

Humic substances mineralization: the variation of pH, electrical conductivity and optical density.

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ABSTRACT: Humic substances mineralization: the variation of pH, electrical conductivity and optical density. **The variation of pH, electrical conductivity and optical density was described during the mineralization of humic substances (humic acid: HA and fulvic acid: FA) extracted from different sources: sediment, dissolved organic matter from 120 days of decomposition of aquatic macrophytes (*Scirpus cubensis* and *Cabomba piauhyensis*) and from water column of Infernão lagoon (21° 33' to 21° 37'S and 47° 45' to 47° 51'W). The assays were incubated in the dark under aerobic and anaerobic conditions at 21.0°C. The initial and final dissolved organic carbon concentrations from FA or HA incubations were measured. The pH, electrical conductivity and optical density values were registered during 95 days. It was possible to verify the presence of a buffer system generated by the humic substances. The decreases of the values of electric conductivity were registered in the two experimental conditions (aerobic and anaerobic), probably because of the prevalence of the biological assimilation (immobilization) in detriment to the ion dissolution. The increase on the optical density values was drive by the elevation of the pH values; the subsequent decrease on the optical density values occurred due to the mineralization of the chromophores groups of FA and HA molecules. The global heterotrophic uptake of carbon was also attributed to the decolorization of the substrata, being observed a positive correlation only for FA. In Infernão lagoon during FA and HA mineralization, the anaerobic process is responsible to 30% of the decolorization of these substances.**

Key-words: pH, electrical conductivity, optical density, humic acid, fulvic acid, aerobic and anaerobic mineralization.

RESUMO: Mineralização de substâncias húmicas: variação do pH, da condutividade elétrica e da densidade óptica. **A variação de pH, condutividade elétrica e densidade óptica foi descrita durante a mineralização de substâncias húmicas (ácido húmico: AH e ácido fúlvico: AF) provenientes de diferentes fontes: sedimento, matéria orgânica dissolvida obtida a partir de 120 dias de degradação de macrófitas aquáticas (*Scirpus cubensis* e *Cabomba piauhyensis*) e da coluna d'água da lagoa do Infernão (21° 33' a 21° 37'S e 47° 45' a 47° 51'W). Os ensaios foram incubados no escuro, sob condições aeróbias e anaeróbias a 21,0°C. As concentrações iniciais e finais de carbono orgânico dissolvido das incubações de AH ou AF foram medidas. Os valores do pH, da condutividade elétrica e da densidade óptica foram registrados durante 95 dias. A partir dos resultados foi possível verificar a presença de um sistema tampão gerado pelas substâncias húmicas. Foram verificadas diminuições dos valores de condutividade elétrica nas duas condições experimentais (aeróbio e anaeróbio), provavelmente devido ao predomínio da assimilação biológica (imobilização) em detrimento da dissolução iônica. O aumento nos valores de densidade óptica foi condicionado pela elevação dos valores de pH; a subsequente diminuição nos valores de densidade óptica ocorreu devido à mineralização dos grupos cromóforos das moléculas de AF e AH. O consumo heterotrófico global de carbono foi relacionado à descoloração dos substratos, sendo observada somente uma correlação positiva para o AF. Na lagoa do Infernão durante a mineralização de AF e AH, o processo anaeróbio é responsável por 30% na descoloração destas substâncias.**

Palavras-chave: pH, condutividade elétrica, densidade óptica, ácido húmico, ácido fúlvico, mineralização aeróbia e anaeróbia.

Introduction

In most natural waters, the major portion of dissolved organic carbon (DOC) is dominated by dissolved humic substances (HS), which corresponds up to 60% of DOC (Thurman, 1985). Aquatic humic substances derived from soil humus and terrestrial and aquatic plants are polar, amorphous, polymeric and straw-colored organic acids (Thurman & Malcolm, 1981). The HS is considered as polymeric products from carbohydrate degradation, lignin, proteins and fats in different age of decomposition. There is a consensus that HS are produced either by biochemical degradation of plant or animal residues or by polycondensation of relatively small organic molecules released during decay, and that microorganisms are related to both processes (Lu et al., 2001). HS may be divided into three fraction: humin, fulvic acid (FA) and humic acid (HA); these compounds are categorized on the basis of its solubility on alkali and acid media. The HS that precipitate in acid is HA and the remaining fraction on solution is the FA; the fraction that is soluble in alkali is the humin (Tate, 1995).

As moderate bacterial activity may occur in humic substances, it is possible that some fraction of aquatic humic substances is available as a primary bacterial substratum (Tranvik, 1988; Hessen, 1992). Bacterial uptake of DOC is the most important biotic sink for DOC in aquatic environments. External factors as temperature, oxygen concentration, pH, electrical conductivity and physical factors also act in the intensity of the mineralization rate. In general, the degradation of an organic resource can be processed under aerobic and anaerobic conditions.

This study aimed to describe the effects of pH, electric conductivity and optical density during the aerobic and anaerobic mineralization of HS. The used substrata were isolated from sediment and dissolved organic matter (DOM) from an 120 days of decomposition of aquatic macrophytes (*Scirpus cubensis* and *Cabomba piauhyensis*) experiment and from the water column of the Infern o lagoon (S o Paulo, Brazil).

