

CELLULASE AND XYLANASE ACTIVITY DURING THE DECOMPOSITION OF THREE AQUATIC MACROPHYTES IN A TROPICAL OXBOW LAGOON

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ABSTRACT

Due to the connection between enzymatic activity and degradation of different fractions of organic matter, enzyme assays can be used to estimate degradation rates of particulate and dissolved organic carbon in freshwater systems. The aim of this study was to quantify and model the enzymatic degradation involving the decomposition of macrophytes, describing temporal activity of cellulases (EC 3.2.1.4 and EC 3.2.1.91) and xylanase (EC 3.2.1.8) during *in situ* decomposition of three aquatic macrophytes (*Salvinia* sp., *Eichhornia azurea* and *Cyperus giganteus*) on the surface and water-sediment interface (w-s interface) of an oxbow lagoon (Óleo lagoon) within a natural Brazilian Savanna Reserve. Overall, the enzymatic degradation of aquatic macrophytes in Óleo lagoon occurred during the whole year and was initiated together with leaching. Xylanase production was *ca.* 5 times higher than cellulase values due to easy access to this compound by cellulolytic microorganisms. Enzymatic production and detritus mass decay were similar on the surface and w-s interface. *Salvinia* sp. was the most recalcitrant detritus, with low mass decay and enzymatic activity. *E. azurea* and *C. giganteus* decomposition rates and enzymatic production were high and similar. Due to the physicochemical homogeneity observed in the Óleo lagoon, the differences between the decay rates of each species are mostly related with detritus chemical quality.

Key words: enzymes, litterbags, mass decay, particulate organic matter.

INTRODUCTION

Aquatic macrophytes play an important role in the detritus food chain and metabolism of aquatic systems (12). These detritus can be responsible for *ca.* 50% of the organic matter and nutrient inputs in these systems (43). After senescence, the bulk of macrophyte tissue enters the detritus pool, where

microorganisms are involved in its breakdown and mineralization (16). The structural compounds (cellulose, hemicelluloses and lignin) represent up to 70% of the aquatic macrophytes dry weight and the remaining mass is represented by the protoplasmatic fraction and hydrosolubles compounds (7).

Bacteria and fungi, with their respective extracellular

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enzyme capabilities, act together in the decomposition and mineralization of plant material in aquatic ecosystems (31), which is an important step in the nutrient and carbon cycle. These microorganisms need to produce extracellular enzymes to break structural compounds into smaller molecules that can be assimilated (8). Abiotic factors such as pH (20), temperature (24) and nutrients (19) influence the decomposition rates together with detritus chemical quality (30). The rates of microbial degradation of lignocellulosic detritus influence both the secondary production and rates of nutrient regeneration in aquatic ecosystems (2).

Cellulose is the major component of lignocellulosic detritus and its complete hydrolysis needs a multicomponent enzyme system comprised of three major enzymes (endoglucanase, exoglucanase and β -glucosidase) (3). In aerobic conditions, the microorganisms secrete a combination of those enzymes into the environment, which acts in synergy on the substrate, known as noncomplexes systems (39). The second major group of cellulolytic systems is the complexed systems wherein the cellulases are organized in relation to each other so that their action can be collective. They are typically produced by anaerobic organisms (22)

Hemicelluloses are the second most common polysaccharides in nature and represent about 20-35% of lignocellulosic biomass (32). In contrast to cellulose, hemicelluloses are easily hydrolysable polymers and do not form aggregates (27). Xylan is the most abundant of the hemicelluloses (14) and its hydrolysis requires a range of xylanases and accessory enzymes (11).

The most important enzymes related to particulate organic carbon decomposition are those involved in the degradation of lignocellulosic materials (35). There is a general consensus that studying extracellular enzymes involved in decomposition may provide valuable information about the nutrient and carbon cycle in ecosystems (30). There are few *in situ* studies in enzymatic decomposition of tropical aquatic ecosystems (30, 26) and deficient knowledge of enzymatic decomposition in

multispecies cultures and mixed communities (22).

Due to the connection between enzyme activity and degradation of different fractions of organic matter, enzyme assays can be used to estimate degradation rates of particulate and dissolved organic carbon in freshwater systems (36). The aim of this study is to quantify and model enzymatic degradation involving the decomposition of macrophytes, describing temporal cellulase and xylanase activity during decomposition of three aquatic macrophytes (*Salvinia* sp. Aubl., *Eichhornia azurea* Kunth and *Cyperus giganteus* Vahl) on the surface and water-sediment interface of an oxbow lagoon.

