Photochemical Effects on Bacterial Degradation of Dissolved Organic Matter in Lake Water

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ABSTRACT

Numerous studies published in recent years emphasize the role of solar radiation in degradation of dissolved organic matter (DOM) in lake and marine waters. The photochemical degradation may act in concert with the activity of heterotrophic microorganisms, transforming recalcitrant DOM into labile organic intermediates that are readily utilized by bacteria. We present results illustrating that the organically bound carbon, phosphorus, and nitrogen may also become increasingly available to bacteria upon exposure to UV radiation. In addition, we summarize recent evidence for an opposite effect of photochemical reactions on freshly produced DOM components, turning them into more recalcitrant forms. In a survey of a large number of lakes, we found both positive and negative effects of photochemical alteration of DOM on bacterial growth potential. The effect on bacterial growth was predominantly positive in oligotrophic softwater lakes, but negative in alkaline lakes with a large indigenous production of DOM, as indicated by high concentrations of algal chlorophyll *a*.

Introduction

Dissolved organic matter (DOM) constitutes a dominant store of energy and several elements in both marine and freshwater environments. In lakes, it mainly consists of dissolved detrital matter from the terrestrial environment, largely of vascular plant origin. Most of the organic matter is recalcitrant, with only a small fraction being readily available for utilization by heterotrophic microorganisms. In a review of published data on the bacterial availability of DOM in lakes, Søndergaard and Middelboe [20] found that on average 14% of the dissolved organic carbon (DOC) was labile towards bacterial degradation. Hence, a substantial amount of potentially available energy is bound in dissolved detritus, but apparently escapes utilization by heterotrophic microbes, and therefore does not contribute to the metabolism of aquatic food webs. Several factors may regulate the availability of DOM, including availability to inorganic nutrients [17, 23, 28] and molecular weight of the DOM [1, 25]. An additional factor that has received considerable recent interest is photochemical transformations of recalcitrant DOM into labile compounds that are utilized by bacteria. In this paper we show that such transformations involve not only the organically bound carbon (DOC), but also affect dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP). In addition, we will demonstrate that photochemical alterations of DOM may also act in the opposite

Microbial Biosystems: New Frontiers
Proceedings of the 8th International Symposium on Microbial Ecology
Bell CR, Brylinsky M, Johnson-Green P (eds)
Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

direction, making some DOM moieties increasingly unavailable to bacterial degradation. Finally, we show that the net outcome of competing stimulatory and inhibitory effects on the bacterial availability of DOM varies among lakes, depending on their water chemistry and on the origin of the DOM.

Photochemical Enhancement of Bacterial Degradability of DOM

DOM, particularly terrestrially derived humic compounds, efficiently absorbs UV radiation. This results in photochemical reactions, such as the cleavage of macromolecules into smaller units [16]. Strome and Miller [22] first suggested that such photochemical reactions increase the ability of bacteria to utilize the DOM. Accordingly, Geller [8] found that certain bacterial strains grew better on isolated lake water DOM after it had been exposed to radiation. Interestingly, this effect occurred upon exposure of the DOM to low levels of visible light in the absence of UV, suggesting that photochemically stimulated degradation of DOM is not limited to high radiation levels in the very surface layer of lakes. Following these early findings, there have been several reports, most of which are reviewed by Moran and Zepp [16]. A variety of monomeric organic molecules, that are photochemically produced from DOM and are highly labile towards bacterial degradation, have been identified. These substrates include carbonyl compounds, such as carboxylic acids, which at times may make up a significant fraction of the carbon demand of heterotrophic bacterioplankton [2].

