



Photo-degradation effect on dissolved organic carbon availability to bacterioplankton in a lake in the upper Paraná river floodplain

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ABSTRACT. Dissolved organic carbon (DOC) is nowadays recognized as the main substrate and source of energy for aquatic microbial community. The great part of available organic carbon for bacterioplankton might be formed after photolytic degradation of humic material, which constitutes the major part of DOC in almost all natural waters. The effects of DOC photo-degradation were evaluated, as was its utilization by bacterioplankton, through a two-step experiment, one involving photo-degradation of DOC and the other bacterial growth on the photo-degraded substrate. Photo-degradation was responsible for the consumption of 19% of DOC, reduced SUVA₂₅₄, an increase in the E2/E3 and E3/E4 ratios, in addition to modifications in the fluorescence spectra that indicated a rise in the labile fraction of DOC. However, these alterations on DOC were not reflected in differences in bacterioplankton growth, as shown by the fact that there were no significant differences in density, biomass, bacterial production, bacterial respiration and bacterial growth efficiency between treatment and control.

Keywords: dissolved organic carbon, bacterioplankton, photo-degradation, experiment.

Efeito da foto-degradação na disponibilidade do carbono orgânico dissolvido para o bacterioplâncton

RESUMO. O carbono orgânico dissolvido (COD) é reconhecido atualmente como o principal substrato e fonte de energia para a comunidade microbiana aquática. Grande parte do COD biodisponível para o bacterioplâncton pode ser gerada após degradação fotolítica do material húmico dissolvido, que constitui a maior parte do carbono orgânico total. Neste trabalho foram avaliados os efeitos da fotodegradação no COD de um ambiente húmico e a sua posterior utilização pelo bacterioplâncton, por meio de um experimento em duas etapas, uma de fotodegradação do COD e outra de crescimento microbiano sobre o substrato fotodegradado. A fotodegradação causou consumo de 19% do COD, diminuição na SUVA₂₅₄, aumento nas razões E2/E3 e E3/E4 e modificações nos espectros de fluorescência apontando aumento da fração lábil. No entanto, as alterações no COD não se refletiram em diferenças no crescimento do bacterioplâncton, não havendo diferenças significativas com relação à densidade, biomassa, produção, respiração e eficiência de crescimento bacteriano.

Palavras-chave: carbono orgânico dissolvido, bacterioplâncton, foto-degradação, experimento.

Introduction

The importance of organic carbon in aquatic environments was for a long time restricted to its interactions with metals and its relationship with ecosystem stability (AZAM et al., 1983; STEINBERG, 2003), being considered biologically inert. However, dissolved organic carbon (DOC) is nowadays recognized as the main substrate and energy source to the aquatic microbial community's maintenance, and it can be considered the base of the planktonic trophic chain (FARJALLA et al., 2006a). The concept of the microbial loop introduced by Pomeroy (1974) and developed by Azam et al. (1983) attributed more importance to DOC and a new role to bacterioplankton, until then a neglected community.

The microbial loop is responsible for transferring the energy from the pelagic system through the DOC → bacterioplankton → protozooplankton route (AZAM et al., 1983). The role of bacteria in this route is to turn dissolved organic matter lost from the herbivorous food web into particulate organic matter, which can be assimilated by protozooplankton (AZAM et al., 1983). In this way, the bacterial community plays a fundamental role in the aquatic trophic chains and has great importance in the biogeochemical processes, even on a biosphere level (COTNER; BIDDANDA, 2002).

DOC availability to heterotrophic bacteria depends on its biochemical composition, molecular size and inorganic nutrients concentration, among other factors (AMON; BENNER, 1996). DOC

from the primary producer's exudates and senescence or from excretes and death of other organisms is considered to be labile and readily available for bacterial consumption (BAINES; PACE, 1994; CARLSON; DUCKLOW, 1996). Compounds of pedogenic origin, i.e., formed in the soil, like most of humic substances (HS), are considered biorefractory (LOVLEY et al., 1996; PATEL-SORRENTINO et al., 2004).

A considerable part of the organic carbon available to bacterioplankton is formed after photolytic degradation of dissolved humic material, which constitutes most of the DOC in almost all natural waters (STEINBERG, 2003; THURMAN, 1985). According to Amon and Benner (1996), HS structures have organic chromophores that absorb radiation in the UV and visible wave lengths. In this way, HS are the compounds that contribute the most to the photochemical reactions in surface waters (ANESIO et al., 2005).