MATERIAL AND METHODS

The Ecological Station of Jataí (21° 33'to 21° 37'S and 47° 45'to 47° 51'W) has 13 oxbow lagoons of the Mogi-Guaçu river in a Brazilian Savanna Reserve of Luiz Antonio, SP. The Óleo lagoon, located at 400 m from the river, is shallow and small ($Z_{\text{máx}} = 5.5$ m and 17.800 m²), and has dissolved oxygen values varying from 0.01 to 7.70 mg L⁻¹ on the surface and from 0.88 to 2.91 mg L⁻¹ on the w-s interface and is an acid lagoon (pH from 4.01 to 6.79) (28). The macrophyte community is well developed in the littoral zone, comprising 13 species (28) of floating, emergent and submersed life forms.

Physicochemical parameters

The physicochemical parameter data were collected every sampling day (1, 3, 5, 30, 60, 100, 120, 150, 200, 250 and 300 days) in the Óleo lagoon. The pH values, electrical conductivity, water temperature and dissolved oxygen were measured in the lagoon with a multi-parameter checker (Horiba – U-10). Water samples were collected and taken to the laboratory to analyze the dissolved organic carbon (TOC analyzer – Shimadzu 5000A), total nitrogen (40) and total phosphorus (38).

Litter bag incubation

The plants were collected in the Óleo lagoon, washed in tap water and dried (45 °C) at a constant weight in the laboratory. The 216 litter bags (20 × 20 cm; mesh size = 0.4 mm) were prepared with 15 g (DW) of *Salvinia* sp., *Eichhornia azurea* and *Cyperus giganteus* fragments previously dried (72 for each species). The litter bags were incubated in the sites where the respective species is most abundant in the lagoon in two incubation depths (surface $Z \approx 0.2$ m and water-sediment interface $Z \approx 3.0$ m) on January 2008. Every sampling day, 6 litter bags (3 from surface and 3 from water-sediment interfaces) were taken to the laboratory to analyze the remaining particulate material.

In order to quantify the remaining organic matter content, the remaining detritus dried at 40-50 °C until the constant dry weight was weighed (mass loss analysis) and incinerated in a muffle furnace at 550 °C for two hours. The cellulose and hemicellulose contents in the detritus were measured using gravimetric methods according to Crampton & Maynard (9) and Han & Rowell (13).

Enzymes activities

For each sampling day, 3 sub-samples (2.0 g FW) were used for enzymes assays. The sub-samples were blended (Ultra-Turrax model T10; Germany) with 10 mL of acetate buffer (50 mM /pH 5.2), sonicated (ultrasound Unique, Brazil) and centrifuged (3,000 × g, 30 min, 4°C; Heraeus 122 Instruments, Megafuge 3.0R, Germany). These enzymatic extracts were used in the cellulase and xylanase activity determination, both determined by spectrophotometric methods (Amersham Biosciences, Ultrospec 2100 pro, Sweden). The cellulase (EC 3.2.1.4 and EC 3.2.1.91) and xylanase (EC 3.2.1.8) activities were determined using the method described by Mandels *et al.* (23), and Highley (15), respectively. The reducing sugars released by the enzyme action on a specific substrate (pure cellulose filter (Whatman n° 1 for cellulase and xylan for xylanase)) were quantified using the Somogy method

(540 nm) (37).

Mathematical modeling of organic matter decay

The temporal variation of remaining particulate organic matter (POM) (ash free) was fitted to a biphasic decay model (21) using non-linear regression, the iterative algorithm of Levenberg-Marquardt (29) according to equation 1:

$$POM = (POM_{LS} \times e^{-k_T t}) + (POM_R \times e^{-k_R t})$$

where: POM_{LS} = initial labile organic matter content (%); POM_R = Initial particulate refractory organic matter content (%); $k_T = k_1 + k_2$; global mass loss coefficient (= labile mineralization coefficient (k_1) + soluble leachate coefficient (k_2))(day⁻¹); k_R = refractory mass loss coefficient (day⁻¹).

