# Effects of detritus chemical composition on the anaerobic mineralization of *Salvinia auriculata* and *Utricularia breviscapa*

## Efeitos da composição química dos detritos na mineralização anaeróbia de *Salvinia auriculata* e de *Utricularia breviscapa*

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**Abstract: Aim**: This study was conducted to evaluate the effect of the detritus composition on the anaerobic mineralization of two species of aquatic macrophytes with different life forms (submerged and free floating). The hypothesis that guided this study was that the carbon concentration derived from detritus hydrosoluble fraction can act as a facilitating factor on its degradation. **Material and Methods:** Incubations containing detritus and water sample from the Óleo Lagoon (21° 33' to 21° 37' S and 47° to 47° 45' to 51' W) for each specie (*Salvinia auriculata* and *Utricularia breviscapa*) were set-up with: (i) integral detritus (sample of dried plant), (ii) lignocellulosic matrix (particulate organic matter (POM) remaining from leachate extraction) and (iii) leachate. The incubations were kept in the dark under anaerobic conditions. Daily rates of gas formation were evaluated and after 138 days, the incubations were fractioned in dissolved and particulate fractions and the mass balances were performed. A mass loss experiment (180 days) was performed for assessment of the dissolved organic carbon, particulate organic carbon and mineralized carbon variations. **Results:** Regardless of the type of detritus (*S. auriculata* and *U. breviscapa*), C-mineralization was faster and higher in the DOC incubations (ca. 85%). For *U. breviscapa* the POM mineralization was slower than the corresponding integral detritus and *S. auriculata* mineralization was slower than *U. breviscapa*. **Conclusions:** The composition of the detritus (i.e. macrophyte type, presence and proportion of leachate) interfered synergistically in anaerobic degradation of these plants. The leachate tends to act as a facilitator, supporting the growth of microorganisms and intensifying mineralization.

**Keywords:** decomposition; aquatic macrophytes; kinetic models; anaerobiosis; gas production.

**Resumo: Objetivo**: Considerando a natureza heterogênea dos detritos vegetais, esse estudo teve por objetivo verificar o efeito da composição química dos detritos na mineralização anaeróbia de duas espécies de macrófitas aquáticas com diferentes hábitos de vida (uma flutuante e outra submersa). A hipótese desse estudo foi que a concentração de carbono da fração hidrosolúvel do detrito pode atuar como um fator facilitador de sua degradação. **Material e Métodos:** Foram preparadas incubações contendo detritos e água da lagoa do Óleo (21° 33' a 21° 37' S e 47° a 45'a 47° 51 O). Para cada espécie (*Salvinia auriculata* e *Utricularia breviscapa*) foram preparadas câmaras com: (i) detritos integrais (amostra de planta seca); (ii) matriz ligno-celulósica (matéria orgânica particulada (MOP) remanescente da extração do lixiviado) e (iii) lixiviado. As incubações foram mantidas em condição anaeróbia e no escuro. Diariamente foram avaliadas as taxas de formação de gases e ao final (dia 138) as incubações foram desmontadas e foram elaborados os balanços de massa. Periodicamente (durante 180 dias), câmaras foram fracionadas em uma fração dissolvida e outra particulada para a avaliação das variações de carbono orgânico dissolvido, carbono orgânico particulado e carbono mineralizado (CM). **Resultados:** Independente do tipo (*S. auriculata* ou *U. breviscapa*) a mineralização do carbono foi mais rápida e elevada para os lixiviados (ca. 85%). Para *U. breviscapa* a mineralização da MOP foi mais lenta que a do respectivo detrito integral e a mineralização de *S. auriculata* foi mais lenta que a de *U. breviscapa*. **Conclusões:** A composição do detrito (i.e. tipo de macrófita,

presença e proporção de lixiviado) interferiu de forma sinérgica na degradação anaeróbia das plantas. O material lixiviado tende a constituir-se em agente facilitador, subsidiando o crescimento dos microrganismos e intensificando a mineralização.

**Palavras-chave:** decomposição; macrófitas aquáticas; modelos cinéticos; anaerobiose; produção de gás.

## 1. Introduction

Carbon moves within biogeochemical cycles through lithosphere, atmosphere and hydrosphere in gaseous (e.g. methane and carbon dioxide) and solid (e.g. coal) forms. The main ecological processes resulting in immobilization and mineralization are weathering, photosynthesis, respiration and decomposition. In freshwater environments, the continuous cycling of carbon is processed according to the dissolved oxygen availability by distinct heterotrophic metabolic routes. The anaerobic carbon decomposition is basically processed within sediments, since this compartment presents the optimal condition for oxygen-free metabolism as methanogenesis (Makhov & Bazhin, 1999).

In tropical and subtropical freshwater systems, the high temperatures and great availability of nutrients (Van Der Heide et al., 2006; Cao at al., 2012) provide a great potential for macrophyte growth both on sediments and the water column. The distinct morphological architecture of macrophytes (floating and submerged) reflects in the plant structure and chemical composition e.g., C, N, P and fibers content.

