Decomposition of dissolved organic matter released by an isolate of *Microcystis aeruginosa* and morphological profile of the associated bacterial community

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(With 2 figures)

Abstract

This study concerns the kinetics of bacterial degradation of two fractions (molecular mass) of dissolved organic matter (DOM) released by *Microcystis aeruginosa*. Barra Bonita Reservoir (SP, Brazil) conditions were simulated in the laboratory using the associated local bacterial community. The extent of degradation was quantified as the amount of organic carbon transferred from each DOM fraction (< 3 kDa and 3-30 kDa) to bacteria. The variation of bacteria morphotypes associated with the decomposition of each fraction was observed. To find the degradation rate constants (k_T), the time profiles of the total, dissolved and particulate organic carbon concentrations were fitted to a first-order kinetic model. These rate constants were higher for the 3-30 kDa fraction than for the lighter fraction. Only in the latter fraction the formation of refractory dissolved organic carbon (DOC_R) compounds could be detected and its rate of mass loss was low. The higher bacterial density was reached at 24 and 48 hours for small and higher fractions, respectively. In the first 48 hours of decomposition of both fractions, there was an early predominance of bacillus, succeeded by coccobacillus, vibrios and coccus, and from day 5 to 27, the bacterial density declined and there was greater evenness among the morphotypes. Both fractions of DOM were consumed rapidly, corroborating the hypothesis that DOM is readily available in the environment. This also suggests that the bacterial community in the inocula readily uses the labile part of the DOM, until this community is able to metabolise efficiently the remaining of DOM not degraded in the first moment. Given that *M. aeruginosa* blooms recur throughout the year in some eutrophic reservoirs, there is a constant supply of the same DOM which could maintain a consortium of bacterial morphotypes adapted to consuming this substrate.

Keywords: eutrophic reservoir, excreted DOM, fractions, bacterial degradation, morphotypes.

Decomposição da matéria orgânica dissolvida liberada por um isolado de *Microcystis aeruginosa* e padrão morfológico da comunidade bacteriana associada

Resumo

Este estudo trata da cinética de degradação bacteriana de diferentes frações de matéria orgânica dissolvida liberada por *Microcystis aeruginosa.* As condições do reservatório de Barra Bonita (SP, Brasil) foram simuladas em laboratório empregando a comunidade bacteriana local associada. A dinâmica da degradação foi quantificada pela quantidade de carbono orgânico transferido de cada fração de MOD (< 3 kDa e 3-30 kDa) às bactérias. A variação de morfotipos bacterianos associados à decomposição de cada fração foi observada. Para encontrar as constantes de degradação (k_T), os perfis temporais das concentrações de carbono orgânico total, dissolvido e particulado foram ajustados a um modelo cinético de primeira ordem. Estas constantes de velocidade foram superiores para a fração 3-30 kDa do que para a fração de menor massa molecular. Apenas na segunda fração pôde ser detectada a formação de compostos de carbono orgânico dissolvido refratário (COD_R) e sua taxa de perda de massa foi baixa. A maior densidade bacteriana foi alcançada em 24 e 48 horas para as frações de alta e baixa massa, respectivamente. Nas primeiras 48 horas de decomposição de ambas as frações, houve um predomínio de bacilos, seguido por cocobacilos, víbrios e cocos, e, do dia 5 ao 27, a densidade bacteriana diminuiu e houve uma uniformidade entre os morfotipos. Ambas as frações foram consumidas rapidamente, confirmando a hipótese de a MOD estar prontamente disponível no ambiente. Isto sugere ainda que a comunidade bacteriana do inóculo utiliza a parte lábil da MOD, até que esta se torne capaz de metabolizar o restante da MOD não degradada no primeiro momento. Já que *blooms* de *M. aeruginosa* se repetem ao longo do ano em alguns reservatórios eutróficos, existe um constante suprimento da mesma MOD que pode manter um consórcio de bactérias adaptadas ao consumo deste substrato.

Palavras-chave: reservatório eutrófico, MOD excretada, frações, degradação bacteriana, morfotipos.