The half-time ($t_{1/2}$) of organic matter decay was calculated by Equation 2 (7):

$$t_{1/2} = \ln(0.5) / -k \quad (2)$$

Mathematical modeling of cumulative enzyme production over time

The temporal variations of cumulative cellulase and xylanase activities were fitted to an exponential curve using non-linear regression (iterative algorithm of Levenberg-Marquardt) following Press *et al.* (29). The coefficient of enzymatic activity (k_p) values were derived from the sigmoid fittings. In the procedures, the time evolution of enzyme production was described by Equation 3:

$$E = E_{\max} \left(1 - e^{-k_p t} \right) \quad (3)$$

where: E = accumulated produced enzyme ($\mu\text{mol min}^{-1} \text{ mL}^{-1} \text{ g}^{-1}$); E_{\max} = maximum enzyme produced ($\mu\text{mol min}^{-1} \text{ mL}^{-1} \text{ g}^{-1}$); k_p = enzyme production coefficient (day⁻¹); t = time (day).

The doubling-time ($D_{1/2}$) of maximum enzyme production was calculated according to Equation 2; in this case used as k_p .

Statistical analysis

All statistical analyses were conducted using a non-parametric variance test (Kruskal-Wallis), values were considered significant at $p \leq 0.05$ and performed by PAST®.

RESULTS

Physicochemical parameters

The physicochemical parameters (Table 1) did not show significant differences between the three incubation sites, only between surface and w-s interface. The pH values varied

around 5.0 during the experiment. Electrical conductivity varied from 0.006 mS.cm^{-1} to 0.008 mS.cm^{-1} on the surface and from 0.014 mS.cm^{-1} to 0.079 mS.cm^{-1} on the w-s interface. The water temperature in Óleo lagoon varied from $17.4 \text{ }^\circ\text{C}$ to $27.8 \text{ }^\circ\text{C}$. Values of dissolved oxygen (DO) on the surface varied from 0.8 mg.L^{-1} to 7.7 mg.L^{-1} and from 0.04 mg.L^{-1} to 2.91 mg.L^{-1} on the w-s interface. Dissolved organic carbon was higher in the *E. azurea* incubation site (6.2 mg.L^{-1} surface and 7.6 mg.L^{-1} w-s interface) than on the *Salvinia* sp. (3.6 mg.L^{-1} surface and 3.2 mg.L^{-1} w-s interface) and *C. giganteus* (3.2 mg.L^{-1} surface and 2.7 mg.L^{-1} w-s interface). Nutrient values were similar in the lagoon incubation sites and depths. Total nitrogen varied from 0.6 mg.L^{-1} to 0.9 mg.L^{-1} and total phosphorus was 0.03 mg.L^{-1} on the surface and w-s interface.

Table 1. Physicochemical parameters of Óleo lagoon

	pH	EC mS.cm^{-1}	T_{max} $^\circ\text{C}$	T_{min} $^\circ\text{C}$	DO_{max} mg.L^{-1}	DO_{min} mg.L^{-1}	DOC mg.L^{-1}	Nt mg.L^{-1}	Pt mg.L^{-1}
surface									
P1	5.3	0.008	25.6	17.4	5.45	0.8	3.6	0.6	0.02
P2	5.1	0.006	26.6	18.8	6.67	1.43	6.2	0.6	0.03
P3	5.3	0.006	27.8	18.6	7.7	0.87	3.2	0.6	0.03
w-s interface									
P1	5.4	0.014	24.7	17.6	1.22	0.04	3.2	0.7	0.03
P2	5.6	0.079	24.5	17.4	1.89	0.05	7.6	0.9	0.03
P3	5.4	0.059	24.9	17.6	2.91	0.12	2.7	0.7	0.03

*where: EC = electrical conductivity, T max = maximum temperature, T min = minimum temperature, DOmax = maximum dissolved oxygen, DOmin = minimum dissolved oxygen, DOC = dissolved organic carbon, Nt = total nitrogen, Pt = total phosphorus.

Detritus decay

Temporal mass loss (Figure 1) follows the bimodal pattern. The initial phase was represented by a fast decay of labile particulate organic matter (POM_{LS}), 11% (*E. azurea*) to 43% (*Salvinia* sp.) (Table 2). The detritus of *Salvinia* sp. had a mass decay which was significantly different ($p \leq 0.05$) from the two other species. These detritus presented the highest percentage of POM_{LS} (41% surface and 43% w-s interface), however, the decay rates (k_t) were the lowest (0.22 day^{-1} surface and w-s interface) and, consequently, the higher half-

times (3.15 days surface and w-s interface). *E. azurea* POM_{LS} percentage was the lowest between the three species (11% surface and 15% w-s interface) and the highest k_T values (1.27 day^{-1} surface and 0.70 day^{-1} w-s interface), together with the lowest half-time (0.55 day surface and 0.99 day w-s interface). *C. giganteus* was the only species to present differences between surface and w-s interface POM_{LS} percentage (15% surface and 26% w-s interface). The decay rates were intermediate; 0.57 day^{-1} surface with a half-time of 1.22 days and a 0.33 day^{-1} w-s interface, with a half-time of 2.10 days.