Aquatic plants represent an important source of energy after death since the detritus transfer large quantities of carbon and nutrients to the aquatic heterotrophic community through decomposition (Waichman, 1996). As a consequence, the decomposition of these plants is a key process in the C-cycle of aquatic ecosystems. Macrophyte community represents an important source of detrital organic carbon (Li et al., 2012). As macrophyte decomposition undergo within aquatic system, the dissolved organic carbon (DOC) is metabolized in the water column and particulate organic carbon (POC) is settled down at the surface of sediments, a reduced medium that often displays a negative redox potential. The average carbon concentration in dry plant matter is 47.8% (Klee and Graedel, 2004) representing the greater element content in plant biomass. The carbon content of *Salvinia auriculata*, a free-floating macrophyte varies from 31.1% (Martins et al., 2003) to 37.7% (Costa & Henry, 2010). The fiber content (i.e. cell wall fraction) for this genus varies from ca. 52%

(Henry-Silva et al., 2001) to 68.2% (Henry-Silva & Camargo, 2002). The cell wall fraction of *Utricularia* represents 39.4% of dry biomass (Henry-Silva et al., 2001). The fiber content for *Utricularia breviscapa* is 31.5% of dry mass (Bianchini Júnior et al., 2010), and its C-content represents 33% of dry mass (Bianchini Júnior et al., 2006).

Considering that macrophytes are responsible for a large input of C-detritus in aquatic systems, the objective of this study was to evaluate the effect of the detritus composition on the anaerobic mineralization of two species of aquatic macrophytes with different life-forms. The hypothesis that guided this study was that the carbon concentration derived from the detritus hydrosoluble fraction can act as a facilitating factor on its degradation. The rates of anaerobic gases production and its different yields (based on chemical structure) in freshwater environments may be useful in the development of predictive mathematical models of C-gas flux providing an indirect measure of the heterotrophic activity on macrophyte detritus.

## 2. Material and Methods

### 2.1. Description of the study site

Óleo Lagoon is an oxbow lakes (Table 1) located in the Mogi-Guaçu River floodplain (State of São Paulo, Brazil; 21° 35' S and 47° 51' W) inside the Ecological Station of Jataí (Luiz Antônio, SP). It is a polymictic lagoon located in a Cerrado biome (Quaresma & Perez Filho, 2012) that exhibits a characteristic acidic soil, the red latosoil (Cavalheiro et al., 1990). The annual temperature is ca. 22 °C varying from 16.0 to 29 °C. The hydrological cycle of the region is defined by high waters period (November to April) showing a mean precipitation of 1.270 mm (Quaresma & Perez Filho, 2012), when the surrounding areas are usually flooded and by a typical dry season (May to October; Cavalheiro et al., 1990). Among a great diversity of macrophytes, *Oxycaryum cubense*, *Salvinia auriculata*, *Egeria najas, Utricularia breviscapa*, and *Ludwigia inclinata* are the most common species of macrophytes in this lagoon (Petracco, 2006).

**Table 1.** Morphological and limnological characteristics of Óleo Lagoon. S = surface; B = bottom.

| Variable | Value | Reference |
|---|---|---|
| Lagoon total area (km²) | 0.0195 | Petracco (2006) |
| $Z_{max}$ (m) | 5.1 | Petracco (2006) |
| $Z_{mean}$ (m) | 2.6 | Petracco (2006) |
| pH | 5.1 (S) - 5.6 (B) | Sciessere et al. (2011) |
| Electrical conductivity (mS/cm) | 0.006 (S) - 0.079 (B) | Sciessere et al. (2011) |
| Dissolved oxygen (mg/L) | 5.45 (S) - 1.22 (B) | Sciessere et al. (2011) |
| Total nitrogen (mg/L) | 0.6 (S) - 0.9 (B) | Sciessere et al. (2011) |
| Total phosphorous (mg/L) | 0.02 (S) - 0.03 (B) | Sciessere et al. (2011) |

## 2.2. Macrophyte preparation

Adult individuals of *Salvinia auriculata* Aubl. and *Utricularia breviscapa* C. Wright ex Griseb. were harvested at the littoral region of Óleo Lagoon. In the laboratory, the macrophytes were washed in distilled water and oven dried (60 °C) until reaching a constant mass. Afterward, *U. breviscapa* was ground (0.2 cm < diameter < 1.32 cm) in order to homogenize the tissues (leaves, roots and stems). *S. auriculata* was used in the experiments as entire individuals. On the day prior to the beginning of the experiment, water samples were collected from three distinct depths (surface = 0.1 m; middle = 2.5 m and bottom = 5.0 m) at the littoral region of Óleo Lagoon using a Van Dorn bottle. The samples were homogenized and filtered (cellulose acetate membrane - pore Ø = 0.45 μm; Millipore) to remove large organisms and course detritus.

## 2.3. DOC formation from leaching process

For DOC hydrosolubilization, 30.0 g of dry *S. auriculata* and dry grounded *U. breviscapa* were immersed into 3.0 L of deionized sterile water and incubated overnight at 4 °C (Mφller et al., 1999). The fragments of particulate organic matter (POM) were separated from the leachate by filtration (cellulose ester membrane - pore Ø = 0.45 μm; Millipore). The carbon of the *S. auriculata* and *U. breviscapa* POM was quantified using a Carlo Erba Model EA1108 Elemental Analyzer. The concentrations of DOC in the leachates of *S. auriculata* and *U. breviscapa* were determined by combustion/non-dispersive infrared gas analysis method (Shimadzu analyzer, model 5000A; precision: 0.1 to 1.0 mg/L and internal standards: sodium carbonates ($NaHCO_3$ and $Na_2CO_3$, proportion 1.65:1 w/w) and potassium hydrogen phthalate).