Besides DOC, inorganic nutrients, mainly dissolved phosphorus, can also influence bacterial growth and can be a limiting factor (MORRIS; LEWIS, 1992; TOOLAN et al., 1991). According to Toolan et al. (1991), phosphorous limitation can occur directly, by a lack of phosphorus to bacteria, or indirectly, by a lack of algal carbon caused by a lack of phosphorus to the growth of phytoplankton. Carvalho et al. (2003) discussed the minor importance of phytoplanktonic carbon to bacterioplankton in the environments of the Paraná river floodplain, shown through the lack of relationship between bacterioplankton density and chlorophyll-*a*.

Since the introduction of the microbial loop concept, many studies have aimed to reveal the role of bacterioplankton and of DOC in aquatic environments, (e.g. TRANVIK; HOFLE, 1987; COLE et al., 1988; THOMAS, 1997; COTNER; BIDDANDA, 2002; STEINBERG et al., 2006), including tropical ecosystems (e.g. FARJALLA et al., 2001a, 2006a and b). The role of photo-degradation in DOC and in its bacterial uptake has also been evaluated in some studies, most of them in temperate environments (e.g. ANESIO et al., 2005; BERTILSSON; TRANVIK, 1998; BERTILSSON et al., 1999; LINDELL et al., 1995; ZAGARESE et al., 2005). In the tropical region, some works have been conducted in the Amazonas river floodplain (e.g. AMADO et al., 2006; AMON; BENNER, 1996; PATEL-SORRENTINO et al., 2004). In the upper Paraná river floodplain, Azevedo et al. (2008) made a study related to the characterization of HS, and Carvalho et al. (2003) evaluated the factors that influence the abundance of bacterioplankton in 20 lagoons. However, so far, there have been no studies

relating DOC quantity and quality with its utilization by bacterioplankton, as the effects of photo-degradation on this process in this floodplain.

This study aimed to evaluate the effects of photo-degradation on DOC, and bacterioplankton growth utilizing photo-chemically degraded DOC as a substrate, in a humic lake from the upper Paraná river floodplain. The hypothesis is that the photo-degradation will cause modifications to the DOC, rendering it more labile. As a result, bacterioplankton growth will be higher or more efficient in the irradiated samples.

Material and methods

Water samples for the experiment were obtained from the Patos lagoon (22°49'33,66"S; 53°33'9,9"W), one of the largest on the Paraná river floodplain. This lagoon is connected to the river Ivinheima, is 3.5 m deep and has dark waters due to the amount of humic substances (AZEVEDO; NOZAKI, 2008; AZEVEDO et al., 2008). The sampling occurred in March 2008, in a low-water phase (Paraná river level lower than 3.5 m), during the Long Term Ecological Research/National Council for Scientific and Technological Development (PELD/CNPq) program campaign, developed by the Nucleus of Research in Limnology, Ichthyology and Aquaculture (Nupelia) at the State University of Maringá (UEM).

The effects of DOC quality, altered by solar radiation, on bacterioplankton were tested through a two-step experiment (Figure 1). In the first step, only the effects of radiation on DOC were evaluated; in the second, bacterioplankton growth on the irradiated substrate was tested. The experiment was elaborated according to a few preview works, such as Amon and Benner (1996), Patel-Sorrentino et al. (2004), Anesio et al. (2005), Farjalla et al. (2006b) and Amado et al. (2006).

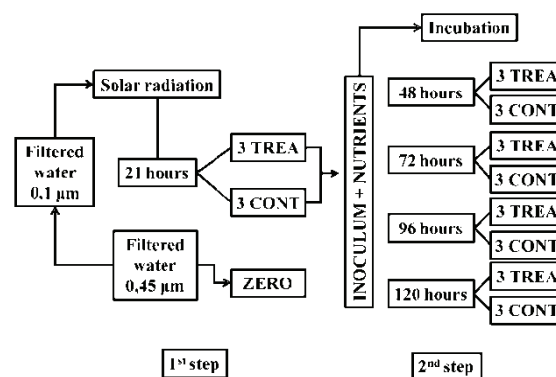


Figure 1. Experimental design.

Forty-five liters of water were collected from the Patos lagoon, around 30 L to simulate the medium and the rest for the photo-degradation itself. Around 12 L were filtered with a cellulose ester membrane with a $0.45\ \mu\text{m}$ pore (Whatman) to remove larger particles and organisms. From this filtrate, one litter was reserved to be used later as the inoculum. The rest was filtered through alumina membranes with a $0.1\ \mu\text{m}$ pore (Wathman) to remove bacteria. The inoculum was stored at a temperature of under 4°C and in the dark until use.