Material and methods

Description of the area studied

Infern o lagoon is one of several oxbow lagoons located at the floodplain of the Mogi-Gua u river (State of S o Paulo, Brazil; 21° 33' to 21° 37'S and 47° 45' to 47° 51'W). Its area is of 3.05 ha; the average and the maximum depth are 2.1 m and 4.9m, respectively (Nogueira, 1989). Due to the intense occupation by *Scirpus cubensis*, *Salvinia auriculata* and *Cabomba piauhyensis* in this lagoon, large amounts of humic compounds are formed. The conversions of humic substances to particulate organic carbon (microorganisms) were, on the average, 13.6% for the fulvic acid and 15.7% for the humic acid (Cunha-Santino & Bianchini Jr., 2002).

Isolation of humic substances

HS were extracted from sediment and DOM from an 120 days of decomposition of aquatic macrophytes (*Scirpus cubensis* and *Cabomba piauhyensis*) experiment and from water column of the Infern o lagoon. HS were isolated using analytic procedures (Malcolm, 1985; Thurman, 1985) that was based on the solubility in acid and alkaline solutions. For the HS from the sediment, the technique consisted of: i) alkaline extraction (NaOH - 0.5 mol.L⁻¹); ii) centrifugation; iii) lowering the pH to » 2 (with HCl) and iv) fractionation of FA and HA by centrifugation (1 h; 978 ´ g). HS from sediment were obtained by successive extractions with NaOH under shaking. After 24 hours, the soluble material in alkali medium was removed and stored.

About 130 L of water lagoon was concentrated by evaporation with a hot plate at low temperature (» 40°C) to a final volume of 2 L. The extraction of HS extraction from DOM was accomplished as mentioned above for sediments.

For the HS production from aquatic macrophytes, the plant material (*Scirpus cubensis* and *Cabomba piauhyensis*) and the water samples were collected from Infern o lagoon.

The plant material was washed with tap water to remove attached matter and oven-dried at 45° C (until constant weight) and pounded. For setup the decomposition experiments, the plant material (2 g D.W.L⁻¹) was placed in acid-washed 5-L flasks with filtered (with glass wool) water from the lagoon. The flasks were maintained at room temperature. After 120 days, the flask contents were fractionated into DOM (whole leachate) and particulate organic matter (POM) by pre-filtration and centrifugation (1 h; 978 × g). HS were isolated from DOM derived from the plants decomposition by the procedures listed above.

Mineralization assays of humic substances

Samples of FA and HA from sediment, DOM from decomposed aquatic macrophytes (*S. cubensis* and *C. piauhyensis*) and from water lagoon were incubated in flasks containing filtered (with glass wool) lagoon water, under aerobic and anaerobic conditions. The anaerobiosis were maintained by bubbling N₂ during 15 min into the chambers every sampling day and aerobiosis by 1 hour bubbling air every time that dissolved oxygen (measured with ODMeter – Metrohm Herisau AGCH-9100/E-6370) reached values below 2 mg.L⁻¹.

The incubations (FA and HA) were maintained at 21.0°C ± 0.6. The solutions were analyzed after 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 56, 65, 80 and 95 days of incubations and pH and electrical conductivity were determined using pH meter (Digimed DMPH-2) and conductivimeter (Marconi CA150). The optical density (OD) was measured at 450 nm using a spectrophotometer (Pharmacia LKB – Novaspec II; the cuvette path length was 1 cm). The decolorization mineralization rates (k_p) were calculated fitting the optical density data to first-order exponential model, using a method of non linear regression, the iterative algorithm of Levenberg-Marquardt (Press et al., 1993). The initial and final (after 95 days) concentrations of carbon from the FA and HA incubations were measured by non-dispersive combustion and detection in infrared gas analysis (Shimadzu, TOC-5000A analyzer).

Results

The time evolution of the pH showed that, independent of the experimental condition (oxygen concentration and substrate type), the variations of the pH values (Figs. 1 and 2) followed the same pattern. The initial phase of mineralization (first day of experiment) showed increments in pH values that were maintained to approximately the 20th and 30th days of experiment, after which period the pH values tended to stabilize or decay.

The values of electric conductivity (Figs. 3 and 4) tended to decrease or to stabilize until the end of the experiment. A quite different behavior was observed for the AH-sediment under aerobic condition (Fig. 4A).

An increase in the values of optical density (Figs. 5 and 6) during the first days of the experiment (until approximately the 2nd day) was observed. After this period these values tended to decrease, until the end of the experiment. The time evolution of optical density during the mineralization had not presented intensive fluctuations, as it was observed for pH and electrical conductivity. However, only small increases were observed in some flasks. In most of cases optical density values were maintained or tended to decrease. The values of optical density recorded in the HA flasks (variation from 0.05 to 0.30) was higher than FA (variation from 0.02 to 0.014).

From fitted optical density data (Figs. 5 and 6), HA and FA losses of color can be evaluated by the decolorization coefficients (k_p). These coefficients were also used to calculate the half-time (t_{1/2}) of the decolorization process. These coefficients (± fitting error) ranged from 0.00162 ± 0.00066 to 0.00277 ± 0.00046 day⁻¹ for HA and 0.000126 ± 0.00073 to 0.00300 ± 0.00089 day⁻¹ for FA (Tab. I). On the average, the higher decolorization coefficients were observed for FA. The t_{1/2} of the decolorization process ranged, on average, from 231 to 5,776 days (HA: 250 to 433 days and FA: 231 to 5,776 days).