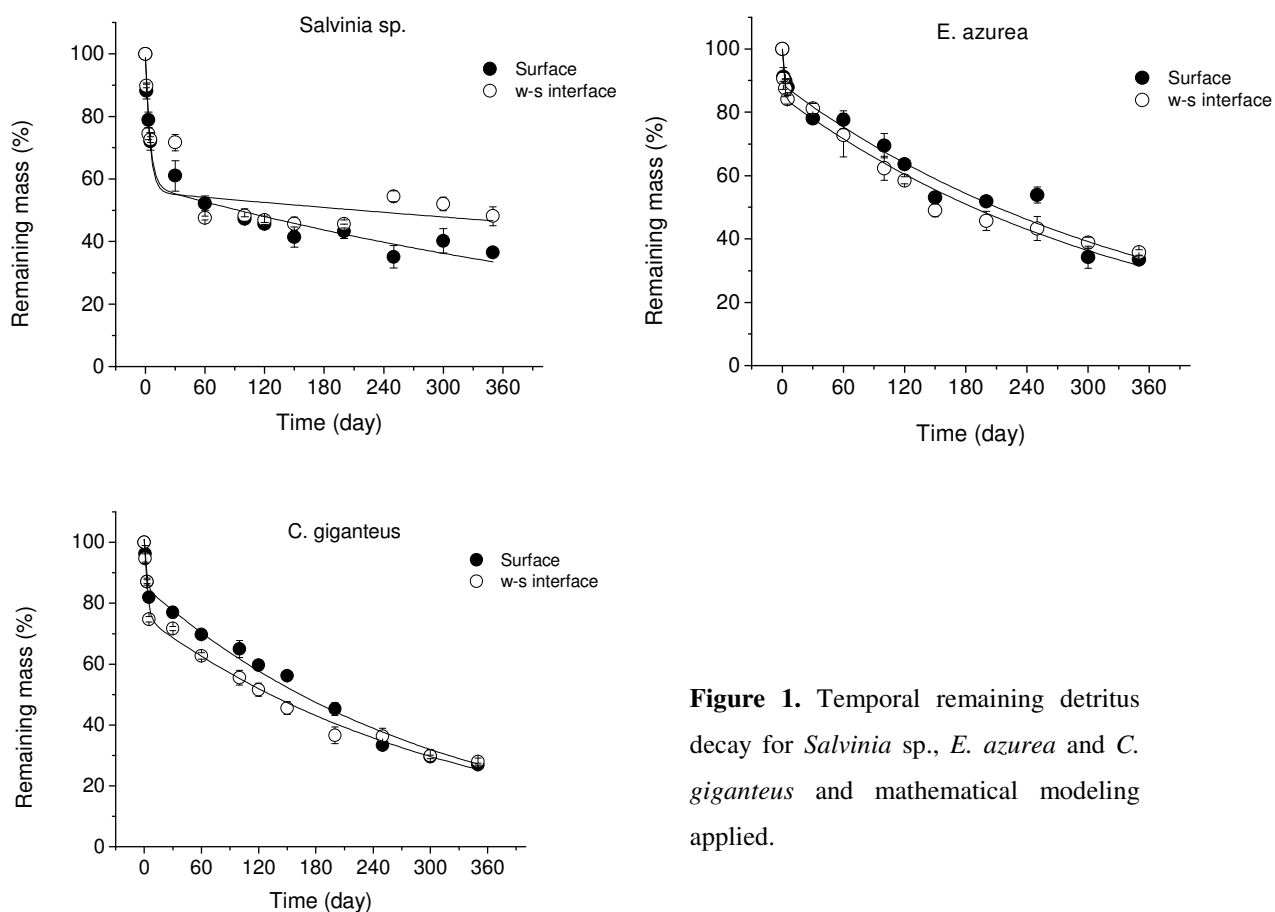


Figure 1. Temporal remaining detritus decay for *Salvinia* sp., *E. azurea* and *C. giganteus* and mathematical modeling applied.