In addition to the photochemical transformation of recalcitrant DOM into labile forms, photochemical reactions may also enhance the bacterial availability of DON and DOP. Bushaw et al. [3] reported photochemical production of ammonium from both isolated humic fractions and whole water DOM from several freshwater sites. Thus, the photochemical ammonification enhanced the ability of bacteria to utilize the nitrogen by transforming recalcitrant DON into highly assimilable ammonium. Similar mechanisms may occur with respect to organically bound phosphorus. Phosphate binds strongly to humic substances, particularly in the presence of high concentrations of ferric iron [5, 6, 10]. In this way microbial access to phosphorus is restricted [21]. The cleavage of the phosphate-iron-DOM complex is catalyzed by solar radiation through the photochemical reduction of the iron to its ferrous form, which then facilitates the microbial utilization of the phosphorus [4, 7].

With this background, we hypothesized that the bacterial utilization of terrestrially derived, humic DOM is facilitated in terms of organically bound carbon, as well as nitrogen and phosphorus. We sampled the interstitial water of a *Sphagnum* bog in southern Sweden at a depth of ca 1 m and brought the sample back to the laboratory protected from exposure to light. Subsequently, we filtered the water through a 0.2 μm filter, and concentrated the DOM with nominal molecular weight >1 kD by tangential flow filtration, using a Millipore Prep-Scale TFF 6 ft^2 tangential flow filtration cartridge. After concentration of the >1 kD fraction of the bog water by 10x, while keeping the volume of retentate constant, we continuously washed it with ten volumes of Milli-Q water, followed by ten volumes of 0.25 M KCl, and finally by five volumes of Milli-Q water. This rinsing scheme theoretically diluted the <1 kD compounds, including inorganic nutrients, by more than three orders of magnitude. KCl was added to remove ammonium adsorbed to the high molecular weight DOM by ion exchange, hence minimizing the inorganic nitrogen in the retentate.

An organic-free, artificial lake water medium, modified from Lehman [14] to exclude inorganic N and P, was supplied with DOM concentrate to a final concentration of 8.9 mg DOC l^{-1}. This solution was incubated in quartz tubes for 17 hr under an artificial radiation source with intensities of UV-B and UV-A similar to the UV radiation of natural sunlight at temperate latitudes. The radiation source is described in Tranvik and Kokalj [26]. Some of the quartz tubes were wrapped in aluminum foil to serve as dark controls.

After irradiation or dark pretreatment, the water was manipulated to achieve bacterial growth conditions where either DOC, nitrogen, or phosphorus would be the limiting nutrient. The appropriate combinations of glucose (10 mg C l^{-1}), nitrate (290 µg NO_3-N l^{-1}added as KNO_3), and phosphate (31 µg PO_4-P l^{-1}added as Na_2HPO_4) were added to produce C-, N-, or P-limiting conditions, while at the same time the only source of the limiting nutrient would be the added DOM concentrate. For each treatment, five irradiated and five dark control-replicates were inoculated with lake water bacteria [24], and bacterial growth was monitored by epifluorescence microscopy of DAPI-stained cells [18] over 6 days.

The bacteria in C-, N- and P-limiting media subjected to irradiation achieved 35-55% higher stationary phase abundance than the bacteria in water that was previously kept in the dark (Fig. 1). These results demonstrate that DOC, DON, and DOP may become increasingly bioavailable upon exposure to solar radiation. During irradiation, we found some production of ammonium, but not in amounts sufficient to explain more than 35% of the photochemical enhancement of bacterial growth (unpublished data). Hence, in addition to the photochemical ammonification previously described by Bushaw et al. [3], DON may become labile due to other transformations of DON. These transformations possibly include the cleavage of N-containing polymers into smaller, more accessible units, or the production of specific compounds, such as amino acids [11]. Likewise, the observed increase in bioavailability of DOP was not in complete accordance with previously described processes. The cleavage of phosphate-iron-DOM complexes is a reversible process [7], i. e. after exposure to radiation the free phosphate returns to the humic-bound state. In our experiment, the enhanced bacterial availability of phosphorus persisted long after the radiation. The bacteria growing in the dark in phosphorus-limited cultures were able to reach higher biomass on pre-exposed DOM, than on DOM that had been kept in the dark several days after the exposure of the DOM took place. Consequently, in addition to the reversible binding of phosphate to DOM, photochemical effects on DOM may include the transformation of DOP from recalcitrant forms into labile forms that are not rapidly returned to the recalcitrant state in the dark. Alternatively, the bacterial cells initially inoculated were able to assimilate a surplus of phosphorus that met the requirements of several divisions.