On the average the initial HA concentrations were expressed on carbon basis (DOC_i), 34.7 mg.L⁻¹; for FA this value was 44.6 mg.L⁻¹. The final carbon concentrations (DOC_f) of

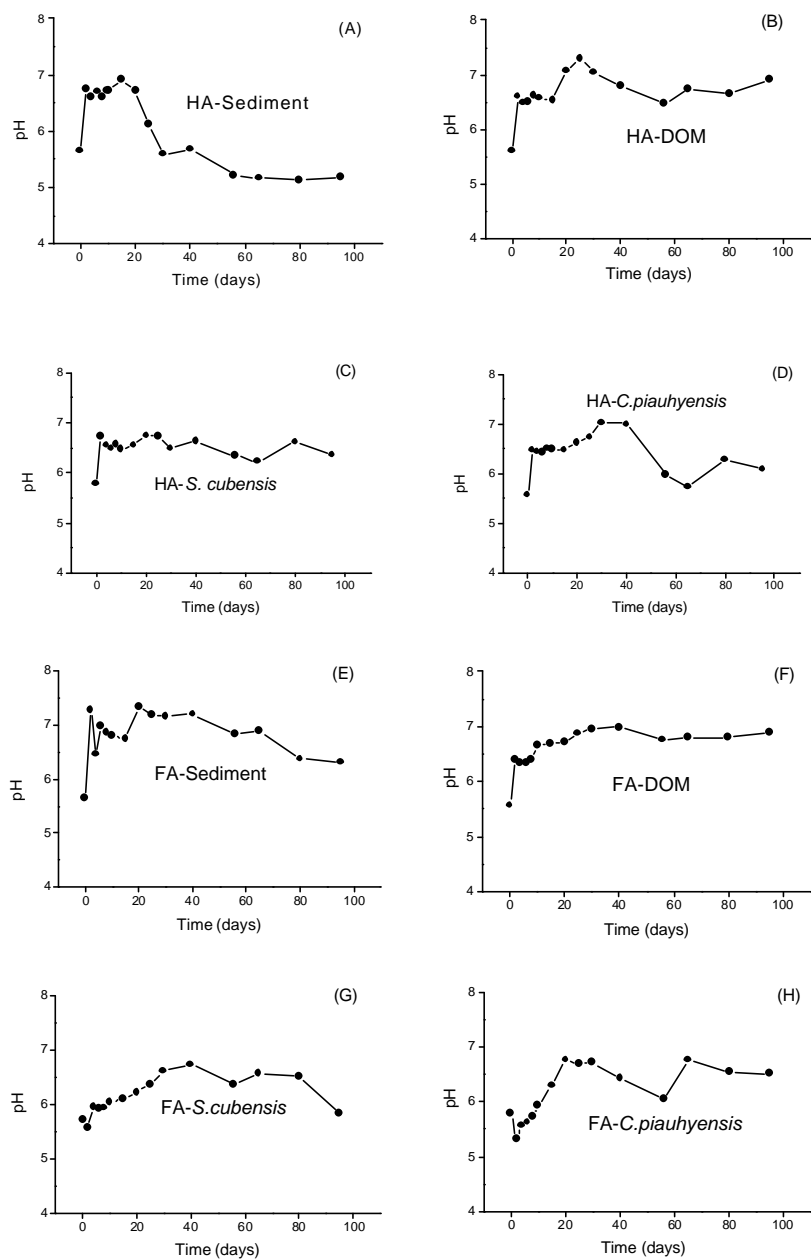


Figure 1: pH time evolution during aerobic mineralization: HA - Sediment (A), HA - DOM (B), HA - *S. cubensis* (C), HA - *C. piauhyensis* (D), FA - Sediment (E), FA - DOM (F), FA - *S. cubensis* (G), FA - *C. piauhyensis* (H).