1. Introduction

Microcystis aeruginosa Kützing (Cyanobacteria) is one of the most frequently cited cyanobacteria in current scientific publications, owing to the problems caused by its frequent blooms and its toxins. This species produces a huge biomass in a diverse range of reservoirs in both tropical and temperate areas (Ha et al., 1999; Matsumura-Tundisi and Tundisi, 2005), and often forms enormous blooms throughout the year. These great quantities of dissolved organic matter (DOM) must be released into the water actively, by excretion, or cell death (Aoki et al., 2008). Although a great volume of data is recorded on the physiology and ecology of this species, little has been published on what happens to the DOM following its release by *M. aeruginosa*.

DOM can affect the physical and chemical properties of water appreciably and influences profoundly the structure of the bacterial community. The DOM excreted by phytoplankton is one of the greatest sources of carbon for heterotrophs in water bodies (Amon and Benner, 1994; Carlson et al., 1994; Lampert and Sommer, 1997; Wetzel, 2001). Furthermore, this DOM plays a vital role in the recycling of organic carbon and nutrients (Azam et al., 1983; Azam and Cho, 1987; Ducklow and Carlson, 1992). By the "microbial loop" (Azam et al., 1983), this DOM acts as an important source of organic carbon at higher levels in the food web. There are reports suggesting that between 40% and 60% of primary production by phytoplankton is recycled by the bacterial plankton, mainly via DOM (Fuhrman and Azam, 1982; Scavia et al., 1986).

Most genera of cyanobacteria, including *Microcystis*, are found together with eubacteria, which associate with both the cells and the DOM released by them (Worm et al., 2001; Eiler and Bertilsson, 2004; Kapustina, 2006). In the DOM released by *M. aeruginosa* there are molecules of high molecular mass, such as polysaccharide chains, exceeding 30 kDa, down to molecules of less than 3 kDa (Bittar, 2005; Huang et al., 2007). Owing to this heterogeneity in the composition of the DOM, its decomposition requires the combined action of diverse groups of bacteria and involves a mixture of strains with a great variety of metabolism. In the literature, some authors have related different cell morphotypes to specific physiological activities, such as the phosphatase activity (Nedoma and Vrba, 2006) or uptake of certain (radiolabelled) substrates (Cottrell and Kirchman, 2004). Therefore, the heterogeneity of DOM released by active excretion or cell death of *M. aeruginosa* (Bittar, 2010) could promote the action of a bacterial consortium with a great variety. In fact, Racy (2004) found a pattern of morphotypes in bacterial consortium associated to blooms of *M. aeruginosa* in a reservoir.

The continuous supply of the same heterogeneous substrate by constant blooms of cyanobacteria in eutrophic or hypereutrophic lentic environments should maintain unchanging this bacterial consortium throughout the year (Eiler and Bertilsson, 2004; Bittar, 2010). If this is true, in laboratorial assays, inocula from this specialised bacterial consortium could repeat the same morphotype diversity found in this kind of environment that would readily use any fraction of DOM released by *M. aeruginosa*. To test this hypothesis, the aims of this study were: 1) evaluate the kinetics of carbon consumption during the decomposition of major fractions of DOM released actively or by death of senescent cells of *M. aeruginosa*; 2) compare the morphometric composition of bacterial consortium in the decomposition of different fractions of DOM.

2. Material and Methods

2.1. Study site

Barra Bonita reservoir has a surface area of 310 km^2 and total volume of 3.2 km^3 and is situated in São Paulo state, Brazil (22° 29' S and 48° 34' W). It is shallow (average depth 10.2 m), hypereutrophic and polymictic. It is formed by the confluence of the Piracicaba and Tietê Rivers, both of which are hypereutrophic and polluted by agricultural and industrial waste (Tundisi et al., 1998). The reservoir lies in a transition zone between tropical and subtropical climatic regions, characterised by dry winters and rainy summers (Calijuri and Tundisi, 1990).

