Photodegradation, chemical and biologic oxidations from mineralization of Utricularia breviscapa leachate.

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ABSTRACT: Photodegradation, chemical and biologic oxidations from mineralization of Utricularia breviscapa leachate. The aquatic macrophytes are important source of organic matter. During the decomposition, this plants release large quantities of dissolved and particulate organic matter that are channeled to the trophic chains. This research aimed at determining the oxidation coefficients of leachate extracted from one aquatic macrophyte species (Utricularia breviscapa), collected in the Óleo Lagoon (21° 36' S and 47° 49' W; Luiz Antonio, SP). The leachate was submitted to photodegradation, chemical oxidation and bacterial activity; for which eight chambers with leachate and lagoon water were used. The incubations were maintained under aerobic conditions, with four kept under solar radiation (two with azide and two without azide) and four kept in the dark (two with azide and two without azide). During 130 days the dissolved oxygen concentrations were measured periodically in the chambers. The results were fitted to first-order kinetics model. The deoxygenation coefficient (k_d) was approximately seven times higher in the incubations without azide and not exposed to solar radiation (biological oxidation) than in the incubations without azide and exposed to solar radiation (photodegradation). It was also approximately four times more elevated than for incubations where only chemical oxidation occurred. Based on the experimental results we concluded that although the chemical oxidation and photodegradation are important processes on mineralization of U. breviscapa leachate; the biological oxidation is more effective process in the cycling of dissolved organic matter of this macrophyte in the Óleo Lagoon.
Key-words: aquatic macrophyte, dissolved organic carbon, decay rates, microbial activity, solar radiation.

RESUMO: Fotodegradação, oxidações química e biológica da mineralização do lixiviado de Utricularia breviscapa. As macrófitas aquáticas representam importante fonte de matéria orgânica em ambientes aquáticos. Durante a decomposição, disponibilizam para as redes tróficas, grandes quantidades de constituintes orgânicos na forma de matéria orgânica dissolvida e particulada. Neste trabalho determinaram-se os coeficientes de oxidação dos lixiviados extraídos de uma espécie de macrófita aquática (Utricularia breviscapa), coletada na Lagoa do Óleo (21° 36' S e 47° 49' W; Luiz Antonio, SP). Os lixiviados foram submetidos à fotodegradação, oxidação química e à atividade microbiana. Para tanto, foram preparadas oito câmaras contendo lixiviado e água da lagoa; foram mantidas sob condições aeróbias: quatro foram incubadas sob radiação solar (duas com azida e duas sem) e quatro foram incubadas no escuro (duas com e duas sem azida). As concentrações de oxigênio dissolvido foram determinadas periodicamente nas câmaras durante 130 dias. Os resultados foram ajustados a um modelo cinético de primeira-ordem. Com base nessas cinéticas verificou-se que o coeficiente de desoxigenação (k_d) foi cerca de sete vezes maior nas incubações sem azida e não submetidas à radiação solar (oxidação biológica) do que para as incubações acrescidas de azida e expostas à radiação solar (fotodegradação). Também foi aproximadamente quatro vezes mais elevado em relação às incubações em que ocorreram apenas oxidações químicas. Com base nos resultados experimentais concluiu-se que apesar da oxidação química e fotodegradação serem processos importantes na mineralização do lixiviado de U. breviscapa, a oxidação biológica é o processo mais efetivo na ciclagem da matéria orgânica dissolvida dessa macrófita na Lagoa do Óleo.
Palavras-chave: macrófitas aquáticas, matéria orgânica dissolvida, taxas de decaimento, atividade microbiana, radiação solar.

Introduction

In aquatic ecosystems, the dominant forms of organic matter are the living biomass and detritus (Odum, 2004). Detritus can be defined as any form of non-living organic matter, including secreted, excreted or exuded products from organisms (Moore et al., 2004). These residues are found as particulate organic matter (POM) and dissolved organic matter (DOM), and represent, frequently, the main sources of energy on aquatic ecosystems (Ziegler & Fogel, 2003).