## 2.4. Kinetics of the decomposition experiment

For each species, carbon mineralization was tested on the integral plant (i.e. unfractionated, Treatment 1), on POM (Treatment 2) and on the leachates (Treatment 3). In Treatments 1 and 2, chambers of decomposition (n = 24), each with 4.0 g of plants or POM and 400 ml of filtered water (cellulose acetate membrane - pore Ø = 0.45 μm; Millipore) from Óleo Lagoon were set up. Initial DOC concentrations in Treatment 3 were 83.77 mg/L for *S. auriculata* and 155.1 mg/L for *U. breviscapa.*

The chambers (volume: 500 ml) were maintained in the dark under anaerobic conditions at 28.0 ± 1.0 °C (n = 97) during 180 days. The anaerobiosis was obtained by bubbling $N_2$ into the chambers until the dissolved oxygen concentration reached zero mg/L (ca. 10 min). In each sampling day (1, 3, 5, 10, 15, 20, 40, 60, 90, 120 and 180 days) the content of two chambers of each treatment were separated into particulate and dissolved fractions (cellulose acetate membrane - pore Ø = 0.45 μm; Millipore) and carbon content were determined in the remaining detritus. Particulate organic carbon (POC) was measured with an Elemental Analyzer (Carlo Erba, model EA1108), and the DOC concentrations were determined by combustion and infrared detection (Shimadzu TOC-5000A).

## 2.5. Gas formation

Replicates for each species and treatment were used to assess gas formation. In each decomposition chamber 5.0 g (DM) of plant fragments were added to 1.0 L of filtered lagoon water (0.45 μm membrane; Millipore). Anaerobiosis inside the chambers was accomplished liked before and the chambers were maintained in the dark at 27.6 ± 0.8 °C (n = 138). During 138 days the daily rates of gas evolved or consumed were measured using the manometric method (Ohle, 1972). The volume of released (or consumed) gas was quantified by the displacement of the water column in the low-pressure manometer (Bianchini Júnior et al., 1997). After determining the daily rates, the gases produced in the chambers were purged in order to

avoid pressurization. At the end of the experiment, pH and redox potential (pH-meter Digimed, model DMPH-2), and electrical conductivity (conductivity-meter Digimed, model DM3) were determined. DOC and dissolved inorganic carbon (DIC) were determined by combustion/non-dispersive infrared gas analysis method (Shimadzu analyzer, model 5000A). The remaining detritus at the end of experiment were oven-dried, gravimetrically quantified and its carbon contents was determined (Elemental Analyzer Carlo Erba, model EA1108).

## 2.6. Calculations

Total inorganic carbon (TIC) deriving from mineralization was calculated as the difference between the initial C contents of resources and the remaining organic carbon measured at the end of the experiment (Equation 1).

$$TIC = (POC_i + DOC_i) - (POC_f + DOC_f) \tag{1}$$

where: $POC_i$ = particulate organic carbon of resource; $DOC_i$ = initial content of dissolved organic carbon; $POC_f$ = organic carbon content of remaining particulate detritus; $DOC_f$ = dissolved organic carbon at the end of the experiment.

The remaining DOC and POC at the end of the experiment were considered as refractory material, referred to as RDOC and RPOC, respectively. The total concentration of C-gas (i.e. $CO_2$ + $CH_4$) was estimated by the difference between TIC and dissolved inorganic carbon at the end of the experiment (DICf); thus the mineralized carbon (MC) = C-gas + DICf. The mineralized carbon (MC) was characterized as labile organic carbon (LOC) and the amount of remaining detritus carbon (POCf + DOCf) was defined as refractory organic carbon (ROC).

The logistic curve model (Equation 2) was used to describe the formation of C-gas (Cunha-Santino & Bianchini Júnior, 2013). According to this model, the losses of matter refer only to the labile (soluble or particulate) fractions of detritus (LSPOC), refractory compounds are considered conservative (i.e. meaning that do not react). Parameterization was obtained by fitting the temporal evolution of C-gas (i.e. numerical integration of positive daily rates obtained by manometric measures) using nonlinear regressions with the iterative algorithm of Levenberg-Marquardt (Press et al., 2007).

$$\frac{dC_{C-gas}}{dt} = \frac{k_G}{k_M} C_{LSPOC} \times \left( \frac{C-gas_{MAX} - C-gas}{C-gas_{MAX}} \right) \tag{2}$$

where: $C_{LSPOC}$ = change per unit time of the amount of POC fractions associated with labile carbon (i.e. particulate and soluble compounds); $C_{C-gas}$ = change per unit time in the amount of C-gas; $k_M$ = rate constant for the total mass loss related to oxidation of labile compounds and dissolved inorganic carbon (DIC) formation (day$^{-1}$); $k_M = k_G + k_D$ ($k_G$ = C-gas formation rate constant (day$^{-1}$); $k_D$ = DIC (e.g. $H_2CO_3$, $HCO_3^-$) formation rate constant (day$^{-1}$); $k_G/k_M$ = C-gas yield coefficient (= C-gas$_{MAX}$/LSPOC); C-gas$_{MAX}$ = maximum amount of C-gas = C-gas yield coefficient × LSPOC (%).

A set of equations was used to describe the mineralization (Equation 3 to 5) of vegetal detritus (Bianchini Júnior & Cunha-Santino, 2011). Parameterization was obtained by fitting the temporal evolution of POC, DOC using nonlinear regressions with the iterative algorithm of Levenberg-Marquardt (Press et al., 2007).