2.2. Cyanobacteria and culture conditions

A strain of *Microcystis aeruginosa* Kützing was isolated from the Barra Bonita reservoir and is currently maintained as an axenic culture (BB05) in the algal culture collection at the Botany Department of the Federal University of São Carlos (SP, Brazil) (World Data Center for Microorganisms 835). Axenic cultures were grown in 4.0L Erlenmeyer flasks, with 2.0 L of ASM-1 culture medium (Gorham et al., 1964) at pH 7.0. Culture flasks received 100-120 $\mu mol.m^{-2}.s^{-1}$ irradiance (Quantameter QSL-100, Biospherical Instruments) during the light period of a 12:12 hours photoperiod, were shaken twice a day and kept at 23 ± 1 °C. The culture was periodically checked for bacterial contamination by transferring aliquots to agar plates containing ASM-1 medium supplemented with 150 $mg.L^{-1}$ each of peptone, glucose and yeast extract.

2.3. Isolation of DOM

DOM was obtained from *M. aeruginosa* in the senescent phase (around 53 days) and included organic matter both excreted by living cells and released by dead cells. The strategy of harvesting DOM from the *M. aeruginosa* culture in senescence was chosen because in this phase a proportion of the cells die and disintegrate, releasing a large quantity of organic matter into the water. At the same time, those cells that remain alive but have not gone into cytokinesis also increase their rate of release of DOM by excretion (Bittar et al., 2005). Cells were separated from culture media aseptically by tangential filtration through hollow-fiber cartridges (Xampler™, G Technology Corp./GE) with pores of 0.65 µm (UFP-6-E-4A,A). The media without cells but with DOM was subjected to tangential ultrafiltration aseptically through hollow-fiber cartridges of 3 kDa (UFP-3-E-4A) to isolate the fraction of DOM

with molecular mass below 3 kDa and through 30 kDa (UFP-30-E-4A) cartridge to obtain fraction of molecular mass of 3 to 30 kDa). After this procedure, autoclaved ultrapure water was added to each of the fractions, to reach the original concentration of the fraction in the DOM released by the alga, and the fraction was finally filtered through polycarbonate membranes with pores of 0.22 µm (Isopore™) to eliminate potential contaminants.

The most homogenous fraction of molecules larger than 30 kDa, the majority composed of extracellular polysaccharides, was excluded from this study, since its degradation has already been investigated and proved to favour the setting up of bacterial consortium and the occurrence of succession phenomena, both in morphotypes and species, at least in the case of DOM released by other microalgae (Freire-Nordi and Vieira, 1996; Giroldo et al., 2007) and cyanobacteria (Colombo et al., 2004; Bagatini, 2008).

2.4. Bacterial inocula

A representative sample of the water column was collected at 0, 1, 5, 10 and 18 m from the surface, at a point in the reservoir 3 km upstream from the dam (22° 32' 34.5" S and 48° 29' 26" W), in June (winter) 2008. The sample was filtered through previously calcined (8 hours at 450 °C) GF/C glass-fibre filters (Whatman, 1.2 µm pores), which removed only algae, bigger protozoa and large particulate fraction from the water. The water-storage and filtration equipment was autoclaved before use.

2.5. Consumption of DOM by bacterioplankton

Bacterial cultures were grown in 2 L glass flasks fitted with vent caps, containing 500 mL DOM fraction, 500 mL reservoir water filtered through 0.22 µm-pore membranes, 100 mL autoclaved ASM-1 medium to supply mineral nutrients and 50 mL bacterial inocula. Two replicate cultures were set up for each DOM fraction (Mw < 3 kDa and 3-30 kDa) and two control cultures, with DOM omitted. All 6 flasks were shaken twice a day and incubated in the dark at 23 ± 1 °C, the average temperature of the water in the reservoir during the dry season (winter) in 2008. Aliquots of 500 mL were taken at 0, 0.5, 1, 2, 5, 8, 12, 15, 19, 23 and 27 days of incubation. These samples were fixed with 4% formaldehyde, stained with DAPI (4',6-diamidine-2-phenylindole, Sigma; final concentration 1 pg.mL^{-1}) and filtered through Isopore™ polycarbonate membranes with 0.22 µm pores, prestained with Sudan Black (Porter and Feig, 1980). Bacterial growth was monitored by counting cells with an epifluorescence microscope (Zeiss Axioplan 2, Jena, Germany) (Racy, 2004).