The water passed through the alumina membrane was distributed in six borosilicate flasks, three of which were protected with aluminum sheets. All the flasks were then immersed in a recipient containing enough Patos Lagoon water to cover them entirely, and the recipient was exposed to sunlight. The flasks were exposed to solar radiation for a total of 21 hours. The controls, however, were always protected with aluminum sheets. The exposure to radiation occurred from 9 am to 4 pm, and from one day to the next the flasks were kept in the dark at under 4°C . Each sample was submitted to measurements of pH (pH-meter Digimed DM2), dissolved oxygen (oxymeter YSI 55), temperature (oxymeter YSI 55) and alkalinity (GRAM method, CARMOUZE, 1994), in the same way as the initial sample (ZERO). Besides these measurements, samples were stored for later DOC, UV-VIS and fluorescence analysis.

Following the first step of the experiment, as described above, treatment (TREA) and control (CONT) received the inoculums (10%) and nutrients ($50\ \mu\text{mol L}^{-1}$ of nitrogen- NO_3NH_4 in addition to $5\ \mu\text{mol L}^{-1}$ of phosphorus- K_2PO_4), and were incubated in closed flasks (no air inside) under a constant temperature (25°C) in the dark. Each TREA or CONT was divided into four flasks that were removed from incubation after 48, 72, 96 and 120 hours (24 flasks total). Of each removed flask, a part was fixated to bacterial counting and then the replicates were carefully mixed to pH, dissolved oxygen, temperature and alkalinity measurements, and an amount was reserved for later DOC, UV-VIS and fluorescence analysis (8 samples in total). Although mixing the samples might have affected the pH and oxygen measurements, the low volume of the samples meant that this procedure was greatly required. Initial bacterial density was calculated through inoculum density, considering the total efficiency of the alumina filtration.

Bacterioplankton density was estimated through epyfluorescence using an Olympus BX51 microscope. Slides were prepared with 0.2 mL of fixed samples colored with 1 mL of 4,6'-diamidino-2-phenyl-indole (PORTER; FEIG, 1980) 0.1% for 15 minutes in

polycarbonate black (colored with *Irgalan black*) membranes with a $0.2\ \mu\text{m}$ pore (Wathman). The cells were counted in groups according to size and shape, and the biovolume of each shape was calculated according to Sun and Liu (2003). Cell measurements were made in sample photographs with the Image-Pro Express image analyzer software, version 4.5.1.3 (Media Cybernetics, Inc.). To estimate biomass based on biovolume, the conversion factor of Thiel-Nielsen and Søndergaard (1998) ($1\ \mu\text{m}^3 = 105\ \text{fg C}$) was used. Of helical cells, rare in the counting, no measurements were made, and the biomass was calculated using the Lee and Fuhrman (1987) conversion factor (1 cel. = $20\ \text{fg C}$). Bacterial production (BP) and bacterial respiration (BR) were calculated using data acquired after 48 hours incubation biomass and CO_2 (calculated by pH) production, respectively. The percentage of labile carbon (LDOC) was estimated by $(\text{BP} + \text{BR}) / \text{IDOC}$, IDOC being the initial DOC. The bacterial growth efficiency (BGE) was also calculated, using $\text{BP} / (\text{BP} + \text{BR})$. The differences between the TREA and CONT parameters (BP, BR, LDOC and BGE) were compared through a T-test, with a significance level of $p < 0.05$.

DOC was determined by using TOC-V CPH (Shimadzu) equipment. The fluorescence analyses were made with a F-4500 (Hitachi) fluorescence spectrophotometer. Emission spectra (excitation wave length of 370 and 314 nm) in the 300 to 600 nm region and synchronous scan with excitation from 250 to 600 nm ($\Delta\lambda = 18\ \text{nm}$) were obtained. All fluorescence spectra were made with $240\ \text{nm min}^{-1}$, 5 nm fence, 1 cm quartz cell and Milli-Q water as blank. Milli-Q water Raman peak intensity was used to monitor changes in fluorescence intensity (FI). A blank spectrum was subtracted from the spectra samples, and DOC (mg L^{-1}) was used to normalize the spectra. The UV-VIS scanning was done using a Carry 50 (Varian) spectrophotometer with 1 cm quartz cell from 250 to 650 nm.

