

O/C Stoichiometry from mineralization of *Montrichardia arborescens* (L.) Schott.

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ABSTRACT: O/C Stoichiometry from mineralization of *Montrichardia arborescens* (L.) Schott. This study aimed at evaluating the oxygen uptake kinetic from aerobic mineralization of *Montrichardia arborescens*, including the monitoring of the temporal evolution of stoichiometric values (O/C) for decomposition of this aquatic macrophyte specie. The plant was collected in the Cantá stream (2° 49' 11" N and 60° 40' 24" W), State of Roraima, Brazil. To measure oxygen uptake, two mineralization chambers with plant fragments and water from the stream were incubated. The chambers were aerated during 104 days, the oxygen and dissolved carbon were monitored and the total mineralized carbon was estimated. The results were fitted to first-order kinetics model, yielding an oxygen uptake of 235.2 mg g⁻¹ (DW) and a deoxygenation rate of 0.0443 day⁻¹ for the mineralization of *M. arborescens*. The stoichiometry coefficients decreased during the course of the experiments. This decrease was probably mainly related to the availability of labile fractions of organic carbon during the early stages of the mineralization process.

Key-words: mineralization, oxygen uptake, *Montrichardia arborescens*.

RESUMO: Estequiometria O/C durante a mineralização de *Montrichardia arborescens* (L.) Schott. Este estudo teve como objetivo avaliar a cinética do consumo de oxigênio na mineralização aeróbia da macrófita aquática *Montrichardia arborescens*. Também foram estimadas as relações estequiométricas entre o oxigênio consumido e o carbono oxidado. A planta foi coletada no igarapé do Cantá (2° 49' 11" N e 60° 40' 24" W), estado de Roraima, Brasil. Para avaliar o consumo de oxigênio foram utilizadas duas câmaras de mineralização contendo fragmentos de plantas e água do igarapé. As câmaras foram aeradas durante 104 dias, o oxigênio e o carbono orgânico dissolvidos foram monitorados e o carbono total mineralizado foi estimado. Os resultados foram ajustados a um modelo cinético de primeira ordem através do qual verificou-se que durante a mineralização de *M. arborescens* o consumo de oxigênio foi 235,2 mg g⁻¹ (PS) e o coeficiente de desoxigenação foi 0,0443 dia⁻¹. Os coeficientes estequiométricos diminuíram com o tempo, provavelmente devido à disponibilidade de fração lábil de carbono orgânico na fase inicial do processo de mineralização.

Palavras-chave: mineralização, consumo de oxigênio, *Montrichardia arborescens*.

Introduction

Many species of aquatic macrophytes contribute substantially to the detritus input in continental aquatic ecosystems (Wetzel, 1995; Bianchini Jr., 2003). The organic matter produced by macrophytes eventually changes to dissolved, colloidal or particulate detrital forms (Brum & Esteves, 2001). With the loss of cellular integrity due to senescence, dead vascular plant material begins to lose organic and inorganic materials shortly after immersion in water (Zozaya & Neiff, 1993; France et al., 1997). The decomposition of the plant detritus occurs under different oxygen levels. When available, oxygen is the main terminal electron acceptor and the end products of decomposition are primarily carbon dioxide, water and new cell material. One way to assess the biodegradability of organic materials

is to measure oxygen consumption, carbon dioxide production and/or the disappearance of dissolved organic carbon (McAvoy et al., 1998). Direct measurement of changes in the concentration of O_2 has been used to estimate rates of heterotrophic process. Implicit in these studies is a quantitative relationship among O_2 consumption, CO_2 evolution and microbial activity (Berman et al., 2001; Strauss & Lamberti, 2002). The relation between the amounts of oxygen consumed per carbon oxidized is referred to as stoichiometric ratio (Brezonik, 1993). This ratio determines the extension of biochemical transformation, representing quantitative information on the consumption of the reagents (e.g. O_2) and the formed products (e.g. CO_2) (Characklis, 1990). On a molar basis, the amount of CO_2 liberated should be approximately equal to the amount of O_2 consumed (Karl, 1986). Overall, the ecological stoichiometry is the study of the balance of energy and multiple chemical elements in ecological interactions (Sterner & Elser, 2002). This study aimed at evaluating the oxygen uptake kinetic from aerobic mineralization of *Montrichardia arborescens*, and monitoring the temporal evolution of stoichiometric values (O/C) for decomposition of this aquatic macrophyte specie.