2.6. Morphological characterisation of the bacterial community

The morphology of the bacteria was observed by epifluorescence microscopy in the slides mounted for cell counts, in images captured by a Zeiss AxioCam HRc camera and processed with the image analysis system Axiovision 4.5 (Zeiss). The morphotypes were classified by the length:width ratio of the cells, as described by Racy (2004).

2.7. Kinetic model of DOM consumption

The results for the consumption of organic carbon were fitted to a first-order model (Equation 1) (Peret and Bianchini Jr., 2004). It was assumed that the mineralisation of DOM proceeded by three competing processes. The first was the direct mineralisation of labile species (IN_1; Equation 2). The second pathway involved the formation of refractory organic carbon (DOC_R; Equation 3) and their subsequent mineralisation (IN_2; Equation 4). The third route (IN_3) consisted of assimilation of organic carbon to form bacteria (Equation 5) and participation of these organisms in the mineralisation of carbon (POC; Equation 6). The time curves of DOC, DOC_R and POC (particulate organic carbon) were used to determine the parameters of the model. This curve-fitting was carried out by nonlinear regression, employing the iterative Levenberg-Marquardt algorithm (Press et al., 1993). All data used to fit the model were corrected by subtracting the values of DOC measured in the controls.

The concentrations of organic carbon (total, dissolved and particulate) were measured with a TOC-V_{CPH} carbon analyser (Shimadzu). POC was estimated as the difference between the total organic carbon and DOC. POC (particle size > 0.22 µm) was assumed to be carbon assimilated by bacteria from the DOC in the DOM fractions released by *M. aeruginosa*.

$$\frac{d[DOC]}{dt} = -k_T[DOC] \quad (1)$$

$$\frac{IN_1}{dt} = \frac{k_1}{k_T} \times k_T\left[DOC\right] \quad (2)$$

$$\frac{d[DOC_R]}{dt} = \left(\frac{k_2}{k_T} \times k_T[DOC]\right) - k_3[DOC_R] \quad (3)$$

$$\frac{IN_2}{dt} = k_3[DOC_R] \quad (4)$$

$$\frac{d[POC]}{dt} = \frac{k_3}{k_T} \times k_T[DOC] - k_5[POC] \quad (5)$$

$$\frac{IN_3}{dt} = k_5[POC] \quad (6)$$

where: k_T = global rate constant for mass loss of DOC; DOC = dissolved organic carbon in the DOM produced by *M. aeruginosa*; DOC_R = refractory organic carbon formed from DOC; POC = bacterial particulate organic carbon; k_1 = DOC mineralisation rate constant; k_2 = DOC_R formation rate constant; k_3 = DOC_R mineralisation rate constant;

k_4 = POC formation rate constant; k_5 = POC mineralisation rate constant.

3. Results

3.1. Consumption of DOM by the bacterioplankton

The model parameters obtained from the experimental results are listed in Table 1. After 27 days of incubation, bacterial degradation of the DOM released by *M. aeruginosa* was faster in the 3-30 kDa (k_T = 0.846 d^{-1} and $t_{½}$ = 19.6 hours) than in the DOM < 3 kDa fraction (k_T = 0.736 d^{-1} and $t_{½}$ = 22.6 hours). Only 44% of the DOC in the small-molecule fraction, and 85% of that in the 3-30 kDa DOM, was consumed rapidly (in the first 48 hours), as shown in Table 1. On the other hand, 22% of the small-fraction DOC became refractory (DOC_R), with a much slower degradation rate, k_3 = 0.00804 d^{-1} ($t_{½}$ = 86 d), while no DOC_R was detected in the 3-30 kDa fraction.

In cultures growing on the < 3 kDa DOM, 34% of the DOC was incorporated into the bacteria (particulate matter), while only 14% was incorporated from the larger molecules in the 3-30 kDa fraction. In its turn, the POC generated from the small-molecule fraction of DOM was mineralized at a faster rate ($t_{½}$ = 2.2 d) than POC generated from the 3-30 kDa fraction ($t_{½}$ = 77 d).

