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Photochemical mineralization of synthetic lignin in lake water indicates enhanced turnover of aromatic organic matter under solar radiation

Anssi V. Vähätalo[1,2,*], Kalevi Salonen[1,3], Mirja Salkinoja-Salonen[2] & Annele Hatakka[2]
[1]*Lammi Biological Station, University of Helsinki, FIN-16900 Lammi, Finland;* [2]*Department of Applied Chemistry and Microbiology, Viikki Biocenter, P.O.Box 56, FIN-00014 University of Helsinki, Finland;* [3]*Section of Hydrobiology and Limnology, Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FIN-40351, Jyväskylä, Finland (* author for correspondence: e-mail: anssi.vahatalo@helsinki.fi)*

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Abstract

The degradation of ^{14}C-[ring]-labelled synthetic lignin (^{14}C-DHP) and dissolved organic carbon (DOC) from lake water were studied simultaneously. ^{14}C-DHP was incubated in humic lake water (colour 173 mg Pt l^{-1}) for 7 d in the dark or under solar radiation. In the dark $<$0.4% of the introduced ^{14}C-DHP label and 4% of the indigenous DOC were mineralized, indicating that the ^{14}C-labelled aromatic rings of DHP and the humic DOC were microbiologically recalcitrant. Under solar radiation (116 MJ m^{-2}), 17–21% of the ^{14}C-labelled carbons in DHP and 18–23% of the indigenous DOC were mineralized in 7 d. Simultaneously the water solubility of ^{14}C-DHP increased. Solar radiation converted the aromatic cores of synthetic lignin to CO_2 and soluble organic photoproducts. The results suggest that solar radiation plays a key role in the decomposition of natural polyaromatic matter.

Introduction

Dissolved organic matter is the largest reactive reservoir of reduced organic carbon on Earth ($\approx 0.6 \times 10^{18}$ g C, Hedges 1992), and a major reservoir of reduced chemical energy in most aquatic systems (Wetzel 1992). In many fresh and coastal waters the bulk of organic carbon consists mainly of allochthonous high molecular weight humic matter (HMW-HM; $>$1,000 g mol^{-1}, Thurman 1985). Photochemical reactions can directly mineralize HMW-HM to CO_2 (Salonen & Vähätalo 1994; Granéli et al. 1996; Gao & Zepp 1998). In addition, photochemical degradation of HMW-HM produces low molecular weight organic compounds (Kieber et al. 1990; Wetzel et al. 1995; Moran & Zepp 1997). These compounds are rapidly consumed by micro-organisms (Miller & Moran 1997; Bertilsson & Tranvik 1998). It is poorly known whether the photoproducts originate from the aromatic cores or from the aliphatic sidechains in HMW-HM. In this study we focused on the solar radiation aided degradation of the aromatic cores of HMW-HM using a ^{14}C-[ring]-labelled lignin model compound as a tool and found that solar radiation is strongly involved in the decomposition of polyaromatic compounds.

Materials and methods

The ^{14}C-[ring]-labelled dehydrogenation polymerizate of coniferyl alcohol (^{14}C-DHP, synthetic lignin, a precursor shown in Fig. 1) was synthesized by Hackman and Odier (see Eriksson et al. 1990) according to Faix et al. (1985). The ^{14}C-DHP had a specific activity of 277 kBq mg^{-1} and a gel permeation chromatography indicated a mean molecular weight of 4,000–10,000 g mol^{-1} (Eriksson et al. 1990). The same ^{14}C-DHP batch from our laboratory was also used to study mineralization of lignin by white-rot fungi (Vares & Hatakka 1997) and their enzymes (Hofrichter et al. 1999).

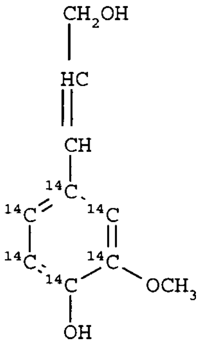

Figure 1. ^{14}C-[ring]-labelled coniferyl alcohol, a precursor for a synthetic lignin (^{14}C-DHP).

Mineralization of ^{14}C-DHP was compared to that of dissolved organic carbon (DOC) of lake water. On August 2, 1996, and between June 14 and August 16, 1994 we collected epilimnetic water from a humic lake Valkea-Kotinen (61°14′N, 25°04′E, water colour 130–173 mg Pt l^{-1}), which is a pristine headwater lake surrounded mainly by coniferous forest. The epilimnetic water was acidic (pH 4.9–5.5), contained 11–38 μM of dissolved inorganic carbon (DIC), 933–1058 μM of DOC, 3.2–5.9 μM of iron, 0.2–1.5 μM of NH_4^+, 0.4–1 μM of NO_3^- and <0.16 μM of PO_4^{3-} (Keskitalo et al. 1998). For a detailed description of the lake see Keskitalo et al. (1998).