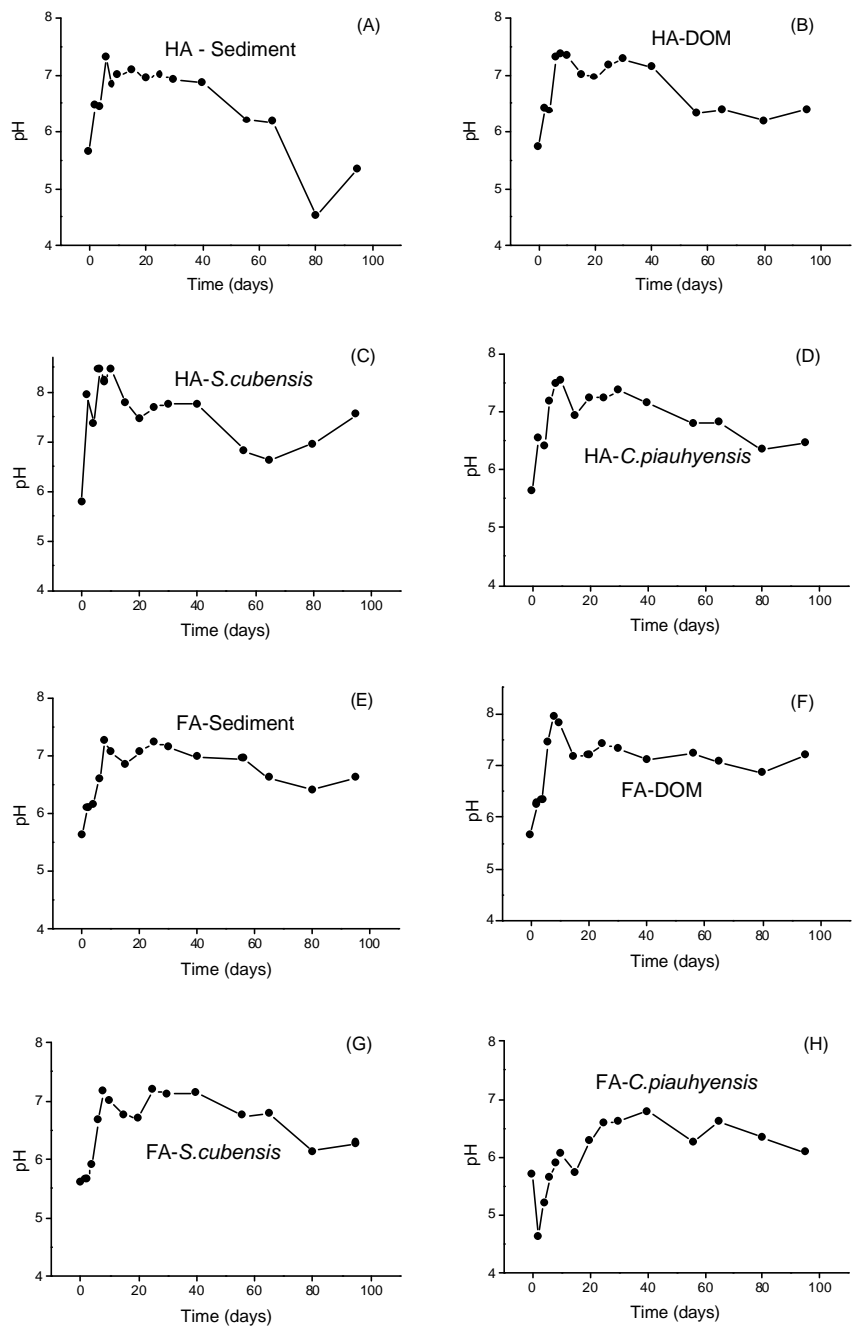


Figure 2: pH time evolution during anaerobic mineralization: HA - Sediment (A), HA - DOM (B), HA - *S. cubensis* (C), HA - *C. piauhyensis* (D), FA - Sediment (E), FA - DOM (F), FA - *S. cubensis* (G), FA - *C. piauhyensis* (H).