3.2. Characterisation of the bacterial community

The bacteria grew rapidly for the first 48 hours, with very similar profiles, on both sources of organic carbon. In cultures supplied with the DOM < 3 kDa fraction, a peak bacterial density of 32.81×10^6 cells.mL^{-1} was reached in 24 hours, while in cultures supplied with 3-30 kDa DOM, the peak density of 32.44×10^6 cells.mL^{-1} occurred after 48 hours (Figures 1 and 2). Between day 5 and day 27 (end of the incubation), the average densities were 7.31×10^6 cells.mL^{-1} for DOM < 3 kDa and 6.73×10^6 cells.mL^{-1} for 3-30 kDa, with little variation over this period (5.87-9.17×10^6 and 6.01-8.14×10^6 cells.mL^{-1}, respectively) (Figures 1 and 2).

Regarding the density of each bacterial morphotype in the first 48 hours, the growth of bacillus predominated in both cultures, representing 60.7% of all cells for DOM < 3 kDa and 56.3% for DOM between 3 and 30 kDa, on average, followed by coccobacillus (24.6% and 23.4%), vibrios (9.5% and 15.3%) and coccus (4.3% and 4.1%). Spirillum and filaments were present at low densities in both cultures at all times. The microscope images indicated that the proportions of the morphotypes were uniform throughout the period from day 5 to 27 in both cultures.

Table 1. Experimental parameters of kinetic model of the degradation of two fractions of DOM produced by *M. aeruginosa*, with molecular masses < 3 kDa and 3 to 30 kDa, where DOC_R = refractory, DOC_L = labile, POC = particulate, k_T = global rate constant for mass loss of DOC, k_3 = DOC_R mineralisation rate constant, k_5 = POC mineralisation rate constant, R^2 = coefficient of determination and $t_{½}$ = half-life corresponding to each rate constant.

DOM Fraction	DOC (mg.L^{-1})	k_T (d^{-1})	$t_{½}$ (h)	DOC_R (%)	k_3 (d^{-1})	$t_{½}$ (d)	R^2	POC (%)	k_5 (d^{-1})	$t_{½}$ (d)	R^2	DOC_L (%)
< 3 kDa	11.714	0.736	22.6	22.5	0.00804	86	0.98	33.8	0.30918	2.2	0.77	44
3 to 30 kDa	3.059	0.846	19.6	-	-	-	0.92	14.0	0.009	77	0.25	85

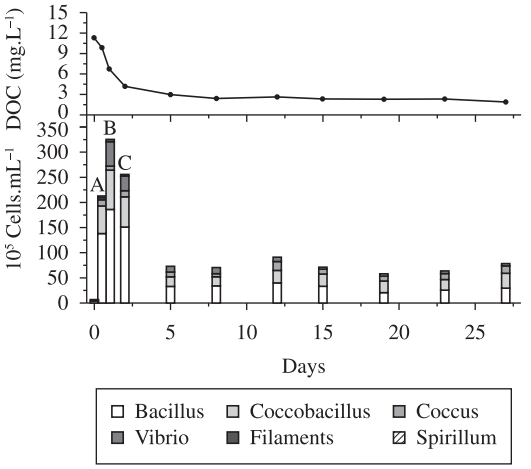

Figure 1. (Top) Bacterial degradation of DOC available in fraction of DOM smaller than 3 kDa and (bottom) bacterial density and distribution into morphotypes, in 27-day cultures.

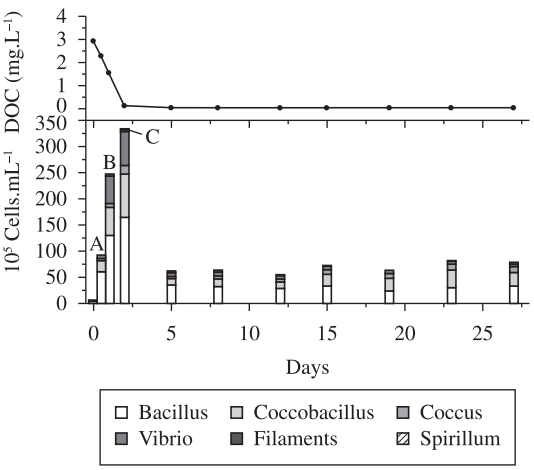

Figure 2. (Top) Bacterial degradation of DOC available in fraction of DOM between 3 and 30 kDa and (bottom) bacterial density and distribution into morphotypes, in 27-day cultures.

