Aerobic mineralization of carbon and nitrogen from Myriophyllum aquaticum (Vell.) Verdc. leachate.

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ABSTRACT: Aerobic mineralization of carbon and nitrogen from Myriophyllum aquaticum (Vell.) Verdc. Leachate. The evaluation of organic matter degradation (carbon and nitrogen compounds) in aquatic systems is extremely important for the understanding of metabolism and functioning of these environments. In this context, this study aimed at describing the kinetics of oxygen uptake during the decomposition of Myriophyllum aquaticum leachate in order to compare the oxygen demand from the mineralization of nitrogen and carbon compounds. This aquatic macrophyte was collected in the littoral zone of the Monjolinho Reservoir (22° 00' S and 47° 54' W). In the laboratory, the plants were submitted to leaching for the acquirement of dissolved organic matter (DOM). Decomposition chambers (n = 13) were prepared with leachate under three different treatments: I: only DOM; II: DOM + nitrapyrin (nitrification inhibitor) and III: DOM + azide. The chambers were incubated in the dark under aerobic conditions and constant temperature (20°C). Dissolved oxygen (DO) concentrations from each chamber were periodically measured during 49 days. The results were fitted to first-order kinetic model. From the results, it was possible to verify that oxygen consumption (CO_{max}) was 2-fold smaller in Treatment I than in Treatment II (CO_{max} = 662 mg DO g^{-1} C), this result demonstrates that nitrapyrin, when inhibiting nitrification process generated a smaller dissolved oxygen demand during mineralization. The Treatment III presented a CO_{max} of 13 mg DO g^{-1} C. The mean deoxygenation coefficient (k_d) in incubations where nitrification was inhibited (k_d = 0.113 day^{-1}) was about 1.2 times higher than k_d where nitrification occurred (Treatment I = 0.139 day^{-1}); the k_d value of Treatment III (0.240 day^{-1}) was the highest among treatments. When M. aquaticum enters in senescence, it releases great amounts of leachate substances that remain directly available in water column of Monjolinho Reservoir. During leachate mineralization, a higher oxygen demand is used to the oxidation of nitrogen compounds in detriment of carbon (i.e. 2-fold) and the chemical oxidations represented only 1% of total oxygen uptake from mineralization process.
Key-words: oxygen demand, inhibition, nitrification, leachate, aquatic macrophytes.

RESUMO - Mineralização aeróbia de carbono e de nitrogênio de lixiviados de Myriophyllum aquaticum (Vell.) Verdc. A avaliação da degradação da matéria orgânica (compostos de carbono e nitrogênio) nos sistemas aquáticos é de extrema importância para o entendimento do metabolismo e funcionamento desses ambientes. Neste contexto, esse estudo visou descrever as cinéticas de consumos de oxigênio durante a decomposição de lixiviados de Myriophyllum aquaticum para comparar as demandas de oxigênio geradas das mineralizações de compostos de carbono e de nitrogênio. Essa espécie foi coletada na região litorânea do reservatório do Monjolinho (22° 00' S e 47° 54' W). Em laboratório, as plantas foram submetidas à lixiviação para a obtenção da matéria orgânica dissolvida (MOD), na seqüência foram preparadas câmaras de decomposição (n = 13) contemplando três tratamentos (I: apenas MOD; II: MOD + nitrapirina e III: MOD + azida). As câmaras foram mantidas sob condições aeróbias, no escuro e em temperatura controlada (20 °C). As concentrações de oxigênio dissolvido (OD) foram determinadas periodicamente nas câmaras durante 49 dias. Os resultados foram ajustados a um modelo cinético de primeira-ordem. Com base nesses resultados, verificou-se que o consumo de oxigênio ($OC_{máx}$) foi cerca de 2 vezes menor no Tratamento I em relação ao Tratamento II ($OC_{máx}$ = 662 mg OD g^{-1} C), demonstran-