1º) POC mass loss (leaching of DOC and mineralization processes of labile (LPOC) and refractory (RPOC) compounds related to particulate carbon):

$$\frac{dC_{POC}}{dt} = -k_T C_{LSPOC} - k_4 C_{RPOC} \tag{3}$$

where: $C_{LSPOC}$ = change per unit time in the amount of POC fractions associated with protoplasmic portions (i.e. labile and soluble compounds); $C_{RPOC}$ = change per unit time in the amount of POC refractory fraction (e.g. cellulose, lignin); $k_T$ = rate constant for the total mass loss related to leaching and oxidation of labile compounds (day$^{-1}$); $k_T = k_1 + k_2$ ($k_1$ = leaching rate constant (day$^{-1}$); $k_2$ = rate constant for the oxidation of labile compounds (day$^{-1}$); $k_4$ = rate constant for oxidation (mineralization) of refractory materials (day$^{-1}$).

2º) Formation and mineralization of DOC:

$$\frac{dC_{DOC}}{dt} = k_1 C_{LSPOC} - k_3 C_{DOC} \tag{4}$$

where: $C_{DOC}$ = change per unit time in DOC concentration; $k_3$ = mineralization rate constant for DOC (day$^{-1}$).

3º) Formation of gases and inorganic substances (mineralization):

$$\frac{dC_{TIC}}{dt} = k_T C_{LPOC} + k_3 C_{DOC} + k_4 C_{RPOC} \tag{5}$$

where: $C_{TIC}$ = amount per unit time of total inorganic carbon (e.g. $CO_2$). The LPOC loss of mass constitutes the first mineralization route (IN1); the losses of DOC comprise the 2$^{nd}$ pathway

(IN2) and the losses related to RPOC include the $3^{th}$ mineralization pathway (IN3).

A specific set of equations was used to describe the DOC (i.e. leachate) mineralization (Equation 6 and 7; Silva et al., 2011). Parameterization was obtained by fitting the temporal decay of DOC using nonlinear regressions with the iterative algorithm of Levenberg-Marquardt (Press et al., 2007).

1º) DOC mass loss (refractory DOC - RDOC) formation and mineralization of labile DOC (LDOC):

$$\frac{dC_{DOC}}{dt} = -k_{T2}C_{DOC} \qquad (6)$$

where: $C_{DOC}$ = change per unit time in the amount of DOC (i.e. macrophytes leachate); $k_{T2}$ = rate constant for the total mass loss related to RDOC formation and oxidation of labile compounds $(day^{-1})$; $k_{T2} = k_F + k_R$ ($k_F$ = RDOC formation rate constant $(day^{-1})$; $k_{LM}$ = rate constant for the mineralization of LDOC $(day^{-1})$.

2º) Formation and mineralization of RDOC:

$$\frac{dC_{RDOC}}{dt} = k_{T2}C_{DOC} - k_R C_{RDOC} \qquad (7)$$

where: $C_{RDOC}$ = Temporal change in the amount of RDOC (e.g. humic substances); $k_R$ = mineralization rate constant $(day^{-1})$ for RDOC (i.e. formation of inorganic substances, $CO_2$ and other gases).

The half-time $(t_{1/2})$ of leaching, oxidation of LPOC, DOC and RPOC (Equations 2 to 6) was calculated from Equation 8.

$$t_{1/2} = \frac{\ln(0.5)}{-k} \qquad (8)$$

where: k = rate constant $(day^{-1})$ for the process (leaching or mineralization).

### 2.7. Statistical analysis

The values of accumulated mineralized carbon (C-gas) from kinetic fittings in each treatment were submitted to the Kolmogorov-Smirnov test to check normality of the distribution and to Bartlett test to verify homoscedasticity. As these conditions were fulfilled, the gas formation from each treatment was submitted to repeated-measures ANOVA. Whenever significant differences among treatments and species were found, post hoc Tukey tests were carried out to identify differences among treatments. Differences were considered significant where $p < 0.01$. The statistical analyses were performed with the software PAST version 2.16 (Hammer et al., 2001).

### 3. Results

Treatments 2 and 3 comprising *U. breviscapa* detritus (i.e. POC and leachate) displayed the highest volumes of C-gas (92.60 to 134.15 ml) during mineralization process (Table 2). Considering the detritus sources used in all treatments (1, 2, and

**Table 2.** Carbon budget and final (138 days) chemical condition of the three treatments of *Salvinia auriculata* and *Utricularia breviscapa*. Where: $POM_i$ = initial particulate organic matter, $C_i$ = initial carbon content, TOCi = initial total organic carbon, $POM_f$ = final particulate organic matter, $C_f$ = final carbon content, $POC_f$ = final particulate organic carbon, DOCf = final dissolved organic carbon TOCf = final total organic carbon, MC = mineralized carbon, C-Gas = percentage of gas content in carbon basis, DIC = RTOC = RDOC = , RPOC = , EC = electrical conductivity, pH = hydrogenionic potential, Eh = redox potential, C-gas = Volume of gas evolved in carbon basis.

| Treatment | $POM_i$ (g) | $C_i$ (%) | $TOC_i$ (g) | $POM_f$ (g) | $C_f$ (%) | $POC_f$ (g) | $DOC_f$ (g) | $TOC_f$ (g) | MC (%) |
|---|---|---|---|---|---|---|---|---|---|
| Salvinia auriculata | 5.015 | 35.89 | 1.800 | 3.090 | 33.55 | 1.029 | 0.044 | 1.073 | 40.5 |
| *S. auriculata* - POC | 5.004 | 35.34 | 1.768 | 3.791 | 37.48 | 1.433 | 0.015 | 1.448 | 18.1 |
| *S. auriculata* - DOC | - | - | 0.196 | - | - | 0.004 | 0.026 | 0.030 | 84.8 |
| Utricularia breviscapa | 5.012 | 32.71 | 1.639 | 1.198 | 30.57 | 0.400 | 0.020 | 0.420 | 74.4 |
| *U. breviscapa* - POC | 5.038 | 34.82 | 1.754 | 2.557 | 30.83 | 0.812 | 0.030 | 0.842 | 52.0 |
| *U. breviscapa* - DOC | - | - | 1.172 | - | - | 0.042 | 0.135 | 0.177 | 84.9 |