4. Discussion

Using cells in the senescent growth phase reproduces as closely as possible what happens in natural blooms of *M. aeruginosa* exposed to intense light on the surface of reservoirs (Bittar et al., 2005).

4.1. Kinetics of the degradation of DOM by the bacterial community

The high coefficients of determination (Table 1) obtained for the fitting of the model parameter to the data show that the proposed model described well the kinetics of DOC consumption.

In the cultures grown on the small-molecule fraction of DOM, two phases of degradation were observed: in the first (fast) phase, the rate constant was 91 times greater than in the second (slow) phase. A similar two-phase pattern was noted in the degradation of polysaccharides excreted by *Staurastrum iversenii* var. *americanum* (Pacobahyba, 2002), and for carbohydrates excreted by diatoms, Ogura and Gotoh (1974). Similarly, Cunha-Santino et al. (2008) found kinetic rate constants (k_D) of the same order of magnitude as the values of k_T obtained here, in experiments to measure the consumption of oxygen in the mineralisation of carbon photosynthesised by phytoplankton species from the same reservoir, including *M. aeruginosa* ($k_D = 0.209$). DOC consumption tended to have a rapid decay in the beginning of the process, in which the first fraction (labile) decayed in a few hours, followed by a period tending to stabilisation of consumption. The decay was less intense after first phase probably due to i) a remaining refractory fraction derived from the cellular excretion that gives to the DOM more resistance to bacterial attacks and ii) the utilisation of the nutrients in the first stage, limiting the growth of microorganisms and turning the decomposition a slow process (Cunha-Santino et al., 2008).

In the cultures provided with 3-30 kDa DOM as sole carbon source, just one (rapid) phase was observed in the decomposition of DOC. One possible explanation for the absence of a slow phase in these cultures could be the low initial carbon content. It might also reflect the wide scatter in the data on consumption of DOC by the bacterial community.

In sum, the higher molecular weight DOM fraction was more readily decomposed by the bacteria than the low molecular weight fraction. Nevertheless, a good percentage (22.5%) of the initial DOC in the low molecular weight fraction was, or became, refractory DOC (DOC_R) in the bacterial culture. In fact, biodegradation by bacteria, as well happens with fotooxidation, can produces new products, among them some refractory to bacterial attack (Bittar, 2010). The author found that 16% of the total excreted MOD mainly aliphatic compounds were refractory.

No DOC_R was detected in the cultures grown on 3-30 kDa DOM. This might be due to competition between mineralisation (conversion of organic forms of carbon to inorganic) and the formation of humic substances. In this case, the reactions involved in humification, and thus in the conversion of compounds into more refractory forms, would have been favoured by bacterial consumption of the low molecular weight DOM. On the other hand, it may be supposed that a higher proportion of the 3-30 kDa molecules of DOM are available for bacterial assimilation and catabolism, as their size confers on them a larger reactive surface (Amon and Benner, 1996).

By comparing the present results for the decomposition of DOM molecular weight fractions up to 30 kDa with those of Cunha-Santino et al. (2008), who recorded the kinetics of oxygen consumption in the degradation of total organic matter excreted by *M. aeruginosa* ($t_{½}$ = 3d), it can be deduced that the lower molecular weight fractions are degraded three times more intensely than the total organic matter present in an algal bloom. This implies that a large part of the organic matter generated by *M. aeruginosa* during a bloom decomposes with a half-life of one day. In other words, much of the organic carbon released into a reservoir as a result of such a bloom is soon mineralised or incorporated into a bacterial biomass that is readily consumable by organisms at higher trophic levels (e.g. heterotrophic nanoflagellates and zooplankton) and thence made available throughout the microbial food web (Azam et al., 1983).