Photochemical Reduction of Bacterial Degradability of DOM

In addition to the transformation of DOM into labile compounds, photochemical reactions may have the opposite effect. It has been suggested that the ubiquity of recalcitrant humic substances in the oceans can be partly explained by a cross-linking of simple compounds, particularly lipids, which gradually transforms them into aromatic, heterogeneous structures resembling humic substances. The cross-linking is triggered by the presence of free radicals photochemically produced [9, 13]. Keil and Kirchman [12] showed that labile protein can

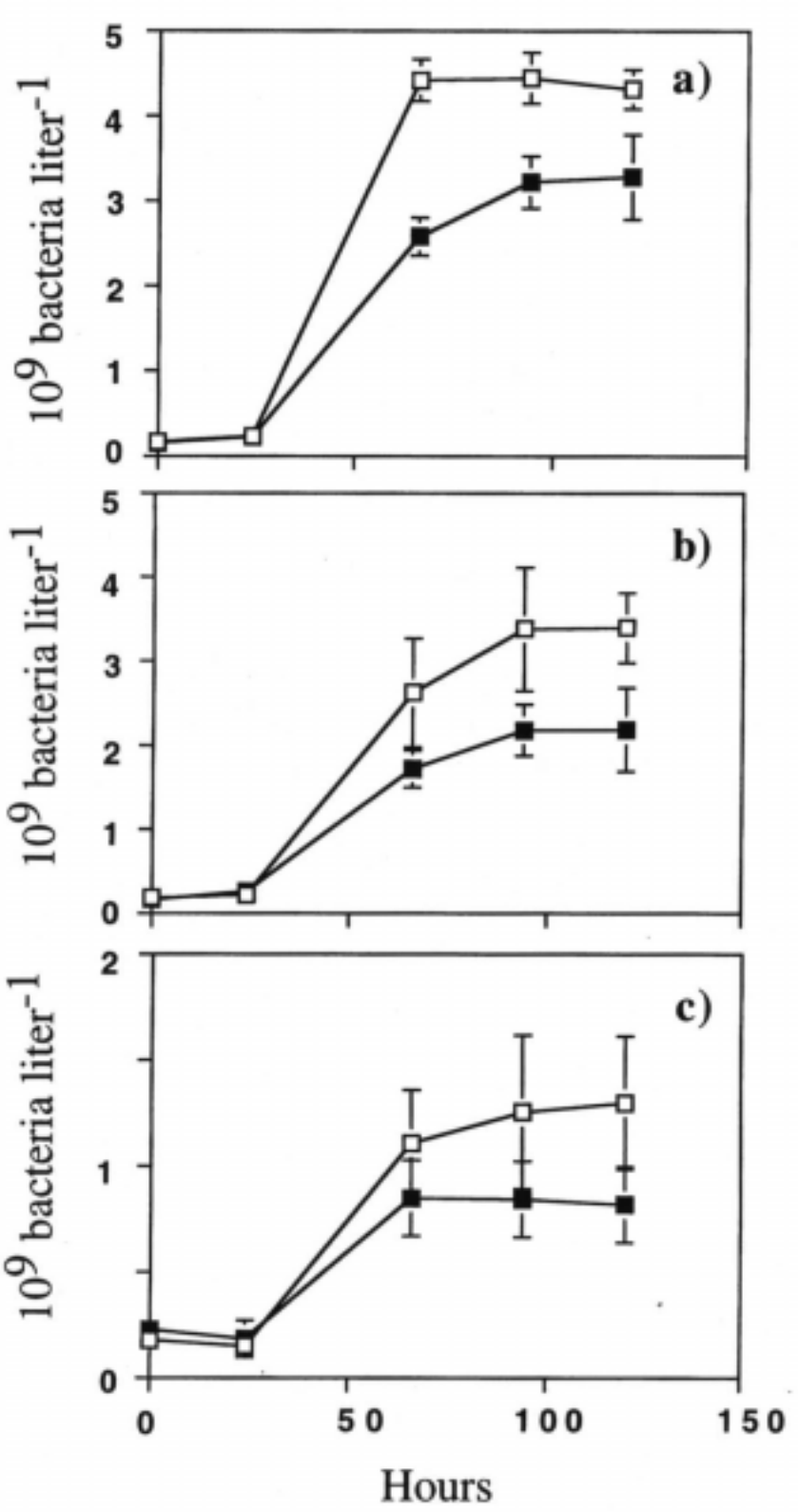

Fig. 1. Bacterial growth on the >1 kD fraction of DOM in artificial lake water with additions of DIN and DIP (a), glucose and DIP (b), or glucose and DIN (c), to achieve C-, N-, or P-limiting growth conditions, with DOM as the only source of the limiting nutrient. The DOM was pretreated under UV-A radiation (open symbols) or in the dark (closed symbols). Error bars are one standard deviation (n=5).

be transformed into more recalcitrant forms in sea water, and that both sunlight exposure and presence of seawater DOM promotes this "aging" process.

We tested whether a similar, UV-catalyzed aging process may occur in freshwaters, with aged bulk DOM promoting the diagenetic change of fresh DOM into recalcitrant forms [26]. We produced a ^{14}C-labeled algal extract and extracted from a lake the humic fraction of the DOM. The algal $DO^{14}C$ was incubated in quartz tubes with organism-free water with and without the presence of humic extract. Some tubes were subject to UV irradiation, and some were kept in the dark. After the abiotic exposure in the dark, or under UV radiation, a bacterial inoculum was added and we followed the microbial mineralization of the algal $DO^{14}C$ into $^{14}CO_2$. The combined presence of humic matter and exposure to UV radiation inhibited the subsequent bacterial mineralization of the algal DOC by 15-20%. Additional experiments demonstrated that the negative impact of humic matter was dependent on its direct presence in the solution during irradiation, and was not caused by altered