Aquatic macrophytes are important source of organic matter since during decomposition they release large quantities of DOM and POM to the trophic chains (Carvalho et al., 2005). The microbial metabolism transformations of DOM and POM are essential for dynamics of carbon, nutrients and energy flow in aquatic ecosystems (Wetzel, 1995). After senescence, with the losses of tissues integrity, large amounts of hydrophilic cellular components are rapidly released (Cunha & Bianchini Jr., 1998). Then, considerable quantities of DOM are produced since the first decomposition stages. The conversions of vascular tissues of aquatic plants into DOM are particularly important once this process transfer carbon to free attached and microorganisms on particulate detritus (Sala & Guide, 1999).

Decomposition is affected by several factors, including temperature (Antonio & Bianchini Jr., 2002), refractory degree of substrates (Wetzel, 1990), available nutrients to microbial activity (Amon & Benner, 1996), oxidant agents, such as oxygen and inorganic and organic electrons acceptors (Kristensen & Holmer, 2001) and solar radiation (Anesio et al., 2000). Achterberg & Van den Berg. (1994) showed that oxidation of organic matter can be fast and efficient under an intensive and continuous source of ultraviolet radiation. This process involves chemical reactions with molecules containing chromophores (e.g. humic and fulvic acids) wich are light absorbing components in natural waters and play an important role in aquatic photochemical processes (Brezonik, 1994).

A positive effect from photodegradation process is the production of labile low-molecular weight organic compounds into bioavailable compounds (Bertilsson & Tranvik, 2000). Wetzel et al. (1995) demonstrated an increase in the efficiency of bacterial growth using DOM exposed to photodegradation. On the other hand, photodegradation changes the quality of DOM (Skoog et al., 1996) because photobleaching enhances light penetration into the water column, thus increasing the solar radiation effects on microorganisms (Herndl et al., 1997).

In this paper we investigate the effects from photodegradation and oxidation on the aerobic mineralization of the leachate of the aquatic macrophyte Utricularia breviscapa, motivated by the importance of the leaching process during senescence of aquatic plants as source of autochthonous DOM to aquatic systems. In addition, the kinetic parameters of photodegradation, biological and chemical processes are estimated.

Material and methods

Sampling Site

Óleo Lagoon (21° 36'S and 47° 49'W) is one of the many oxbow lagoons in the Mogi-Guaçu river floodplain situated within the Natural Reserve of Jataí (21° 33' to 21° 37'S and 47° 45' to 47° 51'W; Luiz Antonio, São Paulo, Brazil). It is a shallow (Z_{mean} = 2.55 m and Z_{max} = 5.10 m) and small (19,470 m²) lagoon; based on the mean average annual variation of limnological variables (mean ± SD), it is an acidic (pH: 5.49 ± 0.65) lagoon with relatively low concentrations of dissolved oxygen (3.57 ± 2.18 mg l^{-1}) and dissolved organic carbon (3.05 ± 0.98 mg L^{-1}); the water temperature usually varies from 18°C ± 2 (July) to 30°C ± 1 (January); (Cunha-Santino, 2003; Petracco et al., submitted). Based on analysis of values to chemical (nitrogen and phosphorous) and biological (chlorophyll) variables of lagoon, it was possible classify this system as oligotrophic (Wisniewski et al., 2000). The relation between the depths of Secchi disc extinction (Z_{ds}) and euphotic zone to the Óleo Lagoon is Z_{eu} = 1.91 x Z_{ds} (Petracco et al., submitted). It has been classified as a seepage lagoon (Santos et al., 1995).

Water and macrophyte sampling

The aquatic macrophytes were collected in distinct points of littoral zone of Óleo Lagoon. The plants were washed within Lagoon water to remove periphyton, sediment particles and coarse material (Osgburn et al., 1987). In laboratory, the plants were washed with tap water, oven-dried (40°) and ground

(Tecnal; model TE-650). Prior to the assays, aqueous extractions were performed to obtain DOM. The extraction comprised addition of 10 g DW of grounded plant in a flask containing 1L of deionized water. The plants and deionized water samples were sterilized by autoclaving during 15 min, 1 atm and 121°C (Ward & Johnson, 1996). After 24 h of cold aqueous extraction (4°C) (Miller et al., 1999), POM was fractionated from DOM centrifugation (1048 x g, 1 h) and filtration through 0.45 mm pore size cellulose ester (Millipore). The solution was frozen (-20°C) in plastic bottles to avoid microorganisms on samples before carrying out the experiment.