Materials and methods

Plant and water sampling

Samples of leaves and stems from different adult individuals of *Montrichardia arborescens* (L.) Schott and water were collected on January/2000 (beginning of flood period) in the littoral zone of the Cantá stream (2° 49' 11" N and 60° 40' 24" W), State of Roraima, Brazil. *M. arborescens* is a typical plant of flooded areas with a height of 2-4 m. This aquatic macrophyte species is popularly known as Aninga and belongs to the Araceae family. It is usually found in the Amazon area and Northeast of Brazil (Rodrigues, 1989). The plant samples were washed with tap water to remove periphyton, sediment particles and coarse material (Osgburn et al., 1987). After washing, the plant material was oven-dried (50° C), ground (0.2 cm > diameter < 1.32 cm) and homogenized. The water samples were prefiltered through 0.4 mm mesh size nylon net and fiberglass wool to remove large organisms and coarse detritus.

Oxygen uptake assay and stoichiometric coefficients

The plant powder (200 mg L⁻¹ DW) was placed in duplicate in acid-washed 1-L flasks with water from the stream. The incubations were maintained under aerobic conditions in the dark at 21.5°C ± 1.0. Considering the lack of limnological data on the Cantá stream, the temperature range adopted followed the used by Brum et al. (1999) in similar experiments. In order to maintain the solutions under aerobic conditions, they were oxygenated (in the initial of experiment) during 1 hour, to keep dissolved O_2 near saturation. After oxygenation, the dissolved oxygen (DO) was measured with an ODMeter (YSI model 58; Yellow Spring Instruments; precision: 0.03 mg L⁻¹). During sampling days whenever the DO concentrations reached 2.0 mg L⁻¹, the solutions were oxygenated again; this procedure were adopted to ensure aerobic condition. The oxygen consumption was estimated during 104 days. To remove the background DO consumption, two blank flasks (with water sample of stream) were also incubated. During the aerobic mineralization, it is usually assumed that the proportionality between the consumed oxygen and CO_2 formed are constant value (e.g. 2.66; Chapra & Reckhow, 1983). We considered, therefore, that consumption of oxygen was directly related to the oxidation of the organic resource, and that first-order kinetics model can represent this process. Using a non-linear method (Levenberg-Marquardt iterative algorithm; Press et al., 1993) the results were fitted to a kinetics model (Peret & Bianchini Jr.; 2004) where the deoxygenation coefficient (k_d) and the total amount of consumed oxygen were estimated.

In order to determine the stoichiometric ratio (oxygen consumed (OC) per carbon oxidized), the amount of consumed organic carbon (COC) was estimated using the kinetic model proposed for this specie (Bianchini Jr. et al., 2002). Using this model the evolution of the net carbon mineralization was obtained according to Equations 1 to 4:

$$IN_1 = \frac{k_M}{k_1} POM_L (1 - e^{-k_1 t}) \quad \dots\dots\dots(1)$$

$$IN_2 = \frac{k_L}{k_1} POM_L \left(1 + \frac{k_2}{k_1 - k_2} e^{-k_1 t} + \frac{k_1}{k_2 - k_1} e^{-k_2 t} \right) \quad \dots\dots\dots(2)$$

$$IN_3 = POM_R (1 - e^{-k_3 t}) \quad \dots\dots\dots(3)$$

$$COM = \sum_{i=1}^3 IN_i \quad \dots\dots\dots(4)$$

where:

- POM_L = Labile/soluble particulate organic matter;
- POM_R = Refractory particulate organic matter;
- DOM = Dissolved organic matter; $DOM = \frac{k_L}{k_1} POM_L$
- COM = Consumed organic matter (mineralized);
- T = Time;
- k_L = POM_L leachate coefficient from the soluble fractions;
- k_M = POM_L mineralization coefficient;
- k₁ = POM_L global decay coefficient (k_L + k_M);
- k₂ = DOM mineralization coefficient;
- k₃ = POM_R mineralization coefficient;
- IN_{1 a 3} = Inorganic compounds produced from three mineralization routes.