radiation as a result of absorption by the humic compounds [26]. We suggested that UV radiation promoted the diagenetic change of fresh, algal DOM into recalcitrant DOM by promoting its reactions with humic compounds. Alternatively, the role of the humic matter was as a source of radicals that could react separately with the algal DOM. In any case, the results imply that recently produced labile DOM components may rapidly be incorporated into the recalcitrant, bulk pool of DOM. Consequently, in addition to the increased biodegradability of bulk DOM described above, UV radiation may also have an opposite effect, turning initially labile DOM into recalcitrant forms.

Lake Survey of Photochemical Effects on the Bacterial Utilization of DOM

Experiments assessing bacterial growth response to irradiation of DOM generally demonstrate a positive effect of radiation on the ability of bacteria to utilize the substrate [15, 19, 22]. Our experimental evidence for negative effects of radiation on the ability of bacteria to utilize DOM of algal origin, together with the fact that previous studies focused largely on waters rich in terrestrially derived organic matter, led us to hypothesize that the net outcome of photochemical transformations of DOM may be different for lakes dominated by algal DOM, as compared to lakes where terrestrial humic matter is the major component of DOM. We set up bacterial regrowth cultures from 32 different lakes, ranging from highly eutrophic lakes dominated by dense algal blooms, to oligotrophic lakes with very low densities of algae, but waters strongly stained with humic matter. From each lake, 0.2-µm-filtered water was kept either in the dark or irradiated with a UV-A source, as described above, for 12 hr. Thereafter, triplicate cultures were set up for each lake and radiation treatment (UV-A exposed, dark), which were amended with inorganic nutrients (1000 µg NO_3-N l^{-1}, 100 µg PO_4-P l^{-1}) and inoculated with bacteria by adding 0.7 µm filtered (Whatman GF/F) water (1% of total volume of culture). The bacterial yield was then compared for cultures based on water that had been kept in the dark or subject to UV-A irradiation.