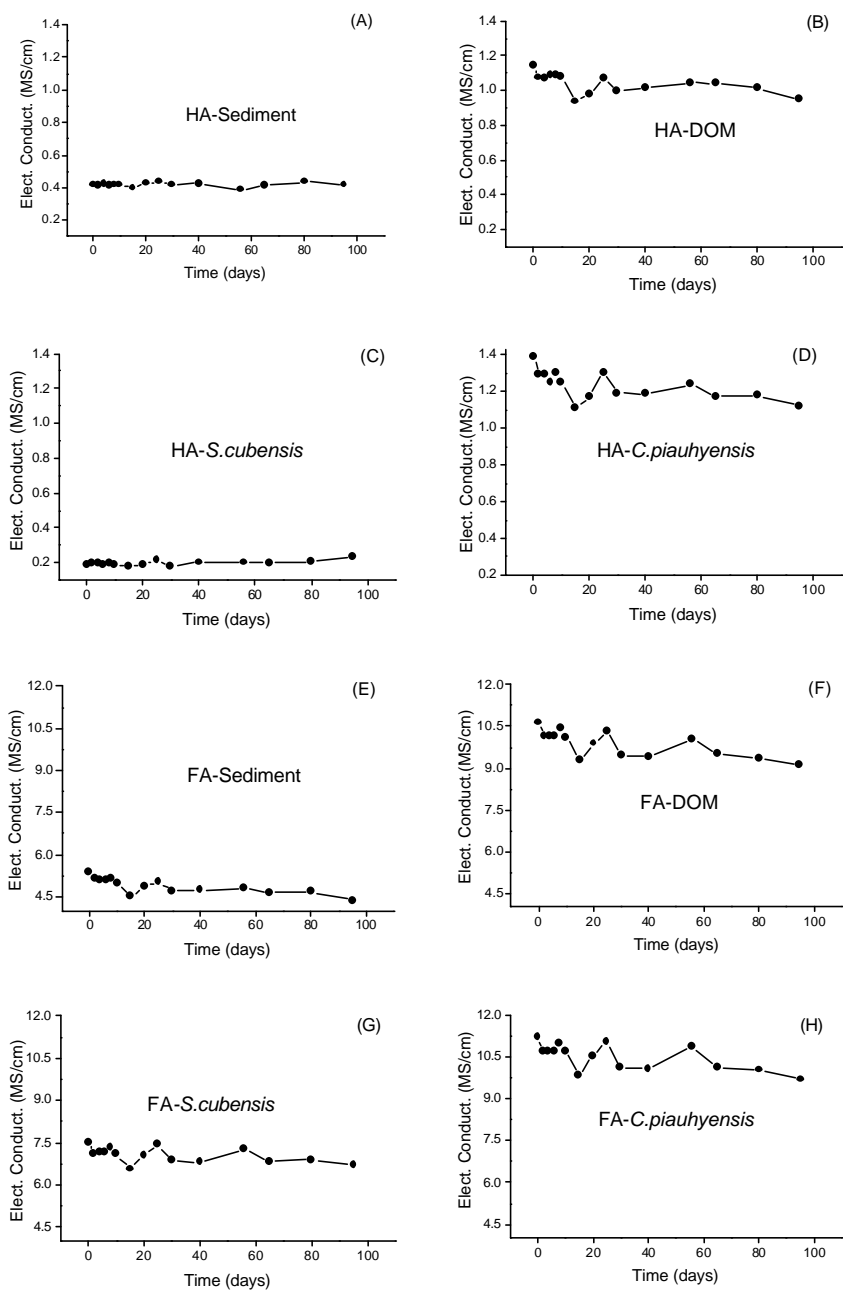


Figure 3: Electrical conductivity time evolution during aerobic mineralization: HA - Sediment (A), HA - DOM (B), HA - *S. cubensis* (C), HA - *C. piauhyensis* (D), FA - Sediment (E), FA - DOM (F), FA - *S. cubensis* (G), FA - *C. piauhyensis* (H).

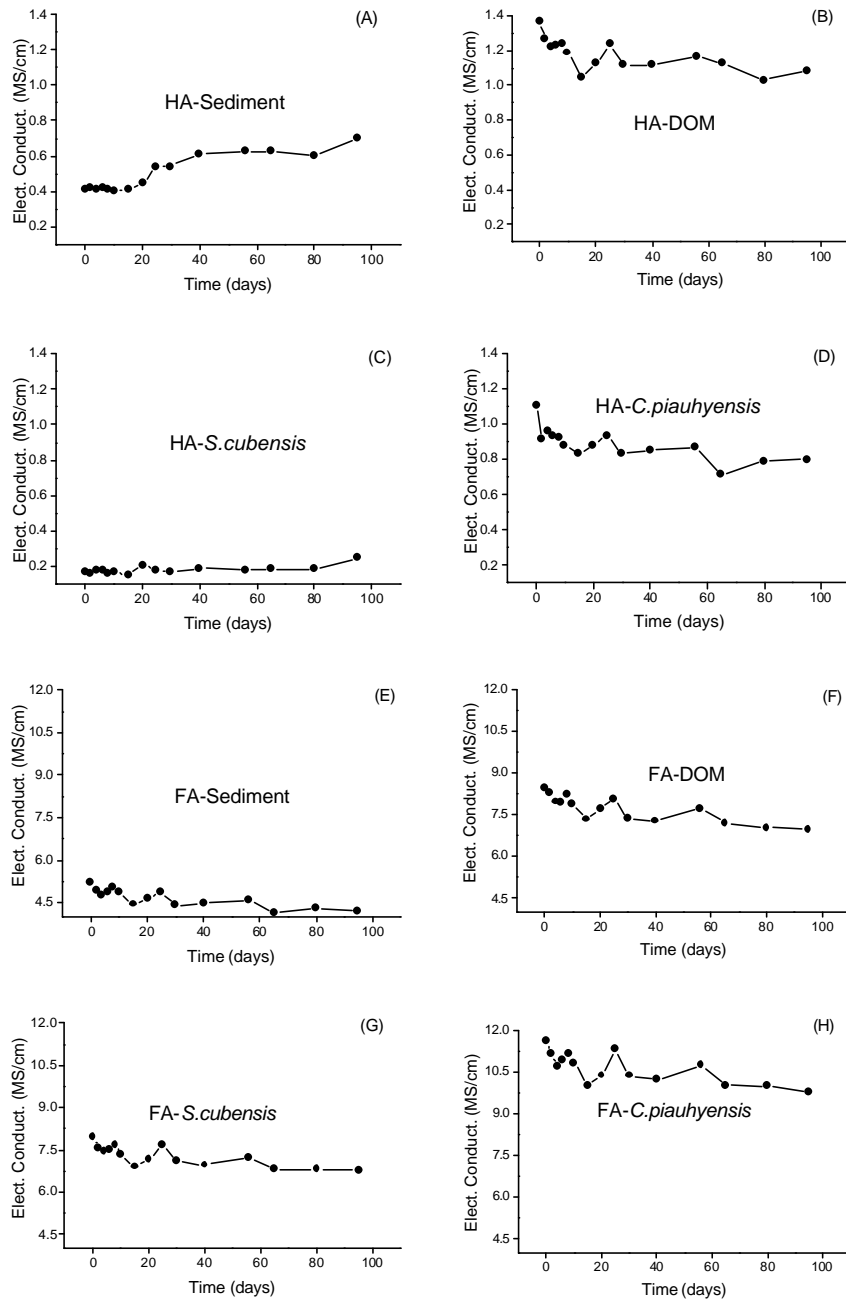


Figure 4: Electrical conductivity time evolution during anaerobic mineralization: HA - Sediment (A), HA - DOM (B), HA - *S. cubensis* (C), HA - *C. piauhyensis* (D), FA - Sediment (E), FA - DOM (F), FA - *S. cubensis* (G), FA - *C. piauhyensis* (H).