The period of POM_{LS} decay was followed by the degradation of recalcitrant organic matter (POM_R). The percentages of POM_R in *Salvinia* sp. detritus was 58% and 56% respectively for the surface and w-s interface and were the lowest values between the three species. The decay rate in the surface (0.001 day⁻¹) was twice the w-s interface value (0.0005 day⁻¹), consequently, *Salvinia* sp. POM_R half-time was twice as fast on the surface (Table 2). *E.*

azurea and *C. giganteus* POM_R presented similar decay patterns and did not show differences between the surface and w-s interface. The percentage of POM_R was 89% (surface) and 85% (w-s interface) in *E. azurea* samples and 86% (surface) and 75% (w-s interface) in *C. giganteus*. The decay rates were 0.002 days⁻¹ to *E. azurea* and 0.003 days⁻¹ to *C. giganteus* (surface and w-s interface). Half-time values were 346 and 231 days, respectively.

Table 2. Parameters obtained from organic matter decay model.

	POM _{LS} (%)	E	kt (day ⁻¹)	E	t _{1/2} (day)	POM _R (%)	E	k _R (day ⁻¹)	E	t _{1/2} (day)	r ²
Surface											
<i>Salvinia</i> sp.	40.6	4.0	0.22	0.05	3.15	57.8	2.7	0.001	0.0002	693	0.97
<i>E. azurea</i>	11.2	4.9	1.27	1.56	0.55	88.7	2.5	0.002	0.0002	346	0.96
<i>C. giganteus</i>	15.3	3.4	0.57	0.32	1.22	85.7	2.2	0.003	0.0001	231	0.98
Sediment-water interface											
<i>Salvinia</i> sp.	43.1	8.1	0.22	0.1	3.15	55.8	5.1	0.0005	0.0002	1386	0.83
<i>E. azurea</i>	14.8	3.8	0.70	0.4	0.99	84.8	2.36	0.002	0.0002	346	0.97
<i>C. giganteus</i>	25.7	3.3	0.33	0.0001	2.10	75.4	2.38	0.003	0.0002	231	0.98

* where: POM_{LS}= labile fraction of organic matter; k_i = Global decay coefficient (labile fraction mineralization coefficient + leaching coefficient); POM_R= refractory fraction of organic matter; k_R = refractory fraction mineralization coefficient; t_{1/2} = half-time; E = error.

Enzymes activity

Cellulase and xylanase activities were detected since the beginning of the decomposition process (Figure 2). The cellulase activity did not show significant differences between the three species ($p \geq 0.05$). Xylanase activity presented significant differences between *Salvinia* sp., *E. azurea* and *C.*

giganteus ($p \leq 0.05$) and was five times higher than the cellulase production. Incubation depths did not influence the enzymatic production of cellulase or xylanase ($p \geq 0.05$). A peak of enzymatic production of *C. giganteus* during the first five days of experiment could be observed.