The time variation of stoichiometric coefficients (OC/COC) was calculated by using the ratio between daily rates of OC (dOC/dt) in this study and COC (dCOC/dt) obtained from aerobic degradation experiment with *M. arborescens* (Bianchini Jr., et al. 2002).

Results and discussion

The total amount of OC, 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 to follow the process of a microbial reaction in aerobic environments (Thomaz et al., 2001). Long-term BOD experiments are practical procedure that expresses OC variation under aerobic conditions (Branco & Rocha, 1977; Farjalla et al., 1999). The kinetic model fitted well (r² = 0.97) the oxygen uptake in aerobic mineralization of *M. arborescens* during 104 days (Fig. 1A). The total oxygen consumed (OC) was 235.2 (± 4.1) mg per gram of detritus (DW) and the deoxygenation coefficient was 0.044 (± 0.002) day⁻¹ (half-time = 16 days).

The experimental and theoretical changes of COC are presented in Fig. 1B. COC increases sharply up to the 20th day, which is followed by a less steep increase. The parameters for the aerobic mineralization of *M. arborescens* obtained by Bianchini Jr. et al. (2002) are presented in Tab. 1. The model pointed to a heterogeneous chemical composition of *M. arborescens*, with at least two fractions: one labile (30.2%) and another refractory (69.8%). The reaction coefficients (k₁, k₂ and k₃) are also shown in Tab. 1 and represent the rate constants for oxidation of the different kinds of organic matter.

With the loss of cellular integrity due to senescence, intensive losses of soluble cellular components occur rapidly, often exceeding 25% of the total organic carbon within the first 24 h (review by Webster & Benfield, 1989). The releases and losses of hydrolyzed components (9.8%) from the tissues during aerobic mineralization of *M. arborescens* are evidenced by the high values of k₁ (0.59 day⁻¹; half-time = 1.16 day) and k₂ (0.012 day⁻¹; half-time = 57.5 day), respectively. During the aerobic mineralization of *M. arborescens*, these two fractions constituted the main provider of organic carbon and inorganic nutrients to the metabolic processes in the microbial community. The heterotrophic respiration is responsible for the higher increments in the OC values during the early stages of the mineralization process (Fig. 1A).

From the changes in oxygen uptake (Fig. 1A) and COC (Fig. 1B), the experimental stoichiometric ratio (O/C) of mineralization was obtained. Theoretical stoichiometric values were estimated (Fig. 1C). Stoichiometry varied over the whole processes, with experimental

Table 1: Parameters of the aerobic mineralization model (Bianchini Jr. et al., 2002), where: k_1 = global decay coefficient for the labile particulate organic matter (LPOM) (from leaching and oxidation processes); k_2 = coefficient of the dissolved organic matter (DOM) mineralization; k_3 = decay rate for the refractory particulate organic matter (RPOM) (from oxidation processes); IN 's = yields of the inorganics compounds; IN_1 = inorganics from LPOM mineralization and IN_2 = inorganics from DOM mineralization and IN_3 = inorganics from RPOM mineralization.

Reaction Coefficients (day ⁻¹)			Half-time (day)	Yields (%)		
k_1	=	0.59	1.16	IN_1	=	20.4
k_2	=	0.012	57.5	IN_2	=	9.8
k_3	=	0.002	345	IN_3	=	69.8

values ranging from 0.13 to 5.03, while the theoretical values ranged from 0.14 to 4.44. Stoichiometric values decreased with time during the experiments, which is mainly associated with the availability of labile fractions of organic carbon (labile particulate and dissolved organic matter) during the early stages of the mineralization process. According to Cunha-Santino (2003), another factor that may contribute to the O/C stoichiometric temporal changes were associated with the alternation of predominant metabolic routes derived from the microorganisms species changes (e.g. pentose phosphate pathway, Embden-Meyerhof-Parnas pathway and Entner-Doudoroff pathway). The chemical composition (oxygen content) can also contribute to the O/C stoichiometric changes by conditioning the oxygen availability during the oxidation reactions (Dagley, 1986).