| Treatment | C-Gas (%) | DIC (%) | RTOC (%) | RDOC (%) | RPOC (%) | EC (µS/cm) | pH | Eh (mV) | C-Gas (ml) |
|---|---|---|---|---|---|---|---|---|---|
| Salvinia auriculata | 82.6 | 17.4 | 59.60 | 2.45 | 57.15 | 872.0 | 6.38 | -162.5 | 65.95 |
| *S. auriculata* - POC | 55.5 | 44.5 | 81.86 | 0.82 | 81.04 | 778.0 | 6.65 | -173.5 | 50.90 |
| *S. auriculata* - DOC | 51.6 | 48.4 | 15.20 | 13.39 | 1.81 | 1787.5 | 6.45 | -154.5 | 40.85 |
| Utricularia breviscapa | 80.9 | 19.1 | 25.59 | 1.19 | 24.40 | 1171.0 | 6.44 | -177.0 | 134.15 |
| *U. breviscapa* - POC | 76.3 | 23.7 | 48.01 | 1.70 | 46.31 | 1053.5 | 6.46 | -173.0 | 92.60 |
| *U. breviscapa* - DOC | 77.7 | 22.3 | 15.09 | 11.54 | 3.55 | 1695.0 | 6.33 | -126.5 | 105.90 |

3) a lineal relationship among evolved C-gas and generated gas volume (Volume (ml) = 134.7 × C-gas (g); $r^2$ = 0.85) and to MC and evolved C-gas (C-gas = 0.785 × MC; $r^2$: 0.98) was observed. Regardless of the mineralization yield, the relation between C-gas and MC indicates that ca. 79% of the mineralized carbon was released in gas form, while 21% were maintained dissolved form (DIC).

After 138 days, for all incubations, the pH varied from 6.33 (*U. breviscapa* DOC) to 6.65 (*S. auriculata* – POC). The redox potential (Eh) of treatments in gas formation experiments varied from -177 to - 126.5 mV (Table 1) showing a reducing medium during breakdown of detritus. The lower EC were always found in *S. auriculata* and *U. breviscapa* - POC (Treatment 2). The EC values were lower in the incubation with entire detritus (Treatment 1 - 872 µS/cm) and POC (Treatment 2 - 778 µS/cm) of *S. auriculata* and higher in incubations with DOC (Treatment 3 - *S. auriculata* DOC: 1787 µS/cm; *U. breviscapa* DOC: 1695 µS/cm).

The daily (expressed by positive values) and the uptake rates (expressed by negative values) of gases are shown in Figure 1. Mineralization exhibited three phases according to the evolved gas accumulated (MC) during decomposition of *S. auriculata* and *U. breviscapa* (Treatments 1 to 3). The first, lasted ca. 10 days and was characterized by instability between formation (positive daily rates) and assimilation (negative daily rates) of C-gas. Except in Treatment 3 with *U. breviscapa*, the second phase lasted ca. 3 months and was characterized by an early high rate of gas formation that tended to decrease slowly. From the 3th month (third phase), the formation rates tended to zero or showed again negative values.

In general, the gases emission experiments displayed the three phases, however the statistical analysis indicated that the kinetics of gas formation (MC in Figure 1) was predominantly different among treatments (p < 0.001, F = 106.53 and df = 5), except for experiment with *S. auriculata* and *U. breviscapa* in Treatment 2 (p = 0.115) and *S. auriculata* and *U. breviscapa* in Treatment 3 (p = 0.999). Assuming the LSPOC as a homogeneous detritus (Equation 2), the half-life of mineralization varied between 2.8 (*S. auriculata* DOC) and 11.2 days (*U. breviscapa* DOC), with average time of 6.1 days (Table 2). According to the parameterization of the kinetic model that considered the C-gas emissions (Equation 2),

RPOC displayed a wide range of detritus, varying from 17.1 to 81.9% (Table 2).

Except for *S. auriculata* POC (Treatment 2), the parameterization of mathematical model used to describe the macrophytes detritus decomposition kinetics (Equation 3 to 5) exhibited different values to RPOC fraction (Table 3), contrasting with the parameterization of gas formation experiments (Table 4). Basically the differences derived from the experimental yields obtained between the set experiments, e.g. the theoretical MC yields at 138th day for the experiment used to describe the kinetic of processes: *S. auriculata*: 17.8%; *S. auriculata* POC: 20.9%; *U. breviscapa*: 41.1%; *U. breviscapa* POC: 44.5%.

Regardless the macrophyte specie, the DOC (i.e. leachates) was the labile organic resources among POC and entire detritus (Figure 2), being ca. 15% of this fraction converted in refractory compounds (Table 2) during the experimental time. Different to verified in Treatments 1 and 2, the labile fractions of DOC in Treatment 3 (*S. auriculata*: 84.0 and 88.5%; *U. breviscapa*: 82.9 and 83.4%) were very close (Tables 3 and 4) in the two experiments of the present study (i.e. gases formation and kinetic experiments). However, the DOC mineralization constant rates ($k_3$) obtained from experiment in Treatment 2 (Equation 4 and 5) were very lower than that measured in incubation with only DOC (Treatment 3 - $k_{T2}$). In the incubations with entire detritus (DOC + POC), the DOC and RPOC mineralization rate constants, $k_3$ tended to be higher, and lower than that for LPOC mineralization (Table 3).