do que a nitrapirina, ao inibir o processo de nitrificação, causou uma menor demanda de oxigênio dissolvido durante a mineralização. O Tratamento III apresentou um $OC_{máx}$ de 13 mg OD g^{-1} C. O coeficiente médio de desoxigenação (k_d) das incubações em que não houve a nitrificação (k_d = 0,113 dia^{-1}) foi cerca de 1,2 vezes maior que o k_d das incubações com nitrificação (Tratamento I = 0,139 dia^{-1}), o k_d para o Tratamento III foi o maior em relação aos Tratamentos I e II (0,240 dia^{-1}). Quando o M. aquaticum entra em senescência, grandes quantidades de lixiviados são disponibilizadas para a coluna d´água do Reservatório do Monjolinho. Durante a mineralização desses lixiviados, uma demanda duas vezes maior por oxigênio foi gerada durante a oxidação de compostos nitrogenados em relação aos de carbono e a oxidação química desses lixiviados representou apenas 1% do consumo total de oxigênio durante o processo de mineralização.
Palavras-chave: demanda de oxigênio, inibição, nitrificação, lixiviado, macrófita aquática.

Introduction

Organic matter in aquatic ecosystems is mainly composed by non-living biomass and detritus. Detritus are constituted of dead organic matter originating from any trophic level (such as amino acids, proteins, cells, tissues) and includes the products resulting from ejection, secretion and excretion processes (Moore at al., 2004). Those residues can be found as particulate organic matter (POM) or dissolved organic matter (DOM). Detritus sources within aquatic ecosystems can be allochtonous or autochthonous (Jonsson et al., 2001; Kritzberg et al., 2004). Most organic matter in aquatic environments appears in a dissolved form, in a proportion of ca. 10:1 to the particulate form (Wetzel, 2001). The largest fraction of DOM consists of high-molecular mass substances (Dalton et al., 2005) such as proteins, polysaccharides, nucleic acids and humic substances (Cabaniss et al., 2005).

DOM is released during active growth of organisms (e.g. exudates) and by the autolysis of cell membranes during senescence and death. During this release, there is a fast utilization of carbon and nitrogen forms by the microbiota (Wetzel, 2001). Thus, microorganisms play a fundamental role on carbon and nutrients (e.g. nitrogen and phosphorous) cycles, besides being important to the energetic balance in aquatic ecosystems (Wetzel, 1995). Heterotrophic bacterioplankton are the main DOM consumers. Despite its importance in the trophic chain, the accurate quantification of their contribution has been hampered by the lack of knowledge of factors that regulate the mechanisms through which DOM are respired by microorganisms or converted into biomass (Giorgio & Cole, 1998).

The carbon and nutrient cycling and its accumulation rate within aquatic systems depend on the balance between immobilization and mineralization processes. During aerobic decomposition, microorganisms convert detritus into smaller organic molecules. These small particles are transformed into inorganic product, such as H_2O, CO_2 and NH_4^+. This conversion of organic to inorganic molecules is known as mineralization. In nitrification, nitrogen reduced compounds (NH_3 and NH_4^+) are oxidized into nitrite (NO_2^-) and later on into nitrate (NO_3^-), these reactions are mediated by some bacteria (Esteves, 1998). The understanding of the nitrogen cycle is important because it is an essential element in protein chains (Esteves, 1998). Considering the leaching process during senescence of aquatic plants as the main autochthonous DOM sources for aquatic systems, this study aimed at describing and comparing the kinetics of oxygen uptake during the decomposition of Myriophyllum aquaticum leachate in order to quantify the oxygen demands during the mineralization of nitrogen and carbon compounds. It also aimed at estimating leachate oxidation kinetic coefficients of this process. This species, which belongs to the Haloragaceae family and Myrtales order, is an aquatic macrophyte original of South America and its occurrence is natural in Brazil. It is perennial and can grow totally submerse in water or project its ramification out of water. It remains rooted in the sediments that reach 2 m of depth (Kissman, 2000). In Monjolinho Reservoir M. aquaticum is the main species that colonizes the littoral zone of this aquatic environment.

Material and methods

Description of studied area

The Monjolinho Reservoir (22° 00' S and 47° 54' W) is a small artificial water system

located inside the campus of the Universidade Federal de São Carlos (SP, Brazil). Its maximum depth is 3 m, the flooded area comprises 46,881 m^2 and the reservoir has a volume of 76,679 m^3 (Angela T. Fushita, personal communication). Depending on the season, the hydraulic retention time of this system varies between 2 and 23 days. Monjolinho Stream provides the waters to the reservoir; the anthropic pressures on this watershed affect the water quality of this ecosystem (Marinelli et al., 2000).