Samples of lagoon water were collected with a Van Dorn bottle (from 0.5m and 5.0m). The water samples were integrated (equivalent volumes of aliquots water were mixed); this procedure was carried out in order to take a broad bacterial sample. In laboratory, this sample was filtered through cellulose ester membrane (Millipore; pore size = 0.45 mm). The macrophyte leachate was added into eight BOD bottles pre-washed with Extran 20% (glasses volume = 1 L). The concentrated leachate was defrosted and dilutions in samples of lagoon water were performed to a final dissolved organic carbon (DOC) concentration of 99.85 mg C^{-1}. The DOC concentrations were determined by catalytic oxidation at high temperature (Shimadzu, model 5000A). It was set up four flasks with addition of aliquots of 0.5% azide; two of theses flasks were exposed to solar radiation (302.7 ± 501.0 mmol s^{-1} m^2; min: 90.07 mmol s^{-1} m^2 and max: 1602.6 mmol s^{-1} m^2; mean photoperiod of October and January = 13.0 hours) and the others (n = 2) were incubated in darkness; in this case the BOD bottles were wrapped in aluminum foil. Two bottles without azide solutions and two control bottles (with lagoon water) were incubated in the dark. The temperature average of incubations were 25.3 ± 1.6°C (min: 20.9°C; max: 28.8°C); it was similar to annual temperature average (25.6°C) of Óleo Lagoon (Cunha-Santino, 2003).

The dissolved oxygen (DO) concentrations were measured periodically during 130 days with a DOmeter (YSI, model 58). After each measurement, the bottles were closed to prevent the loss of oxygen to atmosphere. In order to maintain the solutions under aerobic conditions, when the concentrations of DO had decreased to ca. 2.0 mg L^{-1}, the bottles were oxygenated during 1 hour to keep DO near saturation. The average values of oxygen concentration of control chambers were subtracted of average values of chambers with leachate and no addition of azide to neutralize the quantity effects of organic matter present in Óleo Lagoon.

Considering that oxygen consumption is directly related with an organic resource oxidation and that this process is represented by first-order kinetics models (Bitar & Bianchini Jr., 2002), the temporal variation in the evolved oxygen was fitted to first-order kinetics model (Eq. 1), using a non-linear method (Levenberg-Marquardt iterative algorithm), according to Press et al. (1993).

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

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}); and t = time (day).

The half-time ($t_{1/2}$) of deoxygenation derived from aerobic decomposition of U. breviscapa leachate was calculated by the Equation 2.

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

The estimative of stoichiometric relation between consumed oxygen and oxidized carbon (O/C) were calculated based on the difference between the maximum amount of consumed oxygen (CO_{max}) obtained by kinetic adjustments and mineralized organic carbon (DC = $TOC_{initial}$ – TOC_{final}).

The difference between the values of CO from bottles kept in darkness without azide (referring to biological oxidation + chemical oxidation) and chemical oxidation (flasks in darkness with azide) was performed to obtain the values related to biological oxidation. The photodegradation process results from difference between the CO obtained in bottles exposed to solar radiation evolving the chemical oxidation + photodegradation (incubations submitted to light with azide) and the values obtained in bottles kept in darkness with azide (chemical oxidation). The effect of oxidation from the flasks with leachate was corrected by subtraction of CO values from those control flasks. The CO data were log

transformed and statistically analyzed using analysis of variance (ANOVA) followed by Tukey's studentized test in order to verify for significant differences among treatments ($p < 0.05$).

Results and Discussion

Mineralization experiments with aquatic macrophytes leachates showed changes in stoichiometric between consumed oxygen and oxidized carbon due to the increase of microorganism biomass (Peret & Bianchini Jr., 2004). The determination of accumulated consumption of oxygen has been used to assess the labile fractions during the decomposition of aquatic macrophytes (Farjalla et al., 1999) and mineralization of DOM (Panhota & Bianchini Jr., 2003). In this context, the Figure 1 presents the accumulated consumption of oxygen from chemical (Fig. 1A) and biological oxidation (Fig. 1B) and photodegradation (Fig. 1C) process of leachate from Utricularia breviscapa. The Tab. I presents the parameterization of kinetic model obtained from fittings.