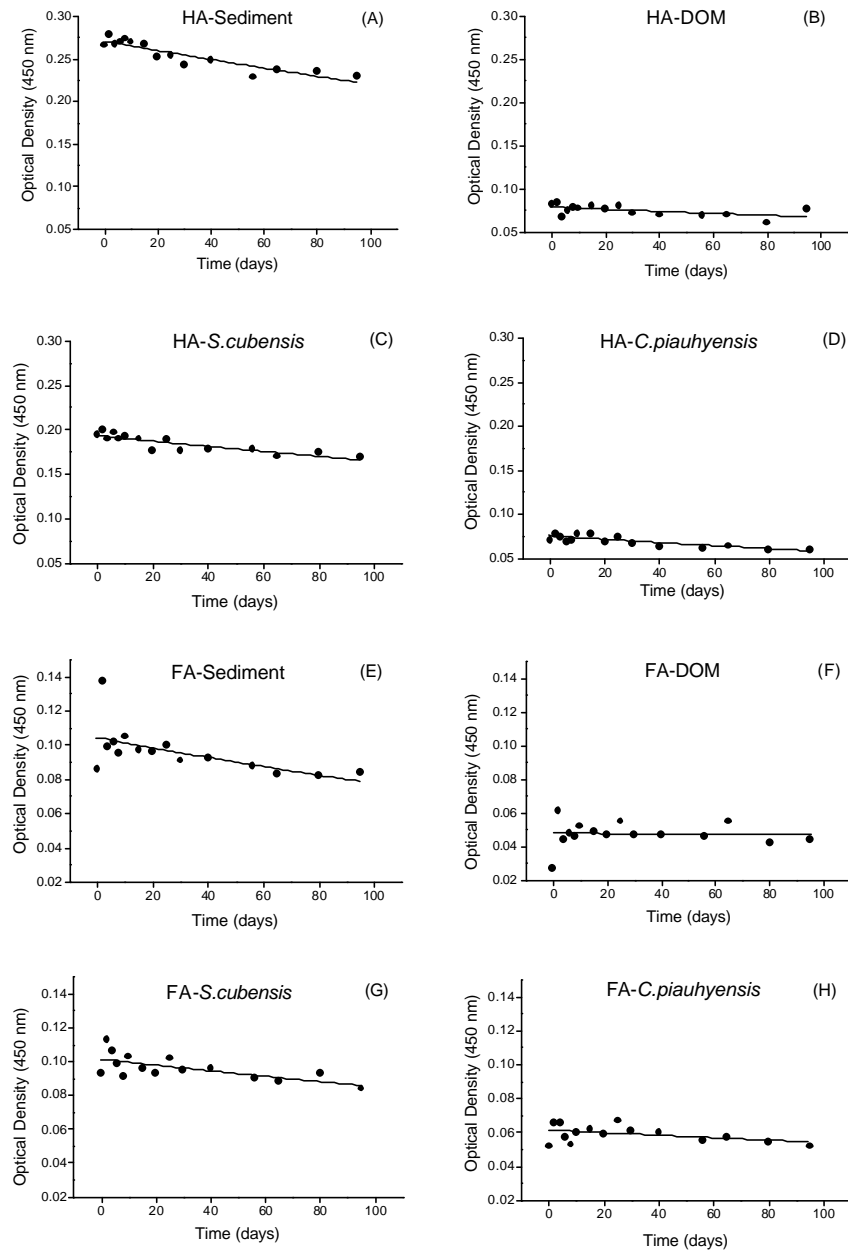


Figure 5: Optical density time evolution during aerobic mineralization: HA-Sediment (A), HA - DOM (B), HA - *S. cubensis* (C), HA - *C. piauhyensis* (D), FA - Sediment (E), FA - DOM (F), FA - *S. cubensis* (G), FA - *C. piauhyensis* (H).