Material sampling and preparation

Samples of M. aquaticum were collected from two distinct sites of Monjolinho Reservoir. In the laboratory, the plants were washed with tap water to remove the periphyton and attached material, and dried at 50 °C to a constant weight. The plants were then grounded and the fragments were fractionated using a pedological sieve set (Tecnal - model TE 650). Water samples (ca. 15 L) from the reservoir were also collected.

Extraction of the leachate and mineralization assays

Prior to the assays, aqueous extractions were performed to obtain DOM. About 10 g DW of plant was added to a flask containing 1 L of deionized water. The plants and deionized water were sterilized by autoclaving during 15 min, 1 atm and 121 °C (Ward & Johnson, 1996). After 24 h of cold aqueous extraction (4 °C) (Mfller et al., 1999), POM was fractionated from DOM centrifugation (1048 g, 1 h) and filtration through 0.45 mm pore size cellulose ester (Millipore). The solution was frozen (-20 °C) until the preparation of the experiment. Samples of the reservoir water were also filtered through 0.45 mm pore size (Millipore) ester cellulose membranes. After these filtrations the leachate was added to lagoon water and the organic carbon concentration (60.2 mg L^{-1}) was measured with a carbon analyzer (Shimadzu - model 5000A).

Mineralization assays

The leachates were incubated in darkness under aerobic conditions. These samples were submitted to three treatments: Treatment 1: 3 incubations with leachate and 2 control incubations containing only reservoir water; Treatment 2: 3 incubations with leachate + nitrapyrin (concentration: 50 mg L^{-1}) according to Nunes et al. (submitted) and two control chambers containing reservoir water + nitrapyrin (concentration: 50 mg L^{-1}) and Treatment 3: 2 chambers with leachate + azide (concentration: 0.05 mg L^{-1}) and 2 control incubations containing reservoir water + azide (concentration: 0.05 mg L^{-1}). All these chambers were incubated at 20 °C and the dissolved oxygen (DO) concentration was measured periodically with a DO meter (YSI, model 58; precision 0.03 mg L^{-1}), during 49 days. To avoid anaerobic processes, each chamber was aerated (with compressed filtered clean air) when the DO concentration reached concentrations below 2.0 mg L^{-1}. After DO concentration determination, the incubations were closed to avoid probable oxygen diffusion. The mean values of DO concentration of the control chambers (for each treatment) were subsequently subtracted from DO concentration mean values of the leachate chambers.

Mathematical modeling

Since oxygen consumption is directly related to oxidation of an organic resource and that this process is represented by first-order kinetics models (Bitar & Bianchini Jr., 2002), the temporal change of the evolved oxygen was fitted to first-order kinetics model (Eq. 1), using a non-linear method (iterative algorithm of Levenberg-Marquardt), according to Press et al. (1993). In these procedures, the time evolution of oxygen consumption was described by Equation 1:

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

where CO = accumulated consumed oxygen (mg g^{-1} C); CO_{max} = maximum oxygen consumption (mg g^{-1} C); k_d = deoxygenation coefficient (day^{-1}); t = time (day). The half-time ($t_{1/2}$) of leachate mineralization was calculated by Equation 2.

$$t_{1/2} = \ln 0.5 / -k_d \qquad (2),$$

The CO values for mineralization of carbon and nitrogen and chemical oxidations were calculated by subtraction from CO values of Treatments I, II and III. The values corresponding to the nitrification process resulted from the subtraction of data between CO from Treatment I (i.e. biochemical oxidation), Treatment II (where nitrification was inhibited) and Treatment III

(only chemical oxidation, since any microbial activity was inhibited with azide). The CO data were log transformed and statically analyzed using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests in order to look for significant differences among treatments ($p < 0.05$).