4.2. Characteristics of the bacterial community

The results of this study confirmed the expectation that bacterial densities would peak early in the experimental incubation, since the bacterial consortium specialised in DOM released by *M. aeruginosa* was ready, in the inocula, to degrade both the fractions of the DOM. Growth curves showed maxima at 24 hours in the cultures provided with low-molecular-weight DOM fraction and at 48 hours in those with the 3-30 kDa fraction. A similar response from the bacterial community was observed by Chróst et al. (1989) in experiments on samples of organic matter collected from a temperate eutrophic lake.

Owing to the difference in the molecular mass and consequently in the dynamics of bacterial degradation of the two fractions of DOM (Amon and Benner, 1996), the corresponding bacterial cultures showed clear differences in their growth profiles during the early rapid phase of decomposition. The slightly delayed bacterial growth seen in the cultures with the 3-30 kDa DOM fraction had already been observed by Pernthaler et al. (1998) in experiments with various types of carbon compound as sole source for bacterial growth. Despite this brief delay in the degradation of the 3-30 kDa DOM, both molecular mass fractions were consumed rapidly, corroborating the hypothesis that DOM is readily available in aquatic ecosystems (Amon and Benner, 1994).

Following the (identical) peaks in bacterial density, the growth curves in the two cultures were similar, as were the degradation rates and half-lives of the two fractions of DOM; in both cultures, the cell density stabilized from day 5. The morphotype profile of the bacterial community growing on the DOM from *M. aeruginosa* remained homogeneous during the degradation of the DOM and similar in the two cultures. Bacillus predominated throughout, followed by coccobacillus, vibrios and coccus, irrespective of the

DOM fraction. Even the percentages of the morphotypes varied little among samples taken at various times, in both cultures, and it is noteworthy that, in the slow phase of degradation of the small-molecule fraction, during which DOC_R is mineralised, the morphotype profile remained similar to that of the culture with 3-30 kDa DOM. This suggests that the microbial community present in the inocula readily uses the labile part of two factions of the DOM, which involves both compounds of low and high molecular mass, until this community be able to metabolise efficiently the remaining of DOM not degraded in the first moment (Docherty et al., 2006).

Various authors have related distinct cell morphotypes to specific physiological activities (Cottrell and Kirchman, 2004; Nedoma and Vrba, 2006), thus, distinct bacterial morphotypes could exhibit particular physiological capacities that enable them to specialise in the degradation of specific organic compounds. In fact, the only notable difference in the pattern of morphotypes of the two cultures was in the total numbers of bacteria in the first few days. These results are very similar to that obtained by Racy (2004) who observed the predominance of coccobacillus, then coccus and bacillus among bacteria in samples of a bloom of *M. aeruginosa* collected in the same reservoir. Thus, as the pattern of morphotypes presents in the inocula is repeated throughout the period of DOM degradation, a stable physiological adaptation of bacterial community towards the consumption of labile and refractory compounds present in DOM released by *M. aeruginosa* is revealed, since there are only slight fluctuations in the proportions of each morphotypes class along the process.

Once *M. aeruginosa* blooms recur throughout the year in some eutrophic reservoirs, there is a concomitantly high and constant supply of DOM as a substrate which could supporting and maintaining a consortium of bacterial morphotypes adapted to consuming this abundant substrate at a constant rate throughout the year (Docherty et al., 2006) which explain the ready use of any fraction of DOM released by *M. aeruginosa.* However, it should take into account that the apparent constancy of the morphotypes diversity found in both fractions, with predominance of bacillus, cannot be more than that, just an apparent characteristic, since the morphotypes found in both fractions almost completely cover the full spectrum of bacterial forms commonly found in aquatic environments (Racy, 2004).

Nevertheless, in environments where substrates are highly variable not only by bacterial degradation but also by competition degradation / photooxidation (Bittar, 2010), its extremely complex correlate microbial morphotypes with degradable substrates, justify this kind of work, as well as requiring additional studies of the bacterial diversity and biodegradability of DOM fractions.

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