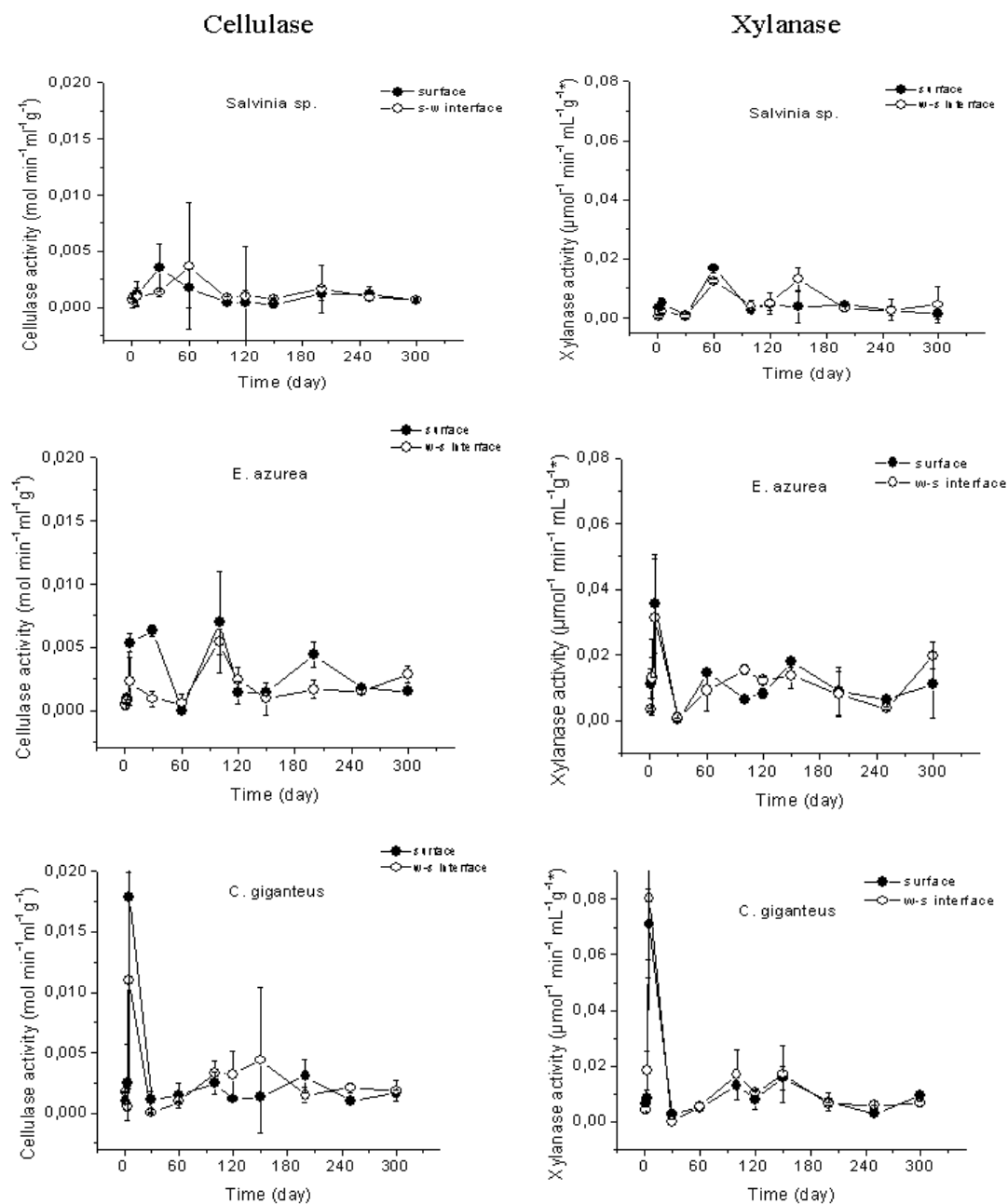


Figure 2. Temporal variation and standard deviation of cellulase and xylanase (surface and water-sediment interface) production during decomposition of *Salvinia* sp., *E. azurea* and *C. giganteus*.

Salvinia sp. had the lowest values of enzymatic production (Table 3), maximum production (E_{\max}) of cellulase was $0.01 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ (surface and w-s interface) with a coefficient production (k_p) of 0.02 day^{-1} (surface) and 0.01 day^{-1} (w-s interface), resulting in doubling times ($D_{1/2}$) of 35 and 69 days, respectively. The xylanase maximum production was $0.05 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ on the surface and $0.07 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ on the w-s interface. The k_p was 0.01 day^{-1} and $D_{1/2}$ of 69 days (surface) and $0.04 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ and $D_{1/2}$ of 17 days (w-s interface).

Detritus of *E. azurea* (Table 3) had a cellulase E_{\max} of $0.03 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ (surface) and $0.02 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ (w-s interface). For this plant, k_d in the surface (0.01 day^{-1}) was twice the w-s interface value (0.005 day^{-1}) with $D_{1/2}$ of 69 and 139 days,

respectively. The xylanase maximum production was $0.12 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ (surface and w-s interface) and, as cellulase, was faster on the surface (0.02 day^{-1}) with a $D_{1/2}$ of 35 days. The w-s interface presented k_p of 0.01 day^{-1} and $D_{1/2}$ of 69 days.

C. giganteus (Table 3) had the highest values of cellulase and xylanase production. The values of cellulase E_{\max} were $0.003 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ (surface and w-s interface). The cellulase production coefficient was 14 times higher on the surface (0.14 day^{-1}) with a $D_{1/2}$ of 5 days than on the w-s interface (0.01 day^{-1}) and $D_{1/2}$ of 69 days. The xylanase maximum production was $0.12 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ (surface) and $0.14 \text{ mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$ (s-w interface). There was no difference between the surface and s-w interface for k_p (0.13 day^{-1}) and $D_{1/2}$ (5 days).

