = 2) were subtracted from average values of chambers with glucose.

Kinetic model: Assuming that oxygen consumption is directly related with the oxidation of organic resources, and that this process could be represented by first-order kinetics models (Bitar and Bianchini Jr., 2002), the temporal variation in DO consumption can be described by Equation 1 (Press *et al.*, 1993):

$$CO = CO_{\max}\left(1 - e^{-k_d t}\right) \quad (1)$$

where: CO = accumulated value of consumed oxygen (mg L^{-1}); CO_{max} = maximum amount of consumed oxygen (mg L^{-1}); k_d = deoxygenation coefficient (day^{-1}); t = time (day).

The half-time ($t_{1/2}$) of deoxygenation derived from aerobic decomposition of glucose was calculated by Equation 2:

$$t_{1/2} = \frac{\ln 0{,}5}{-k_d} \quad (2)$$

Statistical analysis: The CO data were

statically analyzed individually for each treatment using the Kruskal Wallis test, followed by Dunn's Multiple Comparison test, in order to detect significant differences among treatments ($p < 0.05$).

Results

The kinetics of oxygen consumption from the aerobic mineralization of glucose submitted to fractionation in distinct pore size membranes and glass wool are shown in Figure 1, from which the oxygen uptake deriving from water samples from Óleo Lagoon (control) was subtracted. CO_{max} for all 59 days of experiments ranged from 2.10 mg L^{-1} (pore size 0.22 μm treatment) to 32.83 mg L^{-1} (pore size 0.45μm treatment; CO_{max}; Table 1). The mineralization of glucose through 0.22 μm treatment showed the lowest constant rate (k_d: 0.03 day^{-1} and $t_{1/2}$ = days) and the highest values were observed for 0.45 and 2.2 μm treatment (k_d: 0.08 day^{-1} and $t_{1/2}$ = days) as shown in Table 1.

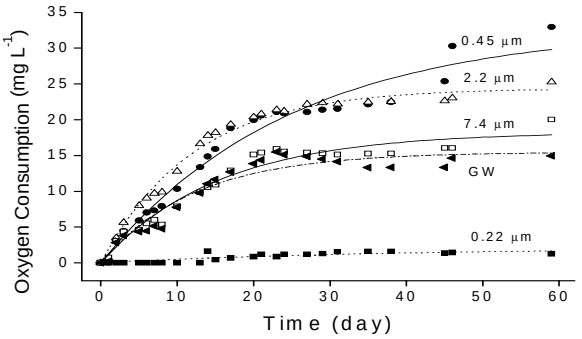

Figure 1. Oxygen consumption kinetics during aerobic mineralization of glucose for all treatments (GW=glass wool).

Table 1. Parameterization of kinetic model during aerobic glucose mineralization: OC_{max} = oxygen consumption from glucose uptake; k_d = DO consumption coefficient; $t_{1/2}$: DO consumption half-time; r^2 = determination coefficient and error = error referred to kinetic fittings.

Treatment	OC_{max} (mg L^{-1})	Error	k_d (day^{-1})	Error	$t_{1/2}$	r^2
Glass wool	15.47	0.66	0.08	0.01	8.66	0.94
7.4 μm	18.26	0.75	0.06	0.01	11.55	0.96
2.2 μm	24.39	0.43	0.08	0.00	8.66	0.99
0.45 μm	32.83	1.97	0.04	0.00	17.32	0.97
0.22 μm	2.10	0.78	0.03	0.01	23.10	0.73

The determination coefficients (r^2) for the kinetic fitting varied from 0.73 to 0.99. Using the kinetics of oxygen consumption, the Kruskal Wallis analysis showed significant differences among glass wool and 2.2 μm treatments ($p < 0.05$) and 0.45 μm ($p < 0.001$) and 0.22 μm treatments ($p < 0.05$). The 7.4 μm treatment was different from 0.45 μm ($p < 0.05$). The statistical analysis did not indicate significant differences between the kinetics of oxygen consumption from glass wool and 7.4 μm ($p > 0.05$).

Discussion

The total amount of CO_{max} 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 CO_{max} to follow the process of a microbial reaction in aerobic environments (Characklis, 1990). The long-term BOD tests are the experimental procedure to obtain this variable (Cunha-Santino and Bianchini Jr., 2003).