Results and discussion

First step - Photo-degradation

In relation to ZERO, DOC diminished $39.8 \pm 0.8\%$ after treatment, while in the control it diminished $20.1 \pm 1.7\%$. This indicates that although some DOC consumption occurred in the protected flasks, radiation caused the mineralization of approximately 19% of the DOC. Dissolved oxygen consumption was nearly the same in TREA and CONT, 24.3 ± 7.8 and $23.1 \pm 6.4\%$, respectively. $\text{CO}_{2\text{TOTAL}}$ also varied in similar way to TREA and CONT, with a small diminishment (Table 1). Alkalinity and pH remained stable during the experiment.

Table 1. Mean (N = 3) and standard deviation (brackets) of variables measured before (ZERO) and after 21 hours of solar exposition (TREA and CONT). *N = 1. †Analytic replicates.

	T °C	DO mg L ⁻¹	pH	Alk. μEq L ⁻¹	CO ₂ TOTAL μmol L ⁻¹	DOC mg L ⁻¹
ZERO	21.60 *	6.73 *	6.67 *	413.20 *	622.30 *	7.02 (0.44) [†]
TREA	34.37 (0.38)	4.87 (0.23)	6.79 (0.19)	420.27 (18.77)	561.07 (35.49)	4.22 (0.06)
CONT	32.80 (1.39)	5.10 (0.53)	6.70 (0.10)	396.07 (4.04)	560.27 (34.99)	5.60 (0.12)

Specific UV absorption (SUVA) values indicate molecular weight, amount of double bonds and aromatic rings on DOC (WESTERHOFF; ANNING, 2000). SUVA values in 254 and 285 nm were higher for TREA and CONT than for ZERO (Figure 2a), indicating an increase in the relative amount of higher molecular weight compounds and higher insaturation degree and aromaticity. Bertilsson et al. (1999) observed the photo-production of carboxylic acids after water sample irradiation. The increase in SUVA may also be attributed to the standardization of absorbance by DOC because the consumption of labile DOC, which does not absorb in UV region, increases the SUVA. Therefore, for the same amount of chromophores, there is a lower amount of DOC.

The ratios between absorbance in 250 and 365 (E2/E3) and 300 and 400 nm (E3/E4) (Figure 2b) indicate a variation in the degree of humification, aromaticity and molecular weight, being inversely proportional to these variables (ARTINGER et al., 2000; CHEN et al., 2002; PEURAVUORI; PIHLAJA, 1997). Both rates rose from ZERO to TREA, indicating a reduction in aromaticity and molecular weight, in part due to radiation (TREA) and in part due to bacterial decomposition (CONT).

Fluorescence intensity (FI) at 450 nm in 314 (IF_{Ex:314/Em:450}) and at 370 nm (IF_{Ex:370/Em:450}) is inversely proportional to the molecules weight (SENESE 1990; SIERRA et al. 1997). The spectra with excitation of 314 nm (Figure 3a) were quite distinct from each other. The maximum FI peak was around 420 nm for all, but the FI rose in the order ZERO < CONT < TREA, indicating degradation of the larger molecules and formation of smaller molecules in TREA and, to a lesser extent, in CONT. With excitation at 370 nm, spectra were more similar (Figure 3b).

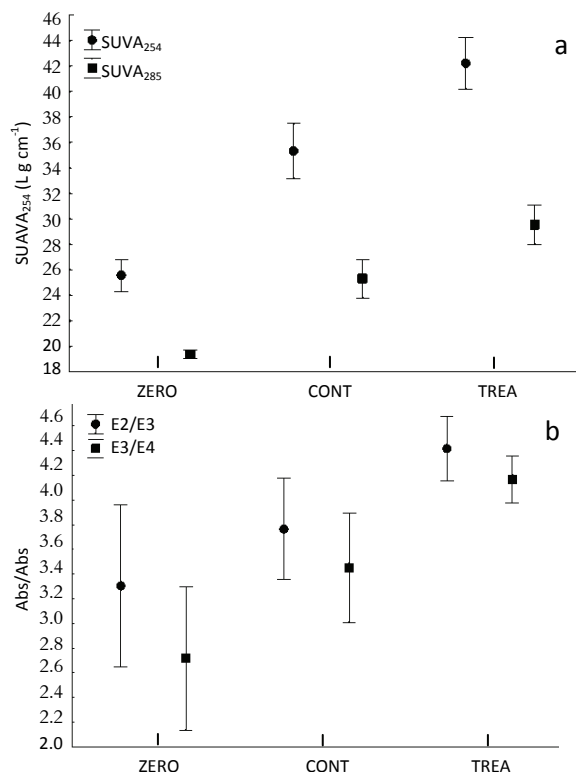


Figure 2. Mean and standard deviation of SUVA values at 254 and 285 nm (a) and the rates between absorbance E2/E3 and E3/E4 (b).