Table 3. Parameters obtained from kinetic model for accumulated cellulase and xylanase production.

	E_{\max} ($\text{mol min}^{-1}\text{mL}^{-1}\text{g}^{-1}$)	E	k_p (day^{-1})	E	$D_{1/2}$ (day)	r^2
Cellulase						
<i>Salvinia</i> sp.	0.01	0.0005	0.02	0.006	35	0.93
	0.01	0.0000007	0.01	0.001	69	0.97
<i>E. azurea</i>	0.03	0.002	0.01	0.002	69	0.95
	0.02	0.002	0.005	0.001	139	0.96
<i>C. giganteus</i>	0.03	0.001	0.14	0.050	5	0.86
	0.03	0.003	0.01	0.004	69	0.84
Xylanase						
<i>Salvinia</i> sp.	0.05	0.0029	0.01	0.002	69	0.96
	0.07	0.0102	0.04	0.001	17	0.97
<i>E. azurea</i>	0.12	0.01	0.02	0.060	35	0.80
	0.12	0.014	0.01	0.003	69	0.87
<i>C. giganteus</i>	0.12	0.007	0.13	0.050	5	0.83
	0.14	0.009	0.13	0.050	5	0.83

*where: E_{\max} = maximum enzyme production, E = error, k_p = enzyme production coefficient and $D_{1/2}$ = doubling-time.

Cellulose and Hemicellulose content

The lowest initial contents of cellulose (32%) were observed in *Salvinia* sp. and in *C. giganteus*, the highest content (57%) (Table 4). It was not possible to detect decay in the cellulose content of *Salvinia* sp., *E. azurea* had a loss of about 10% and *C. giganteus* a loss of about 20% (Table 4). The hemicellulose content (Table 4) was similar between the 3 species ($\approx 30\%$) and a loss of this content was not observed.

Table 4. Cellulose and hemicellulose initial and final content (%) of *Salvinia* sp., *E. azurea* and *C. giganteus*.

	Cellulose (%)	Hemicellulose (%)
		initial
<i>Salvinia</i> sp.	32	26
<i>E. azurea</i>	43	33
<i>C. giganteus</i>	57	26
		final
<i>Salvinia</i> sp. surface	31	36
w-s interface	32	36
<i>E. azurea</i> surface	32	33
w-s interface	34	21
<i>C. giganteus</i> surface	38	36
w-s interface	36	32

DISCUSSION

In general, there were no physicochemical variations between the three incubation sites. The variations observed between the surface and w-s interface were, mainly, dissolved oxygen and electrical conductivity. Even with the low depth of Óleo lagoon (Z_{\max} 3.5 m), the DO values on the w-s interface were lower than $1.0 \text{ mg}\cdot\text{L}^{-1}$ most of the year, showing anaerobic processes. The electrical conductivity (EC) values were nearly seven times higher on the w-s interface than on the surface. The release of ions from the sediment increases these EC values on the w-s interface.

Leaching is a physical process, which occurs during the first 7 days after the macrophyte senescence (10). During this period, *Salvinia* sp. detritus had the higher values of decay coefficient and half-time (0.22 day^{-1} and 3.15 days), compared with *E. azurea* and *C. giganteus*. Since the physical parameters show no significant differences between the incubation sites, the slow decay of *Salvinia* sp. is related with this plant chemical composition. *E. azurea* and *C. giganteus* POM_L decay was faster on the surface (1.27 day^{-1} and 0.57 day^{-1}) than on the w-s interface (0.70 day^{-1} and 0.33 day^{-1}) and this could be related with the lower temperature at the w-s interface samples which were decomposed.

Parallel to leaching, an enzymatic activity peak was observed in the first days of *E. azurea* and *C. giganteus* decomposition (Fig. 2). This could be related with the release of nutrients during the leaching process. Many authors have suggested that most nutrient elements in aquatic macrophytes are rapidly leached in the initial period of litter decomposition (34): labile compounds like sugars, amino-acids, aliphatic acids (42) and nutrients such as dissolved nitrogen (e.g. ammonia), ortho-phosphate and total phosphorus (43). These released nutrients can increase the bacterial production (33), causing the enzymatic production peak. Nunes *et al.* (25) also observed an initial peak in cellulase and xylanase production in an *in vitro* anaerobic experiment with 3 other macrophytes of Óleo lagoon

(*Ricciocarpus natans* (L.) Corda, *Oxycaryum cubense* (Poepp. & Kunth) and *Cabomba furcata* Schult. & Schult).