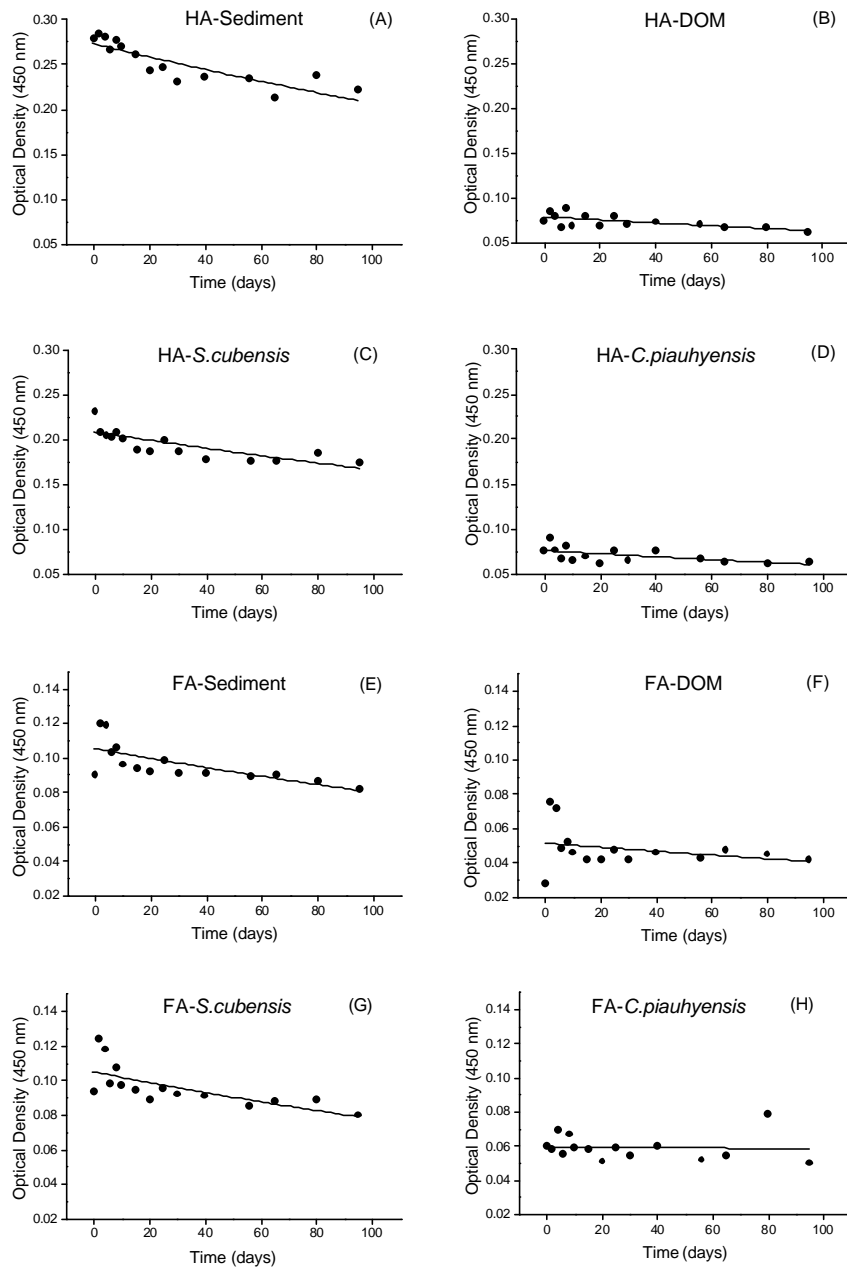


Figure 6: Optical density time evolution during anaerobic mineralization: HA -Sediment (A), HA - DOM (B), HA - *S. cubensis* (C), HA - *C. piauhyensis* (D), FA - Sediment (E), FA - DOM (F), FA - *S. cubensis* (G), FA - *C. piauhyensis* (H).

these substrata (HA and FA) were, on the average, 25.91 mg.L⁻¹ and 34.83 mg.L⁻¹, respectively. The difference between DOC_i and DOC_f (= dC) represents the global heterotrophic uptake of carbon (Tab. I). Fig. 7 shows simple linear analysis, relating the dC and k_D for HA and FA mineralization under aerobic and anaerobic conditions. A negative relation between for HA was observed (r² = -0.11) whereas for FA a positive correlation was found (r² = 0.58).

Table I: Decolorization coefficients (k_D) during HA and FA mineralization, under aerobic and anaerobic conditions. Error derived from the kinetic decolorization fittings, the half-time of process and the concentrations of initial (C_{initial}) and final carbon (C_{final}) = dC of HA and FA solutions.

Source	k _D (day ⁻¹)	Error	Half-time (day)	C _{initial} (mg.L ⁻¹)	C _{final} (mg.L ⁻¹)	dC (mg.L ⁻¹)
AEROBIC CONDITION						
HA - Sediment	0.00207	0.00026	335	46.34	11.19	35.15
HA - DOM	0.00162	0.00066	428	21.91	5.420	16.49
HA - <i>S. cubensis</i>	0.00160	0.00025	433	46.29	11.83	34.46
HA - <i>C. piauhyensis</i>	0.00269	0.00052	258	24.90	5.121	19.78
FA - Sediment	0.00295	0.00111	235	45.74	6.852	38.89
FA - DOM	0.00012	0.00146	5776	44.51	11.70	32.81
FA - <i>S. cubensis</i>	0.00175	0.00055	396	43.57	11.18	32.39
FA - <i>C. piauhyensis</i>	0.00126	0.00073	550	43.95	10.59	33.36
ANAEROBIC CONDITION						
HA - Sediment	0.00277	0.00046	250	44.17	13.35	30.82
HA - DOM	0.00229	0.00076	302	21.81	4.642	17.17
HA - <i>S. cubensis</i>	0.00227	0.00048	305	47.23	13.72	33.51
HA - <i>C. piauhyensis</i>	0.00235	0.00094	295	24.73	4.827	19.90
FA - Sediment	0.00278	0.00082	249	46.16	6.859	39.30
FA - DOM	0.00243	0.00229	285	45.83	10.49	35.34
FA - <i>S. cubensis</i>	0.00300	0.00089	231	42.72	9.885	32.84
FA - <i>C. piauhyensis</i>	0.00024	0.00120	2888	44.19	10.45	33.74

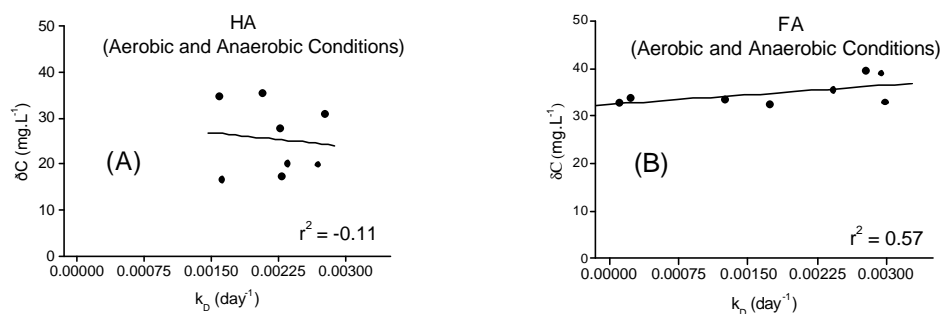


Figure 7: Decolorization coefficients (k_D) and dC (DOC_i - DOC_f) correlation from HA (A) and FA (B) aerobic and anaerobic mineralization.