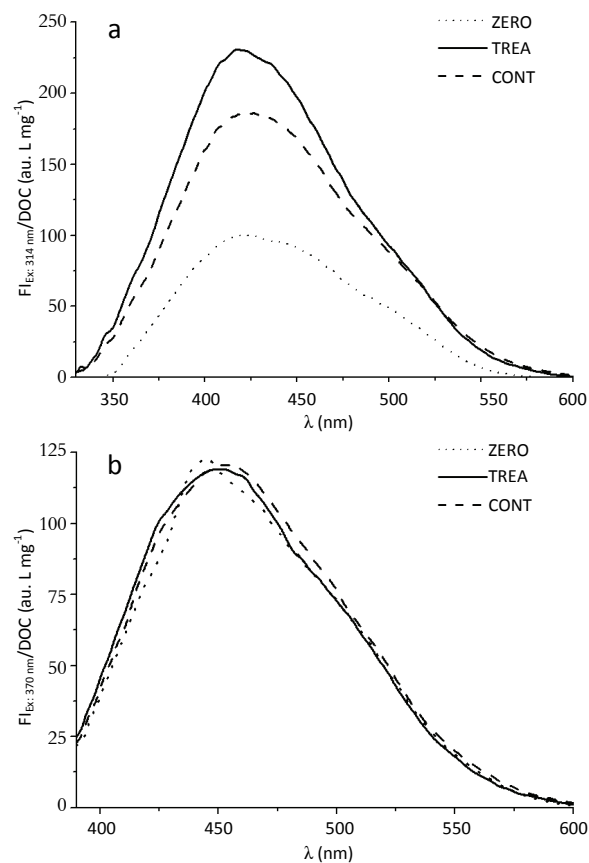


Figure 3. Fluorescence spectra with excitation at 314 (a) and 370 nm (b).

Although the larger molecules were broken, mainly in their more accessible functional groups, it is possible that some high molecular weight and high aromatic content structures remained, and these were responsible for most of the fluorescence emission at lower energy wave lengths.

The ratio between the fluorescence intensities at the 450 and 500 nm wave lengths with an excitation of 370 nm (denominated FR) can characterize DOC in relation to its humification degree (MCKNIGHT et al., 2001; WESTERHOFF; ANNING, 2000; WU et al., 2007). These values were 1.8 ± 0.3 for ZERO, 1.9 ± 0.0 and 2.1 ± 0.0 for CONT and TREA, respectively. FR values are inversely proportional to the humification degree of DOC, indicating that a formation of more labile compounds took place.

In synchronous spectra, Peuravuori et al. (2002) and Ferrari and Mingazzini (1995) identified regions that might indicate some HS structures. In general, the higher the excitation and wave length emission is, the higher the complexity of the molecules that emit fluorescence. Fluorescence intensity diminishes as the molecular weight of compounds emitting fluorescence rises (SENESE, 1990; SIERRA et al., 1997). Both TREA and CONT reached their peak between within 300 and 350 nm (Figure 4), which corresponds to the emission of two conjugated aromatic rings and their derivatives (PEURAVUORI et al., 2002) or to the emission of simpler compounds, indicating the formation of more labile molecules. At around 400 nm, the compounds that emit fluorescence are those with five benzene rings and close to 450 nm, those with more than five rings, some lignin derivatives or fulvic acids (FERRARI; MINGAZZINI 1995; PEURAVUORI et al., 2002; SIERRA et al., 1997). TREA showed higher FI in both regions, followed by CONT and ZERO, indicating that the molecular weight or structure complexity is higher in ZERO than in TREA.