Results

Time variations of CO during the aerobic mineralization of M. aquaticum leachate submitted to different treatments are shown in Figure 1. In the beginning of the experiments consumption was higher for Treatments I and II (varying from 15th to 20th day). After this period, a gradual decrease tending to stabilization was observed. In Treatment III this tendency was not observed. From the fitting with Eq.1, the CO_{max} values were 13 mg DO g^{-1} C (Treatment III), 316 mg OD g^{-1} C (Treatment I) and 662 mg OD g^{-1} C (Treatment II). Referring to the total oxygen demand, the chemical oxidation (Treatment III) represented 1% of the total OC in the mineralization process, oxidation of nitrogen compounds represented 67% (Treatment II) and carbon compounds represented 32% (Treatment I). Deoxygenation coefficients (k_d) ± kinetic fittings errors from Treatments I, II and III were respectively 0.139 ± 0.007 day^{-1}, 0.113 ± 0.008 day^{-1} and 0.240 ± 0.028 day^{-1}. Half-times ($t_{1/2}$) from these treatments were respectively 6, 5 and 3 days (Tab. 1). k_d for

Table 1: Parameters from oxygen consumption model during aquatic macrophytes leachate aerobic decomposition.

Species	CO_{max} (mg g^{-1} C)	k_d (day^{-1})	Period (days)	References
WITHOUT INHIBITORS				
Myriophyllum aquaticum	991	0.122	49	This study
Utricularia breviscapa	1280	0.076	130	Santos et al. (2006)
Cabomba piauhyensis	1578	0.054	80	Peret & Bianchini Jr.,(2004)
Cyperus giganteus	1098	0.031	80	Peret & Bianchini Jr., (2004)
Egeria najas	1239	0.041	80	Peret & Bianchini Jr., (2004)
Eichhornia azurea	1151	0.042	80	Peret & Bianchini Jr., (2004)
Salvinia auriculata	256	0.044	80	Peret & Bianchini Jr., (2004)
Scirpus cubensis	1220	0.049	80	Peret & Bianchini Jr., (2004)
Utricularia breviscapa	1414	0.034	80	Peret & Bianchini Jr., (2004)
Salvinia auriculata (15°C)	2029	0.093	60	Panhota et al. (2006)
Salvinia auriculata (20°C)	2051	0.103	60	Panhota et al. (2006)
Salvinia auriculata (25°C)	2017	0.144	60	Panhota et al. (2006)
Salvinia auriculata (30°C)	1999	0.134	60	Panhota et al. (2006)
Utricularia breviscapa (15°C)	2123	0.057	60	Panhota et al. (2006)
Utricularia breviscapa (20°C)	1822	0.072	60	Panhota et al. (2006)
Utricularia breviscapa (25°C)	1890	0.077	60	Panhota et al. (2006)
Utricularia breviscapa (30°C)	2043	0.064	60	Panhota et al. (2006)
Salvinia molesta	1051	0.056	45	Panhota et al. (in press)
Myriophyllum aquaticum	1187	0.060	45	Panhota et al. (in press)
Mean	1496	0.071	-	
SD	507	0.034	-	
WITH INHIBITORS				
Utricularia breviscapa (with azide)	63	0.019	130	Santos et al. (2006)
Myriophyllum aquaticum (with azide)	13	0.240	49	This study
Myriophyllum aquaticum (with nitrapyrin)	316	0.139	49	This study

Treatment III (DOM + azide) was 2.2 the k_d from Treatment II and 1.7 the value for Treatment I.

The determination coefficients, r^2, varied from 0.90 to 0.98 for the fitting of the data in Figure 1 with the kinetics model of Equation 1, which indicates that this model was adequate to represent oxygen consumption from M. aquaticum during aerobic degradation processes. The statistical analysis of the kinetics model pointed to differences between Treatments I and II ($p < 0.001$) and III ($p < 0.01$).