Discussion

HA and FA are weak polyprotic acids and the existence of carboxyl (COOH) functional groups and negative charges (e.g. imide; phenolic OH, enolic OH) in the chemical structure of these substances resulted in an initial acid incubation medium (pH variation from 5 to 6). The increase on pH values recorded in the initial phase of the experiment (c.a. 10 days) during the mineralization was expected, since microorganisms processing the substrate release catabolic compounds to the solution. Thus a change from the initial acid medium to a neutral to alkaline (pH variation from 7 to 8) was observed. The variability in pH values can be attributed to the transformation of the substrate form reduced to oxidized

material during anaerobic mineralization and to the intense liberation of CO₂, predominantly during aerobic mineralization.

The predominance of a neutral to alkaline pH suggest that the HS established a buffer system, which is characteristic of the FA and HA molecules. HS exhibit buffering capacity over a wide range of pH and the maximum buffering capacity for HA, as well as FA, depends upon ions concentration of the solution (Stevenson, 1982). As the degradation proceeds it is also proposed that a second buffer system, the carbonate system (CO₂ - HCO₃⁻ - CO₃²⁻), coming from the CO₂ released by the mineralization, is also important. Such system is responsible for the stabilization of pH during the experiment. The relative proportion of CO₂, HCO₃⁻ and CO₃²⁻, is pH-dependent and according to the pH values in the mineralization chambers that ranged between 7 and 8, the HCO₃⁻ was the predominant chemical ionized specie. The pH is an important physiological factor for development of the heterotrophic microbial communities. In general, microorganisms cannot thrive at extremes pH values, in such conditions exposed microbial cells components can be hydrolyzed or proteins denatured (López-Archilla et al., 2001). The neutral to alkaline pH of the mineralization chambers could not have strongly affected the progress of the mineralization process.

The decrease in electric conductivity verified under both incubation conditions probably is related to the dominance of the biological assimilation of nutrients over the ion dissolution and losses of ions such as OH⁻ and H⁺ and the formation of neutral inorganic ions pairs (e.g. CaCO₃). On the other hand, for the HA-sediment, the increase in electrical conductivity is probably related to the dominance of ion dissolution over biological assimilation or ion pair formation (Antonio et al., 1999). Both aerobic and anaerobic mineralization generates CO₂ as end products. This molecule would establish equilibrium and react to form the carbonic acid, that would ionize and it would form bicarbonate and carbonate ions. The formed ions are part of the inorganic carbon pool of the solutions and contribute to the increase of the electrical conductivity.

The kinetic fitting obtained with the optical density data showed the decolorization process along the mineralization of HA and FA molecules. The anaerobic process was, on average, 30% higher than aerobic process. In spite of the aerobic process energetic yield is more efficient than anaerobic process, the microbiota of Infernão lagoon are well adapted to anaerobic conditions (Antonio & Bianchini Jr., 2002) becoming the anaerobic process more effective in the removal of HS molecules. When considering the type of resource, the HA decolorization process presented, on the average, a k_D 12% higher than FA. Probably this difference is a consequence of the chemical characteristic of the HS molecules. In comparison with HA, the FA molecule presents low content of carbon and nitrogen (Stevenson, 1982).

The melanoidin solubility is pH-depend and is less soluble in acidic pH than alkaline (Migo et al., 1993). Therefore, the increase in the pH due to the buffer system established HS and carbonate system generates an increase on optical density. The subsequent decrease of optical density is, probably, a result of the carbon mineralization in the labile fraction of FA and HA molecules which accounts for approximately 25% of these substances (Cunha-Santino & Bianchini Jr., 2002). During the decolorization process, there is a specific attack to the cromophores groups of the HS molecules; these cromophores are color centers with both phenolic quinones and conjugated double bounds (Thrumann, 1985).

The linear regression between dC and k_D for HA and FA mineralization under aerobic and anaerobic conditions (Fig. 7) showed a positive correlation for FA; for the HA no relation were obtained. The usual definition of humic and fulvic acid based on the difference of aliphaticity and reactivity, indicates the FA fraction as being more aliphatic and reactive and less aromatic than the HA fraction (Schulten & Schnitzer, 1995). Thus, the HA chemical properties contributed to inhibition of the decolorization process due to the aromaticity and less reactivity of this molecule. In relation to HA the resynthesis reactions lead to a new colored compound. HS resynthesis refers to condensations or polymerization, by enzymatic reaction mediated by microorganisms or chemical reactions (Hatcher & Spiker, 1988). For instance, the non-enzymatic reaction of a reducing sugar

(e. g. fructose, glucose) with an amino compound (e. g. amino acids) with the maximum absorbance wavelength ranging between 420 to 480 nm, corresponding to the development of brown melanoidins, knowing as the Maillard reaction (Manzocco et al., 2001).

In conclusion, the anaerobic process is about 30% higher on the decolorization of these substances than aerobic process at the Infernão lagoon during de FA and HA mineralization. HA molecules presented higher decolorization coefficients (» 12%) than FA molecules. HS affect the buffer system of the lagoon by maintained the pH levels at a neutral level and during the mineralization large amounts of ions are liberated to the lagoon; the decrease in the total electrical conductivity of the water column is probably related to the assimilation process of these ions by microorganisms.

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