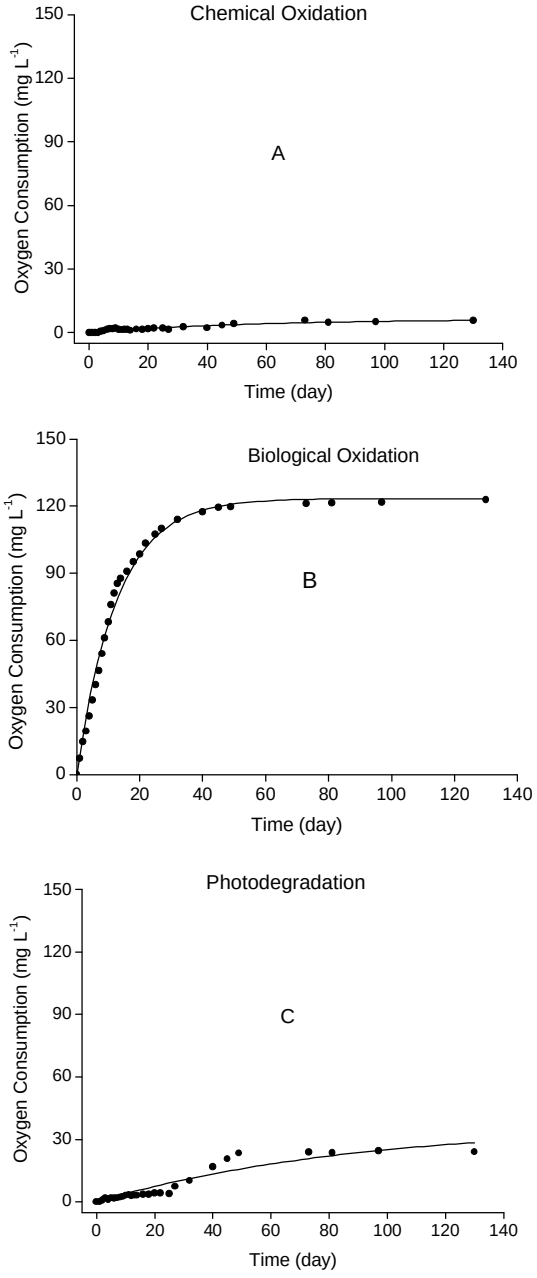

Figure 1: Accumulated oxygen consumption from aerobic mineralization of Utricularia breviscapa leachates.

The high determination coefficients (r^2 = 0.89 to 0.99) indicated that the mathematical model was robust to describe the kinetics of aerobic process. The kinetics of oxygen consumption (bottles exposed to solar radiation or darkness) was similar to that reported in other studies (Bitar & Bianchini Jr., 2002; Cunha-Santino, 2003; Romeiro, 2005). High oxygen consumption occurred in the beginning, especially until the 15th day, and then decreased in later stages of the experiment until reaching a stable value. The consumed oxygen (CO_{max}; Tab. I) varied from 6.30 mg L^{-1} for chemical oxidation to 123.41 mg L^{-1} for biological oxidation, corresponding to 63.1 and 1234.1 mg DO g^{-1}C of leachate from U. breviscapa, respectively.

Table I: Parameterization of kinetic model and organic carbon budget of the incubations with U. breviscapa leachate; DC: mineralizad organic carbon amount; O/C: stoichiometric relation between consumed oxygen and mineralized carbon; CO_{max} = oxygen consumption; k_d = DO consumption coefficient; $t_{1/2}$: DO consumption half-time; r^2 = determination coefficient and error = error referred to the kinetics fittings.