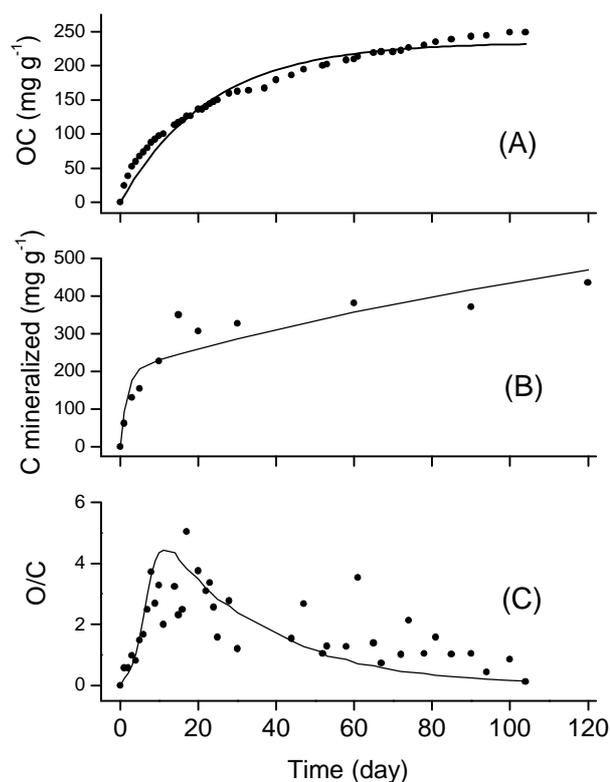


Figure 1: Cumulative oxygen consumed (A), cumulative C mineralized (COC) (B) and O/C stoichiometric ratio (C) from aerobic mineralization of *M. arborescens*. The fittings of the kinetics model are shown as a solid line.

The detritus (dissolved and particulate) consist of several organic compounds, many of which may serve as microbial substrates (Cunha-Santino et al., 2003). This mixture encompasses both low and high molecular weight compounds which will be easily assimilated and metabolized by microorganisms through several different pathways (Chróst, 1991). These compounds comprise monomers such as dissolved free amino acids and carbohydrates (Bunte & Simon, 1999). It is well known that bacteria in particular utilize DOM, partly for their energy requirements and partly for the synthesis of new cell material. The time evolution of stoichiometric values obtained in the initial stage was probably related to the chemical composition of DOM. These molecules may be inferred to belong to compounds with relatively high oxidisable potential and availability to microbes. DOM represents a large pool of energy for organisms adapted to the uptake of these compounds.

The low stoichiometric values at the end of experiments were probably related to the RPOM oxidation fractions, which are composed of lignocellulolytic residues (Cunha & Bianchini Jr., 1998). Since the remaining detritus is formed by lignin and cellulose, probably its refractory characteristics are associated with the low O/C values (Fig. 1C). Another factor to be considered is the formation of humic substances. The low coefficients observed by Cunha-Santino & Bianchini Jr. (2002a) during the decay of these substances (mean decay coefficient = 0.01 day⁻¹; half-time = 70 days), may also contribute to the low stoichiometric coefficients observed in the late stages of mineralization.

Studying the decomposition of *Utricularia breviscapa* in four different temperatures, Cunha-Santino (2003) observed an increment in the O/C values in the earlier stage of decomposition and a subsequent decrease in the values. The maximum values were observed on the 5th day being 9.5 (at 15°C), 16.2 (at 20°C), 16.0 (at 25°C) and 20.2 (at 30°C).

The stoichiometric ratio during decomposition of branches, barks, litter and leaves also presented the pattern of high ratios in the initial stage of decomposition and tended to decrease with time (Cunha-Santino & Bianchini Jr., 2002b). In this study the maximum stoichiometric values were observed on the 3th day and these values were: 6.73 (barks), 1.05 (branches), 2.45 (leaves) and 3.88 (litter).

Taken together with mass conservation, the stoichiometric coefficients allows us to infer that in the Cantá stream, during the earlier stages of decomposition of this aquatic macrophyte, high oxygen deficits will be generated compared to later stages. The intensity and extent of dissolved oxygen deficits are driven by the chemical composition of plant tissues (amount labile and refractory fractions) and the environmental heterotrophic capacity.

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