Among the 32 lakes, the bacterial yield in irradiated water was from 45% less up to 139% higher than the yield in dark controls. In 14 of the lakes, the bacterial yield was reduced by the UV-A treatment. Hence, this survey of a wide range of lakes suggests that stimulated bacterial growth due to photochemical transformations of DOM is not a general phenomenon. A plausible hypothesis explaining this would be that, during irradiation,

various DOM components are made labile (e. g. transformed into carbonyl compounds; 16, 27], while simultaneous processes turn other DOM moieties into more recalcitrant forms (e. g. polymerization and condensation reactions [9, 12, 26]). The net outcome of these competing pathways depends on intrinsic (e.g., age, origin, composition) or extrinsic (e.g., inorganic water chemistry) properties of the DOM. We compared some basic water chemistry parameters for the lakes where the bacterial growth was either enhanced or reduced after UV-A treatment (Table 1). Conductivity, pH, and acid neutralizing capacity were different in the two categories of lakes, suggesting that in acidic lakes with low ionic strength the net effect would be an enhancement, while in alkaline lakes the result would tend to be negative. In addition, in lakes with high concentrations of chlorophyll *a*, suggesting that the DOM originates to a large extent from phytoplankton, the photochemical reactions tended to act negatively on bacterial substrate utilization. With the exception of one lake with a extremely high chlorophyll *a* concentration (504 µg/liter), the concentration of chlorophyll *a* in lakes where radiation caused enhanced bacterial growth never exceeded 10 µg l^{-1}. On the other hand, average water color (i. e. humic content) was higher (although not significantly) in lakes where bacterial growth was enhanced by radiation).

Table 1. Water chemistry of lakes where UV-A irradiation of DOC resulted in either a reduction or an enhancement of subsequent bacterial growth (average ± standard deviation). Probability (p) of no difference between lakes where the bacterial growth was enhanced vs. reduced was assessed by Mann-Whitney U-test. Average and standard deviation of chl *a* concentration in lakes with enhancement excluding one extreme lake (504 µg chl *a* l^{-1}) in parentheses (see text for details).

	Photochemical effect on bacterial growth		
	Reduction (14 lakes)	Enhancement (18 lakes)	p
Acid neutralizing capacity (mequiv.l^{-1})	0.78 ± 0.52	0.24 ± 0.41	0.001
PH	8.42 ± 0.75	6.69 ± 1.22	0.000
Conductivity (µS cm^{-1})	165 ± 97	88 ± 82	0.017
Water color (absorbance at 430 nm cm^{-1})	0.018 ± 0.016	0.029 ± 0.025	0.400
Chlorophyll *a* (µg l^{-1})	51.1 ± 64	30.5 ± 118 (2.7 ± 2.5)	0.001

Conclusions

In conclusion, a picture of a complex, important role of photochemistry in the biogeochemistry of DOM is emerging. Our results illustrate several important details of this picture, including the ”competing” positive and negative effects of radiation on bacterial growth. Other nutrients, not only organic carbon, are affected by the photochemical reactions. In addition to the example given here of photochemically facilitated utilization of organic nitrogen and phosphorus, radiation may also play important roles in the cycling of trace elements. The results cited and reported here are based mostly on experimental systems where complexity is reduced and processes such as mixing are largely neglected. Future challenges include the assessment of the importance of DOM photochemistry in nature in terms of microbial ecophysiology as well as ecosystem level effects.

Acknowledgments

This work was supported by grants from the Swedish Natural Science Research Council.