Process	CO_{max} (mg L^{-1})	Error	k_d (day^{-1})	Error	$t_{1/2}$	r^2	ΔC (mg L^{-1})	O/C
Reactions in darkness								
Chemical Oxidation	6.30	0.69	0.019	0.003	36.5	0.89	1.17	5.38
Biological Oxidation	123.41	1.49	0.079	0.003	8.8	0.99	84.83	1.45
Chemical + Biological Oxidation	127.84	1.42	0.076	0.002	9.1	0.99	86.00	1.49
Reactions in light								
Chemical Oxidation + Photodegradation	42.96	6.79	0.012	0.003	57.8	0.92	71.39	0.60
Photodegradation	36.95	7.52	0.011	0.003	63.0	0.89	70.22	0.53

Aerobic mineralization experiments incubated in the dark (equivalent to biological + chemical oxidation) present the following values to CO: 1190.0 and 1240.0 mg DO g^{-1} C of glucose (samples of water from an eutrophic reservoir; Panhota & Bianchini Jr., 2003); 139.9 mg DO g^{-1} C of humic acid and 581.9 mg DO g^{-1} C of fulvic acid (humic substances from an oxbow lake; Cunha-Santino & Bianchini Jr., 2004); 573.5 mg DO g^{-1} C of tannic acid (sample of water from Monjolinho Reservoir; Cunha-Santino et al., 2002); 1060.0 and 879.9 mg DO g^{-1} C of glycine and lysine (sample of water from Monjolinho Reservoir; Cunha-Santino & Bianchini Jr., 2003) and 595.2 mg DO g^{-1} C of glucose (sample of water from an oxbow lake; Antonio & Bianchini Jr., 2002). The CO_{max} from U. breviscapa leachate in dark chambers were ca. two (biological oxidation; 1235.3 mg DO g^{-1}C of leachate) and nine times (biological + chemical oxidation; 1280.3 mg DO g^{-1} C of leachate) higher than that obtained for fulvic and humic acids, respectively. These points to the labile nature of the compounds in the leachate produced by dissolution of polar compounds (Moorhead et al., 1996) from the aquatic macrophytes cytoplasm (Webster & Benfield, 1986). In this context, the leachates are probably constituted by carbohydrates, polyphenols and nutrients such as nitrogen, phosphate, potassium and calcium (Suberkropp et al., 1976; Best et al., 1990; Gupta et al., 1996; Mun et al., 2001).

The deoxygenation coefficient (k_d) derived from parameterization of Equation 1 varied between 0.011 ± 0.003 day^{-1} (photodegradation; $t_{½}$ = 63.0 days) to 0.079 ± 0.003 day^{-1} (biological oxidation; $t_{½}$ = 8.8 days; Tab. I). The k_d was seven times higher for chambers without azide and not exposed to solar radiation (biological oxidation; figure 1B) than that ones without azide and maintained under light conditions (photodegradation; figure 1C). On average, it was four fold higher than the treatment regarding to chemical oxidation.

The values of k_d obtained in aerobic mineralization of leachate extracted from aquatic macrophyte were: 0.054 day^{-1} (Cabomba furcata); 0.031 day^{-1} (Cyperus giganteus); 0.041 day^{-1} (Egeria najas); 0.042 day^{-1} (Eichhornia azurea); 0.049 day^{-1} (Oxycaryum cubense); 0.044 day^{-1} (Salvinia auriculata) and 0.034 day^{-1} (Utricularia breviscapa; Peret & Bianchini Jr., 2004). Values obtained to some organic

compounds were: 0.016 day^{-1} (glucose), 0.025 day^{-1} (sucrose), 0.050 day^{-1} (starch) and 0.048 day^{-1} (lysine; Cunha-Santino & Bianchini Jr., 2003). The value obtained in this study (0.076 day^{-1} = chemical + biological oxidation) was higher than the values observed in experiments of Cunha-Santino & Bianchini Jr. (2003) and Peret & Bianchini Jr. (2004). These low values of k_d compared with the value obtained in the present study can be related with the incubation temperature, that was 20°C for leachate of various species of aquatic macrophyte and for organic resources and 25°C in the present study. The increase of k_d as a consequence of temperature variation was suggest by Romeiro (2005) in studies of oxygen consumption during the degradation of Ludwigia inclinata (Q_{10} = 2.11). The intensity of macrophyte leaching depends on plant species (size, morphological structure), that determining the MOD quality (Park & Cho, 2003).