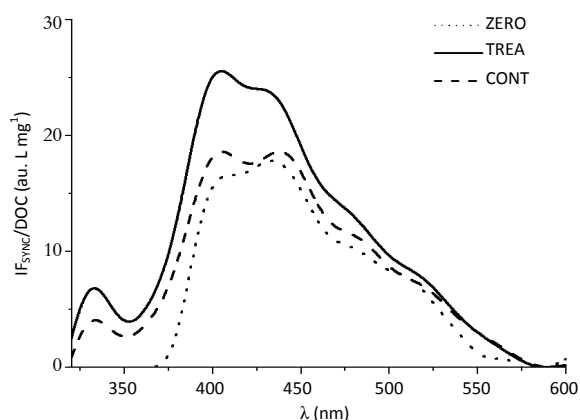


Figure 4. Synchronous emission spectra ($\Delta\lambda = 18$ nm).

FI in wave lengths longer than 500 nm indicates that more complex compounds still remain after photo-degradation and are not significantly altered by bacteria.

DOC variation and spectroscopic analysis showed that photo-degradation actually transformed organic matter from more complex molecules to more labile ones.

Second step – DOC utilization by bacterioplankton

Bacterial growth after incubation occurred faster than expected, and as the first samples were removed after 48 hours, it was not possible to observe the complete growth curve. However, it is likely that in 48 hours the bacteria were in their final exponential phase or at the beginning of the stationary phase because they had higher density values. Initial density was $0.14 \pm 0.03 \cdot 10^9$ cel. L^{-1} and the maximum achieved was $1.03 \pm 0.30 \cdot 10^9$ cel. L^{-1} in CONT and $0.88 \pm 0.08 \cdot 10^9$ cel. L^{-1} in TREA in 48 hours of incubation. TREA and CONT showed a significant difference (T test, $p < 0.05$) only in relation to LDOC percentage ($t = 4.725$; $p = 0.018$), confirming the production of more labile carbon through photo-degradation.

BP and BR values (Table 2) did not differ significantly between TREA and CONT, but CONT showed a higher BP value while TREA showed a higher BR value.

Table 2. Bacterial Production (BP), respiration (BR), labile DOC (LDOC) and bacterial growth efficiency (BGE) after 48 hours of incubation for TREA and CONT. * CO_2 measurements in a mix of the three replicates. *Significant difference (T test, $p < 0.05$).

	BP ($\mu g C L^{-1} h^{-1}$)	BR ($\mu g C L^{-1} h^{-1}$)	LDOC (%)	BGE (%)
CONT	3.12 (0.75)	2.09 #	8.51* (0.59)	28.71 (4.98)
TREA	2.53 (0.30)	2.71 #	10.71* (0.26)	20.25 (1.94)

Amado et al. (2006) found significant differences in BP between irradiated samples and controls only in one (drawdown) of four tested cases (drawdown, low-water, filling-up and high-water) in Lake Batata (Pará State, Brazil), with higher BP in the irradiated sample. In the same work, BR was significantly different only during the filling-up phase, being higher in the irradiated sample (AMADO et al., 2006).

According to Anesio et al. (2005), BP on its own is not enough to evaluate the effect of photo-degraded DOC on microbial activity, and BGE should be considered instead because it expresses the relation between BP and BR. There was no significant difference between CONT and TREA BGEs but CONT showed higher values, as in other

similar works (AMADO et al., 2006; FARJALLA et al., 2001b; PULLIN et al., 2004). Obernosterer and Benner (2004) suggested that photo-degradation, besides acting on the larger molecules, can also remove a considerable part of labile DOC, which could result in a drop in bacterial activity in irradiated samples. Bertilsson et al. (1999) registered organic substrate loss after photo-degradation but did not find differences in BGE values. Anesio et al. (2005), however, found BGE values to be significantly higher for irradiated samples, and cited many processes which could have influenced, or even competed with photo-degradation, such as photo-production of inhibitory substances, like the formation of hydroxyl radicals (PULLIN et al., 2004), balance between formation and degradation of substrate and changes in the nutritional value of DOC. Considering that the photo-degradation and bacterial growth were performed separately, some of the effects that could prevent bacterial growth, such as the formation of radicals, can be discarded. However, the balance between formation and degradation of substrate and nutritional value of DOC certainly have to be considered.

Conclusion

Both steps of the experiment showed the importance of photo-degradation in the transformation of organic matter. After irradiation of the water samples, there was production of lower molecular weight and lower aromaticity compounds. The presence of more labile organic matter in irradiated samples was not reflected in a more efficient utilization of DOC by bacterioplankton, and showed no visible effects on its growth. Although the parameters used in this study were based on previous works, it is possible that specific characteristics of the environment require more adequate parameters so that the effects of radiation over DOC and its utilization by bacterioplankton can be assessed more accurately. Irradiation and incubation time are parameters that should be better adjusted.

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