From the kinetics point of view, the oxygen consumption was similar to that observed by Borsuk and Stow (2000). There was an increase in oxygen demand in the beginning of the experiment, followed by a decrease in oxidation, tending towards stabilization of the process (Figure 1). The stabilization of oxygen uptake was frequently associated with the mineralization of refractory fractions (Cunha-Santino and Bianchini Jr., 2004) or, as verified in the present study, to the depletion of carbon sources that were oxidized. Considering the high determination coefficients (r^2 varied from 0.73 to 0.99) obtained from the fittings using the kinetics model (Figure 1), it was possible to verify that the proposed model (Equation 1) was adequate to represent the kinetics of oxygen consumption.

Experiments on aerobic decomposition that comprised organic resources presented the following values for oxygen consumption: 476 and 496 mg g^{-1} of glucose (water samples from an eutrophic reservoir; Panhota and Bianchini Jr., 2003); 139.9 mg g^{-1} C of humic acid and 581.9 mg g^{-1} C of fulvic acid (water samples from an oxbow lake; Cunha-Santino and Bianchini Jr., 2004); 307.4 mg g^{-1} of tannic acid (water samples from the Monjolinho reservoir (Cunha-Santino *et al.*, 2002); 339.2 and 386.3 mg g^{-1} of glycine and lysine (water samples from the Monjolinho reservoir; Cunha-Santino and Bianchini Jr., 2003); 238.1 mg g^{-1} of glucose (water samples from an oxbow lake; Antonio and Bianchini Jr., 2002). In the present study, the values in mg g^{-1} C of glucose were: 175.0 for 0.22 μm treatment; 2735.8 for 0.45 μm treatment; 2032.5 for 2.2 μm treatment; 1521.7 for 7.4 μm treatment and 1289.2 for glass wool treatment. These values were higher than those obtained for glucose by Antonio and Bianchini Jr. (2002) and Panhota and Bianchini Jr. (2003). However, the CO_{max} could be influenced by extrinsic factors, such as microbial composition and

number; origin of indigenous inoculum, procedure used in experiment preparation (*i.e.* filtration) and temperature of incubation.

The lower value of CO_{max} (2.10 mg L^{-1}; Table 1) suggests the predominance of chemical oxidation (Santos, 2005) or bacterial-free activity due to filtration through a 0.22 μm pore size filter; in this context, it should be considered the incubations not were maintained under sterile conditions. Bacteria–free water has conventionally been obtained by filtration through 0.22 μm pore size filters, but a recent study has shown that these may not always have uniform pore diameters, which could result in the passage of particles of considerably larger size into the filtrate (Stockner *et al.*, 1990), suggesting that in order to obtain a bacteria-free medium, a filtration through 0.1 μm pore size filter must be performed.

Oxygen consumption was higher in water samples filtered in 0.45 μm membrane pore size; suggesting that predation occurred in glass wool, 2.2 and 7.4 μm treatments; nanoflagellates range in size from 2 to 20 μm and most are capable of bacterivory (Saunders *et al.*, 1989; Boenigk and Arndt, 2002). From our results, it is clear that the removal of consumers > 2.2 μm had a significant positive effect on the net respiration of bacteria. Bouvy *et al.* (2006) showed that the removal of large bacterivorous predators (fraction < 3 μm) increased the growth rate of heterotrophic bacteria (0.682 day^{-1}) compared with the growth rates in the presence of predators (fraction < 60 μm; 0.437 day^{-1}).

The fractionation method was successful in separating groups of the microbial food web (bacteria, flagellates, ciliates) and to elucidate trophic links among microbial components (Samuelson and Anderson, 2003; Vaqué *et al.*, 2004). However, this method may cause cell damage and may then increase the amount of dissolved organic matter (Gasol and Moran, 1999), including a possible increase of bacterial growth (Vaqué *et al.*, 2004).

An experimental protocol defines DOM operationally as all dissolved material that passes through a 0.45 μm pore size filter; this procedure also selects the organisms that act directly on organic matter cycling. Successive fractionating was applied to verify the influence of organisms on aerobic mineralization of glucose; the results suggest that filtration in 0.45 μm pore size is the convenient procedure to assess microbial respiration. Pore size > 0.45 μm appeared to have favored bacterivory, and consequently the CO_{max} for these treatments were slow.

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