## 4. Discussion

Carbon fluxes into the detrital pool are strong dependent on primary production (Cebrián & Duarte, 1995). The detritus organic matter derived from living biomass represents a structural component of the ecosystems (Neagoe et al., 2012) and also acts as a control variable in decompositions models since organic carbon is the precursor of C-gas mineralization products ($CO_2$ and $CH_4$). The C-gas fluxes from benthic metabolism were comprised mainly by $CO_2$ and $CH_4$ (Hamilton et al., 1994; Repo et al., 2007). Considering the anaerobic mineralization, the DIC measured in all treatments with the slightly acid medium of the decomposition chambers indicated that DIC comprised mainly carbonates and bicarbonates derived from speciation of $CO_2$ released from biogenic metabolism. Fermentation liberates as end-product $CO_2$ that
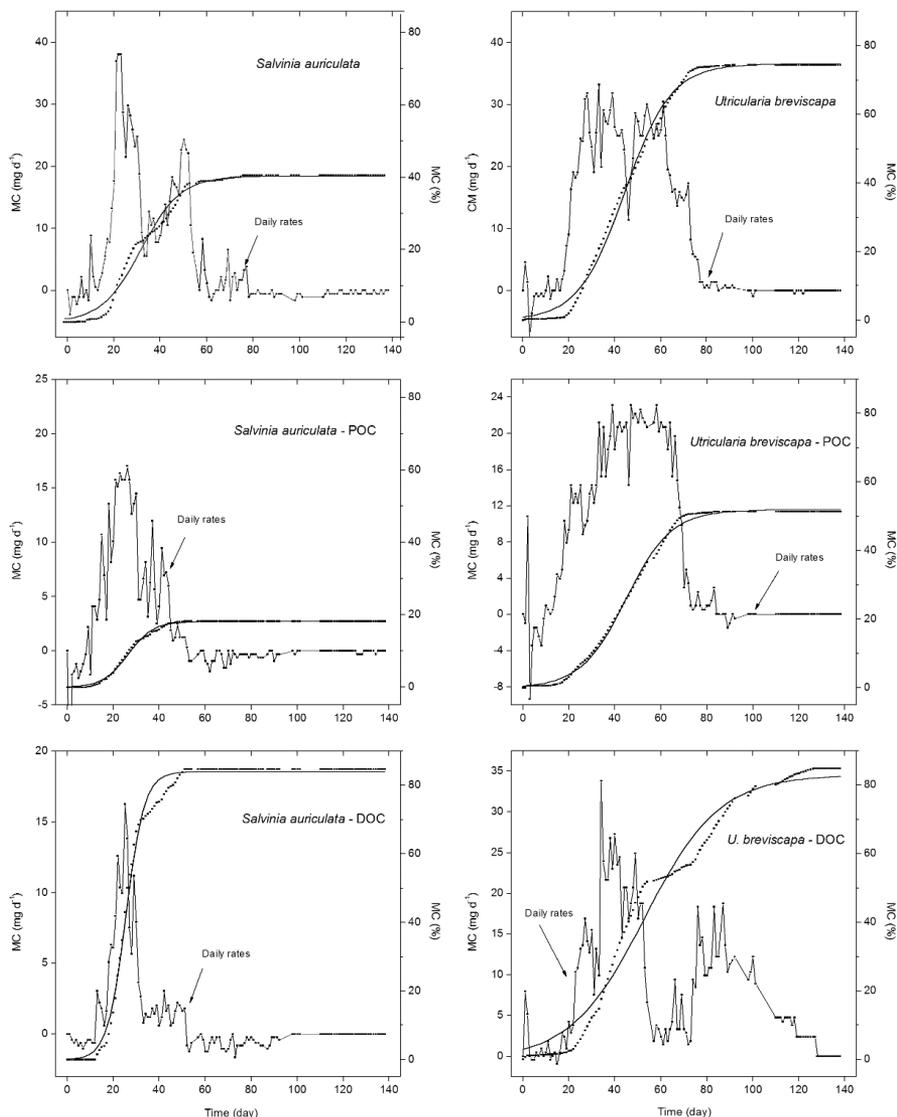
**Figure 1.** Kinetic of mineralization of the three treatments: Treatment 1 - integral detritus (top), Treatment 2 - POC (middle) and Treatment 3 – DOC (bottom) of *Salvinia auriculata* (left) and *Utricularia breviscapa* (right) according to manometric measures.

**Table 3.** Parameterization of *Salvinia auriculata* and *Utricularia breviscapa* decomposition. Where: LPOC = labile fraction of particulate organic carbon; DOC = dissolved organic carbon ($\equiv$ leachate); RPOC = refractory fraction of particulate organic carbon; LDOC = labile fraction of DOC; RDOC = Refractory fraction of DOC; $k_T$ = loss mass rate constant owing leaching and mineralization of labile POC ($k_T = k_1 + k_2$); $k_3$ = DOC mineralization rate constant; $k_4$ = RPOC mineralization rate constant; $k_{T2}$ = loss mass rate constant owing RDOC formation and mineralization LDOC ($k_{T2} = k_M + k_F$); $k_R$ = RDOC mineralization rate constant; $r^2$ = determination coefficient of model fit.