References

1. Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr 41:41-51
2. Bertilsson S, Tranvik LJ (In press) Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. Limnol Oceanogr
3. Bushaw KL, Zepp RG, Tarr MA, Schulz-Jander D, Bourbonniere RA, Hodson RE, Miller WL, Bronk DA, Moran MA (1996) Photochemical release of biologically available nitrogen from dissolved organic matter. Nature 381:404-407
4. Cotner JB, Heath RT (1990) Iron redox effects on photosensitive phosphorus release from dissolved humic materials. Limnol Oceanogr 35:1175-1181
5. De Haan H, Jones RI, Salonen K (1990) Abiotic transformations of iron and phosphate in humic water revealed by double isotope labeling and gel filtration. Limnol Oceanogr 35:491-497
6. Francko DA, Heath RT (1979) Functionally distinct classes of complex phosphorus in lake water. Limnol Oceanogr 24:463-473
7. Francko DA, Heath RT (1982) UV-sensitive complex phosphorus: Association with dissolved humic material and iron in a bog lake. Limnol Oceanogr 27:564-569
8. Geller A (1986) Comparison of mechanisms enhancing biodegradability of refractory lake water constituents. Limnol Oceanogr 31:755-764
9. Harvey GR, Boran DA, Chesal LA, Tokar JM (1983) The structure of marine fulvic and humic acids. Mar Chem 12:119-132
10. Jones RI, Salonen K, De Haan H (1988) Phosphorus transformations in the epilimnion of humic lakes: abiotic interactions between dissolved humic materials and phosphate. Freswater Biol 19:357-369
11. Jørgensen NOG, Tranvik L, Edling H, Granéli W, Lindell, M (1998) Effects of sunlight on occurrence and bacterial turnover of specific carbon and nitrogen compounds in lake water. FEMS Microbiol Ecol 25:217-227
12. Keil RG, Kirchman DL (1994) Abiotic transformation of labile protein to refractory protein in sea water. Mar Chem 45:187-196

13. Kieber RJ, Hydro LH, Seaton PJ (1997) Photooxidation of tricglycerids and fatty acids in seawater: Implication toward the formation of marine humic substances. Limnol Oceanogr 42:1454-1462
14. Lehman JT (1980) Release and cycling of nutrients between planktonic algae and herbivores. Limnol Oceanogr 25:620-632
15. Lindell, MJ, Granéli, W, Tranvik, LJ (1995) Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. Limnol Oceanogr 40:195-199
16. Moran MA, Zepp RG (1997) Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol Oceanogr 42:1307-1316
17. Morris DP, Lewis WM (1992) Nutrient limitation of bacterioplankton growth in lake Dillon, Colorado. Limnol Oceanogr 37:1179-1192
18. Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:843-948
19. Reitner B, Herndl GJ, Herzig A (1997) Role of ultraviolet-B radiation on photochemical and microbial oxygen consumption in a humic-rich shallow lake. Limnol Oceanogr 42:950-960
20. Søndergaard M, Middelboe M (1995) A cross-system analysis of labile dissolved organic carbon. Mar Ecol Prog Ser 118:283-294
21. Stewart AJ, Wetzel RG (1981) Dissolved humic materials: Photodegradation, sediment effects, and reactivity with phosphate and calcium carbonate precipitation. Arch Hydrobiol 92:265-286
22. Strome DJ, Miller MC (1978) Photolytic changes in dissolved humic substances. Verh Internat Verein Limnol 20:1248-1254
23. Toolan T, Wehr JD, Findlay S (1991) Inorganic phosphorus stimulation of bacterioplankton production in a mesoeutrophic lake. Appl Environ Microbiol 57:2074-2078
24. Tranvik LJ (1988) Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb Ecol 16:311-322
25. Tranvik LJ (1990) Bacterioplankton growth on fractions of dissolved organic carbon of different molecular weights from humic and clear waters. Appl Environ Microbiol 56:1672-1677
26. Tranvik LJ, Kokalj S (1998) Decreased biodegradability of dissolved organic carbon of phytoplankton origin due to interactive effects of UV radiation and humic matter. Aquat Microb Ecol. 14:301-307
27. Wetzel RG, Hatcher PG, Bianchi TS (1995) Natural photolysis of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. Limnol Oceanogr 40:1369-1380
28. Zweifel UL, Norrman B, Hagström Å (1993) Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. Mar Ecol Prog Ser 101:23-32