Litter quality as defined by chemical composition markedly influences the decomposition rates of different types of plant litter (34), mainly the POM_R degradation. *Salvinia* sp. was the more recalcitrant species, with the highest half-time values for POM_R (693 and 1386 days). The recalcitrance of *Salvinia* generous was also observed in previous study; in a decomposition experiment with macrophytes of an Italian wetland, *Salvinia natans* had the lowest decay rates (18). Barik *et al.*, observed low decay rates for *Salvinia cullata* ($0.28\% \text{ day}^{-1}$) compared to *Eichhornia crassipes* ($1.49\% \text{ day}^{-1}$) samples (6).

There was a direct relation between the mass loss and enzymatic activity shown by higher detritus mass loss and higher k_R values in samples with great enzymatic activity (*E. azurea* and *C. giganteus*). Fiber disposition is a dominant structural feature limiting the rate and extent of using biomass materials (22) and can be the cause of different enzymatic productions between the three species. The higher enzymatic degradation activity on *E. azurea* and *C. giganteus* detritus caused a greater POM_R loss ($t_{1/2}$ values were twice the *Salvinia* sp. values in the surface and 4 times the w-s interface values - Table 2).

Cellulose and hemicelluloses are connected in the plant structure and decomposition. There is an inter-relationship between the systems that regulate these compounds (39). Cellulose fibers are embedded in a matrix of, primarily, hemicelluloses (22), filling the voids around cellulose fibrils and providing coupling to lignin (5). This disposition facilitates microorganism degradation. Thus, the higher xylanase activity observed in detritus of *Salvinia* sp., *E. azurea* and *C. giganteus* (nearly, 5 times higher (Table 3)) could occur due to the easy access of the microorganisms in this component in relation to cellulose. Furthermore, hemicelluloses must be degraded, at least partially, before cellulose (17).

The results observed for cellulose and hemicelluloses

contents seem to contradict the detritus mass decay and enzymatic activity, since it was not possible to observe decay in hemicelluloses contents, even with a great xylanase activity. The microorganisms involved in cellulose degradation produce multiple enzymes to degrade plant cell materials, known as enzyme systems (39) and can co-produce enzymes active specifically on hemicelluloses (22). Therefore, a greater decay in hemicelluloses contents was expected.

The general low enzyme activity observed during the experiment could be related not only to the detritus quality but with the system characteristics, such as low nutrient levels (Table 1). In permanent tropical aquatic systems, where temperature and moisture are not constraining, nutrient availability and decomposer activity determines the decomposition rates together with chemical properties of the decomposing material (4). Low decomposition rates are common in plants inhabiting poor nutrient sites, characterized by high nutrient absorption (1). Nutrient inputs can increase decomposition rates (30). The slight increase in enzymatic activity during the dry season (Fig. 2) can be related to the macrophyte senescence and nutrients release by the leaching of those detritus. Thus, in addition to constraints imposed by the structure of fiber itself, limitations imposed by diffusion and transport of the cellulolytic agent to the site of the enzymatic attack, may severely limit the use of fibers in some habitats (44).

Even with the occurrence of anoxia periods on the w-s interface (28) and possible release of nutrients, the proximity of the two incubation depths (less than 3.0 m) could influence the results of statistical analysis, that did not show differences between the surface and w-s interface in detritus mass decay and enzymatic production, even with an increase in xylanase maximum production on the w-s interface in *E. azurea* and *C. giganteus* detritus.

Overall, the enzymatic degradation of aquatic macrophytes in Óleo lagoon occurs during all seasons and initiates together with leaching. After senescence, the aquatic

macrophytes can be decomposed in the floating detritus bulk or on the w-s interface. Enzymatic production and detritus mass decay are similar in these two sites. Due to the environmental homogeneity in this lagoon, it can be inferred that the chemical composition of the macrophytes detritus dominate the influence on decomposition rates (mass loss and enzymatic production) of aquatic macrophytes in this system. Xylanase production was higher due to the easier access to this compound by cellulolytic microorganisms.

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