| Treatment | LPOC (%) | DOC (%) | RPOC (%) | $k_T$ (day$^{-1}$) | $k_3$ (day$^{-1}$) | $k_4$ (day$^{-1}$) | $r^2$ |
|---|---|---|---|---|---|---|---|
| *S. auriculata* | 8.8 | 5.2 | 86.0 | 0.284 | 0.0005 | 0.0008 | 0.87 |
| *S. auriculata* - POC | 6.2 | 7.8 | 86.0 | 0.389 | 0.0021 | 0.0012 | 0.93 |
| | | | | | | | |
| *U. breviscapa* | 19.7 | 15.3 | 65.0 | 0.116 | 0.0110 | 0.0012 | 0.97 |
| *U. breviscapa* - POC | 23.1 | 25.9 | 51.0 | 0.195 | 0.0083 | 0.0006 | 0.93 |
| | LDOC (%) | RDOC (%) | $k_{T2}$ (day$^{-1}$) | $k_R$ (day$^{-1}$) | $r^2$ | | |
| *S. auriculata* - DOC | 88.5 | 11.5 | 0.083 | 0.001 | 0.99 | | |
| *U. breviscapa* - DOC | 83.4 | 16.6 | 0.120 | 0.007 | 0.99 | | |

**Table 4.** Parameterization of the C-gas formation from anaerobic decomposition of *Salvinia auriculata* and *Utricularia breviscapa*. Where: LSPOC = labile/soluble particulate organic carbon (%); RPOC = refractory particulate organic carbon (%); C-gas$_{MAX}$ = maximum amount of C-gas; DIC = dissolved inorganic carbon; $k_M$ = rate constant for the total mass loss related to oxidation of labile compounds and DIC formation; $k_M = k_G + k_D$ ($k_G$ = C-gas formation rate constant; $k_D$ = DIC formation rate constant).

| Treatment | LSPOC (%) | RPOC (%) | C-gas$_{MAX}$ (%) | DIC (%) | $k_M$ (day$^{-1}$) | $t_{1/2}$ (day$^{-1}$) | $r^2$ |
|---|---|---|---|---|---|---|---|
| *S. auriculata* | 40.3 | 59.7 | 33.3 | 7.0 | 0.118 | 5.9 | 0.99 |
| *S. auriculata* - POC | 18.1 | 81.9 | 10.0 | 8.1 | 0.184 | 3.8 | 1.00 |
| *S. auriculata* - leachate | 84.0 | 16.0 | 43.4 | 40.6 | 0.251 | 2.8 | 0.99 |
| *U. breviscapa* | 74.7 | 25.3 | 60.4 | 14.3 | 0.100 | 6.9 | 1.00 |
| *U. breviscapa* - POC | 51.8 | 48.2 | 39.6 | 12.2 | 0.102 | 6.8 | 1.00 |
| *U. breviscapa* - leachate | 82.9 | 17.1 | 64.4 | 18.5 | 0.062 | 11.2 | 0.98 |
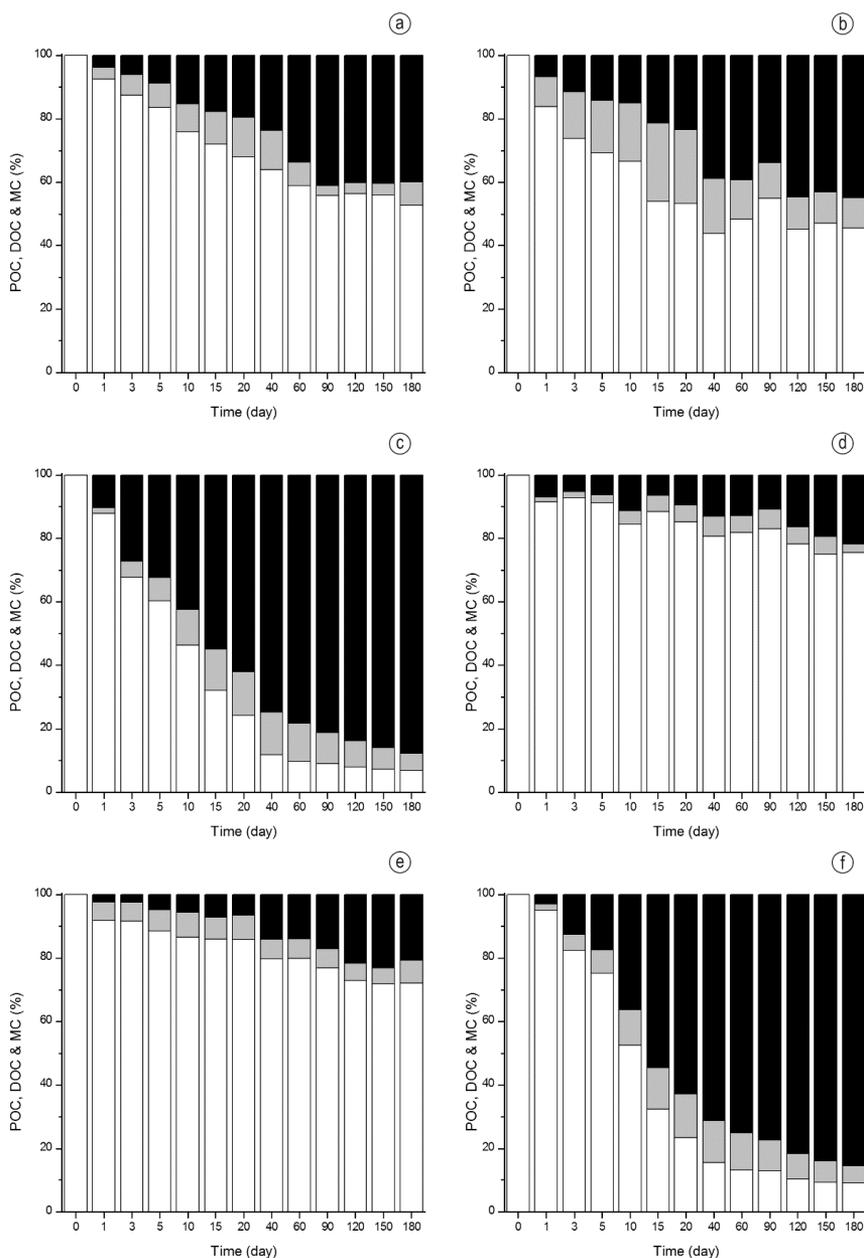