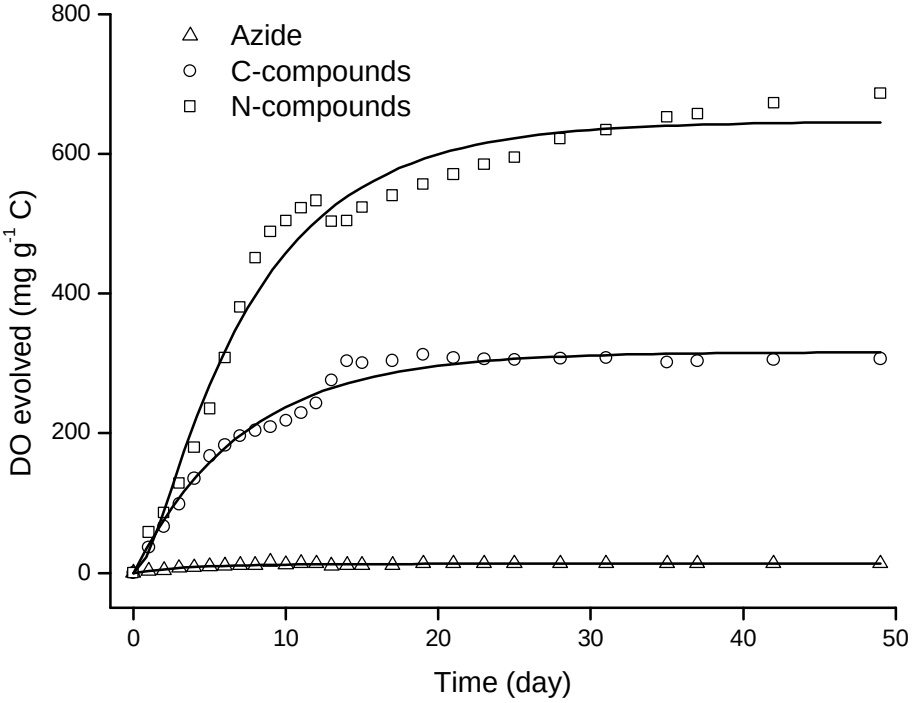

Figure 1: Kinetic fitting of consumed oxygen during mineralization and decomposition of M. aquaticum DOM under different treatments.

Discussion

Accumulated oxygen consumption has been used to evaluate the oxygen demand during aerobic decomposition processes of aquatic macrophytes (Farjalla et. al., 1999; Brum et al., 1999), amino acids and sugars (Cunha-Santino & Bianchini Jr., 2003), humic substances (Cunha-Santino & Bianchini Jr., 2002), polysaccharides excreted by alga (Antonio, 2004), alga cells (Pacobahyba et al., 2004), glucose (Panhota & Bianchini Jr., 2003), aquatic macrophytes leachates submitted to photodegradation (Santos et al., 2006), grass leaves (Branco & Rocha, 1977) and lignocellulosic detritus (Sciessere et al., 2006). In addition, the dissolved oxygen concentrations have been frequently used to evaluate the heterotrophic metabolism in aquatic ecosystems (Berman et al., 2001). In these studies the quantitative relations between oxygen consumption, CO_2 production and microbial activity are implicit (Characklis, 1990); in the molar basis, CO_2 concentration is approximately similar to consumed oxygen amount (Karl, 1986).

From the kinetics results, oxygen consumption in Treatments I and II was similar to results from other experiments (Borsuk & Stow, 2000; Bitar & Bianchini Jr., 2002). There was high DO consumption in the beginning of the mineralization processes, after which DO tended to stabilize. Since aquatic macrophytes detritus are chemically heterogeneous, presenting a label and a refractory fraction (Antonio, 2004), it is supposed that in the beginning the label fraction predominated, once this fraction demands a higher amount of DO for its oxidation than the refractory fraction. This tendency was not observed in Treatment III, because CO_{max} due to chemical oxidation represented 1% of the total CO_{max}

(i.e. nitrogen and carbon compounds oxidation). Aerobic mineralization assays from Utricularia breviscapa leachate showed a CO_{max} from chemical oxidation of 4.9% (Santos et al., 2006). These chemical oxidation processes can be exemplified by oxidative decarboxylation of hydrolyzed tannins (i.e. polyphenols) such as gallic and ellagic acids (Queiróz et al., 2002). With regard to k_d, its values were of the same order of magnitude for the 3 treatments. According to the $t_{1/2}$ presented in M. aquaticum leachate mineralization the oxygen consumption is characterized as short term processes (ca. 6 days).