The accumulated oxygen consumption represents the production of CO_2 derived from DOM oxidation. Therefore, higher oxygen consumption was observed with the treatment mediated by heterotrophic process (Fig. 1B). In these chambers the consumption of carbon (DC = 84.83 mg L^{-1}; Tab. I) was higher than in those with the treatment with azide and keeping the samples in the dark (DC = 1.17 mg L^{-1}; Tab. I). This chemical process can be exemplified by hydrolysable tannins decarboxylation such as gallic acid and ellagic acid (Queiróz et al., 2002). The treatment involving solar radiation and with azide also led to a high carbon consumption (DC = 70.22 mg L^{-1}). In this case the production of hydrogen peroxide by photodegradation (Brezonik, 1994) can be acting as an oxidant agent for the organic matter from the leachate of U. breviscapa. When excited, photosensitive molecules transfer energy to other molecules in the dissolved organic matter, thus forming highly reactive species, e.g.: peroxide, superoxide, and hydroxyl radicals (Campos et al., 2001).

The chemical + biological oxidation and biological oxidation (Fig. 1B) were similar ($p > 0.05$); the chemical oxidation (Fig. 1A) was significantly different ($p < 0.05$) of all treatments; this is inferred from the analysis of O/C for the various treatments in Tab. I. The lowest stoichiometry relation (average = 0.56) was obtained for samples exposed to solar radiation and with addition of azide, while the chamber maintained in darkness with azide (chemical oxidation) displayed the highest stoichiometry coefficient (5.83). On the other hand, the bottles without azide regardless of the exposure to light had practically the same O/C (1.45 and 1.49). Hence, in the process with heterotrophic organisms, the stoichiometric values are an indirect form to identify the metabolic routes used by these microorganisms (Cunha-Santino & Bianchini Jr., 2003). This fact suggests that, despite the difference in the reaction velocity (k_d), similar processes are involved in the biological oxidation and biological + chemical oxidation ($p > 0.05$). In treatments exposed to solar radiation, the hydrogen peroxide produced causes the values of OC_{max} to be underestimated (Cunha-Santino & Bianchini Jr., 2003), generating low stoichiometric coefficients.

The O/C for the biological + chemical oxidation and biological oxidation obtained in the present study is similar to those for leachates of other macrophyte species from Óleo Lagoon. Indeed, Peret & Bianchini Jr. (2004) reported the following O/C values: Cabomba furcata = 1.57; Cyperus giganteus = 1.09; Egeria najas = 1.24; Eichhornia azurea = 1.15; Oxycaryum cubense = 1.22; Salvinia auriculata = 0.25 and Utricularia breviscapa = 1.41. Therefore, the metabolic routes by decomposition microorganisms are similar.

The DOM released during the degradation of U. breviscapa can be an important process in Óleo Lagoon. Indeed, since it is a submerged aquatic macrophyte and the senescent biomass is in permanent contact with the water, the leachates quickly become part of DOM pool. The leaching is responsible for ca. 23% of detritus mass loss of U. breviscapa (Cunha-Santino, 2003; Peret & Bianchini Jr., 2004). The leachate has a high potential to be utilized (high values of k_d) by autotrophic (e.g. mineral nutrients) and heterotrophic organisms (e.g. organic matter). From a systemic point of view, based on the $t_{1/2}$ from Tab. I, we infer that the oxygen consumption was fast (4.8 to 63.0 days). Within a short time within Óleo Lagoon, the leachate from U. breviscapa can support the processes involved with carbon consumption (e.g. microbial uptake) and indirectly the availability of oxygen. Werh et al. (1998) showed that depending on the source of DOM, the planktonic production, the composition of species and the microorganisms activity can be affected. In

addition to chemical and biological oxidations, in the upper layers of Óleo Lagoon photodegradation can also improve oxygen consumption. The stoichiometric values of oxidations were similar for process involving microbial activity, suggesting that even though these processes were mediated by distinct populations the catabolic routes were similar. In this context, based on the experimental results we concluded that although the chemical oxidation and photodegradation are important processes on mineralization of U. breviscapa leachate, the biological oxidation is more effective process in the cycling of dissolved organic matter of this macrophyte in the Óleo Lagoon.

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