**Figure 2.** Carbon budget from the anaerobic mineralization of *Salvinia auriculata* (a = integral detritus; b = POC and c = DOC) and *Utricularia breviscapa* (d = integral detritus; e = POC and f = DOC). Where: particulate organic carbon (POC) = white bar; dissolved organic carbon (DOC) = gray bar and mineralized carbon (MC) = black.

in water, reduced pH of the medium by formation of $H_2CO_3$. Also, the DIC concentration derived from ionization of bicarbonate contributed to the elevated EC observed.

The three stage of decay process refers to the type of molecular components of detritus: (i) in the early stage, low weight-molecular (as carbohydrates, amino acids, and amino sugars) components were dominant, (ii) in the second stage, the contents sugar (mono-, oligo-, and polysaccharides) increased and, (iii) at the later stage, aromatic products were dominant once these compounds showed strong recalcitrance for microorganisms (Chen & Zhou, 2010).

In the early phase of decomposition of gases formation kinetics null gas volume was observed, due mainly to dissolution of $CO_2$ in the medium. Thus, only after the solution was saturated the method became more accurate from experimental point of view. To *S. auriculata* and *S. auriculata* POC (Treatment 2), the reaction rate constants ($k_1$, $k_3$ and $k_4$) were higher for the decomposition of fibers (i.e. cellulose, lignin and hemicellulose). For *U. breviscapa* these results were inverse ($k_3$ and $k_4$), except for $k_T$ (Table 4). As decomposition undergo, recalcitrant compound is biochemically synthesized by secondary reactions (i.e. humification) that include the transformation of biomolecules originating from dead organisms and the interaction of microbial activity. The chemical intricate molecule (secondary synthesis reactions) of the humic substance (HS) make turnover of these compounds a slow (Grinhut et al., 2007) but continuous process. Growth of decomposing microbiota can be suppressed by the high resistance to degradation of HS. Decay of HS molecules showed a slight decrease in carbohydrate content and some modifications in carboxyl content (Kontchou & Blondeau, 1990).

The most LSPOC is represented by the small molecules directly usable by organotrophic microorganisms. As macrophyte detritus decay proceeded, small molecular compounds polymerized around the aromatic rings (e.g. phenols), producing a refractory DOC that became more stable with decay time. Lower turnover rates can be associated either with RPOC (as humic and fulvic acids).

Independently of treatment, for decomposition of *S. auriculata* (entire detritus or fibers) the absence of leachate enhanced the decomposition coefficients, indicating that the previous extraction of leachate (i.e. DOC from macrophyte biomass) induced physical changes in the remaining fibrous

material that facilitates further biochemical reactions. The chemical structure of fibers in considering rate-regulating factors, and the fraction of lignified tissue may vary among litter species. The degradation pattern is related to the arrangement of the structural components in the fiber (Berg & McClaugherty, 2008) by an inducible enzyme system (Boschker & Cappenberg, 1998).

In nature, decomposition of entire plant (Treatment 1) is a decoupled process, in fact the POC (Treatment 2) settles and accumulated over sediments and, the hydrosoluble fraction is usually (DOC - Treatment 3) is processed in water column. The consequence of the interactions between detritus (POC and DOC) and microorganisms is the effectiveness of decomposition process. Chemical composition of detritus is known to be a key factor affecting C-gas flux rates, but the interplay between extrinsic factors may affect the chemical benthic fluxes by decomposition processes from anaerobic sediments. Studying decomposition processes and chemical composition of sediments allows us to understand the fate of the autochthonous and allochthonous organic matter and also the factors controlling the geochemistry of sediments. In tropical areas methanogenesis derived organic matter decomposition from macrophyte-dominated aquatic systems is a complementary process (as energy source) in the diagenesis of sediments (Cunha-Santino & Bianchini Júnior, 2013). Considering the gas production in tropical areas, studies that address the biochemical aspects of the decomposition of autochthonous organic matter sources in the sediment will be indispensable for understanding the formation of C-gas in the floodplain aquatic systems, as Óleo Lagoon.

## 5. Conclusions

The detritus plays a key role as a structural component of an aquatic system since this component support the functioning of the integrated system. Based on the responses from the bioassays simulating the anaerobic decomposition of the *S. auriculata* and *U. breviscapa*, we conclude that regardless of the type of detritus (*S. auriculata* and *U. breviscapa*), C mineralization was faster and more intense in DOC, i.e. Treatment 3 (ca. 85%). For *U. breviscapa* the POC mineralization (Treatment 2) was slower than entire detritus (Treatment 1) and *S. auriculata* mineralization was slower than *U. breviscapa*. The composition of the detritus (i.e. macrophyte type, presence and proportion of DOC) interfered synergistically in anaerobic degradation

of these plants. The DOC tends to consist in a facilitator, supporting the growth of microorganisms and intensifying mineralization.

## Acknowledgements

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