Table I summarized the parameters obtained in this study and in others that adopted the same kinetics model. One notes that the CO_{max} mean value in assays without any inhibitor (i.e. assays that consider C and N mineralization) was 1,406 ± 507 mg DO g C^{-1}. The minimum CO_{max} value was reported for Salvinia auriculata (Peret & Bianchini Jr., 2004), while the maximum one was recorded for Utricularia breviscapa at 15°C (Panhota et al., 2006). Comparing the sum of these demands (from Treatments I, II and III) of M. aquaticum leachate with the other studies presented in Table I, the CO_{max} between these three treatments was smaller than the mean value registered (n = 19; 1496 mg DO g C^{-1}), but the values are within the same order of magnitude.

In another experiment with mineralization of M. aquaticum leachate collected from the same reservoir as in the present study, CO_{max} was 1,187 mg DO g^{-1} C (Panhota et al., in press), which is 1.2 the CO_{max} measured in the present study. This difference can be attributed to some qualitative and quantitative differences of the microorganism community in the reservoir, since these experiments were conducted on distinct periods.

k_d from M. aquaticum was 1.7 the average value of studies summarized in Table I. Despite these differences between k_d from aerobic leachate mineralization, this constituted a short-term process ($t_{1/2}$ varying from 5 to 22 days).

The CO_{max} for the sum of Treatments I, II and III of M. aquaticum leachate mineralization in this experiment was 7 times the values of humic acids mineralization found by Cunha-Santino & Bianchini Jr. (2004). This fact suggests the label characteristic of leachate compounds, mainly presented in the protoplasmatic fractions and structural tissues of plants (Webster & Benfield, 1986). Leachates are formed by dissolution of polar composites (Moorhead et al., 1996) and generally are constituted by carbohydrates, polyphenols and nutrients, such as nitrogen, orthophosphate, phosphor, potassium and calcium (Suberkropp et al., 1976; Best et al., 1990; Gupta et al., 1996; Mun et al., 2001).

Nitrogen cycling is mostly of microbial nature. Transformations that occur in N cycling (i.e. amonification, nitrification and denitrification processes) are coupled to each other and also influenced by carbon availability and microbial metabolism. Amonification is controlled by the C:N ratio; if in some environments this ratio is high, nitrogen is basically converted into microorganism biomass; if the ratio is low, a NH_4^+ production can be observed (Strauss & Lambert, 2002). As for nitrapyrin as nitrification inhibitor, it inhibits the ammonia monooxigenases enzymes, thus inhibiting ammonia oxidation. Relating to nitrapyrin as nitrification inhibitor, it inhibits ammonia monooxigenases enzyme, and consequently inhibiting ammonia oxidation and according to Strauss & Lambert (2000) this inhibitor do not affect directly the microbial community, once it was not observed great changes in biomass and respiration rates.

In aquatic systems, microbial degradation affects indirectly the regulation of carbon and nutrients (e.g. N and P) from aquatic macrophytes detritus. These effects are related to k_d, i.e. metabolic processes with high k_d usually have shorts half-life periods. Hence, chemical elements associated with these processes do not tend to accumulate in ecosystems. As the half-life period found for C and N compounds of M. aquaticum leachate are low (ca. 6 days), these elements tend to be metabolized within the water column or exported, depending on the residence time of the reservoir, that varies with the season (rainy season = 2 days and dry season = 23 days; Nogueira & Masumura-Tundisi, 1994).

When M. aquaticum reach a senescence phase, it releases great amounts of leachate that remain directly available for heterotrophic microorganisms in the water column of Monjolinho Reservoir. Despite the lack of studies focusing on mineralization of DOM nitrogen from leachates of aquatic macrophytes, the results presented here showed an oxygen

demand for nitrogen compounds 2-fold higher than carbon compounds mineralization. This demonstrates the importance of M. aquaticum leachate nitrification in the budget of dissolved oxygen of Monjolinho Reservoir once it is the main specie that colonizes the littoral zone of this environment. Thus for the metabolism and functioning of Monjolinho Reservoir, the M. aquaticum is the major responsible for the autochthonous detritus input and oxygen budget that originated from aquatic macrophyte decomposition.

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