The water was filtered through 0.2 μm (Millex-GS, Millipore, later referred as sterile filtrate), through Whatman GF/D (nominal pore size of 2.7 μm) or through 1 μm Nuclepore (two latter referred as microbial filtrates) filters. Filtered lake water spiked or not amended with ^{14}C-DHP in dimethylformamide (DMF) was poured without headspace into three glass stoppered preignited (2 h at 450 °C) quartz or borosilicate tubes (diameter 16 mm, length 150 mm). The final concentrations were 54 μg DHP l^{-1} (^{14}C-label of 900 dpm ml^{-1}) and 0.28% DMF (v/v). One tube of each set was used for the determination of the initial conditions; the two others were incubated for 1 or 7 d in the dark or under solar radiation. The quartz tubes were exposed to solar radiation in a water bath or *in situ* at the depth of 0.8 to 2 cm and were accompanied by the borosilicate tubes covered with aluminium foil (the dark conditions). The water bath contained epilimnetic water of Valkea–Kotinen, and the exposure simulated the conditions in the lake just below the surface. Global radiation (0.3–3 μm) at the surface of Earth was measured with a global radiation meter equipped with a BPW 20 photodiode (Hortimic Oy) and the dose was 116 MJ m^{-2} during 7 d of the exposure. Air temperature was 4.4–25.4 °C during 7 d of the exposure. *In situ* incubations were done at weekly intervals from June 14 to August 16, 1994 in the middle of Lake Valkea-Kotinen, and the samples were exposed to global radiation of 16 ±3.7 MJ m^{-2} d^{-1} (mean ± SD, range 9–20, n = 10).

Mineralization of DOC was measured as an increase in the concentration of DIC. The concentration of DIC was measured according to Salonen (1981) from seven (0.46 ml) subsamples. The CO_2 from the carbon analyzer was trapped in 9 ml of absorption solution (ethanolamine : ethanol, 1 : 7, v/v) and the ^{14}C-activity was measured with a Wallac 1400 liquid scintillation counter (Turku, Finland), after introduction of Optiphase HiSafe 3 liquid scintillator (Wallac; Salonen & Hammar 1986). A 0.2 μm nitrocellulose filter (Type 11307-025N, Sartorius) was used to fractionate the ^{14}C into a retentate and to a water soluble permeate. One ml of tube content was taken for the determination of the total ^{14}C-radioactivity before a fractionation by filtration.

Results

Partitioning of ^{14}C-DHP before solar radiation exposure

Before solar exposure ^{14}C-DHP in the sterile (<0.2 μm) and microbial (<GF/D) filtrates of lake water partitioned similarly (t-test, $t_4 < 1.58$, $P > 0.19$) and hence these results were combined (initial, Table 1). Most (81%) of ^{14}C-DHP was retained on the nitrocellulose filter (retentate, Table 1). The permeate and inorganic parts (Table 1) contained 8.5% and 0.27% of the introduced ^{14}C-label. The sum of ^{14}C-label in the retentate, permeate and inorganic fraction yielded 89% recovery of the introduced ^{14}C-DHP (Table 1).

Mineralization of ^{14}C-DHP and lake water DOC under solar radiation

After 7 d of exposure to solar radiation inorganic ^{14}C increased almost two orders of magnitude in the sterile and the microbial (<GF/D)-filtrates of the lake water (Table 1, Figure 2). Altogether 18.9 ± 1.3% (mean ± SD, range 17.0–20.9%, n = 6) of the added ^{14}C-DHP was found as inorganic carbon (sterile and microbial, <GF/D-filtrates combined, Table 1, Figure 2). In the

Table 1. Absolute and relative partition of radioactivity of ^{14}C-[ring]-DHP in the sterile (<0.2 μm) and the microbial ($<$GF/D) filtrates of lake water before and after 7 d of incubation in the dark or under solar radiation. Different letters (superscripts) at each row show statistically different distribution of ^{14}C between treatments (t-test, $P < 0.05$). Same letters indicate no statistically significant differences between treatments

Fraction	^{14}C-dpm ± SD in 10 ml				
	Initial	After 7 d of incubation			
		In the dark		Under solar radiation	
	$n = 6$	$n = 3$		$n = 3$	
		Sterile	Microbial	Sterile	Microbiol
Inorganic	24 ± 13^{a}	50 ± 2^{b}	53 ± 22^{ab}	1724 ± 120^{c}	1566 ± 59^{c}
Unfiltered[1]	8947 ± 451^{a}	8852 ± 167^{a}	8705 ± 103^{a}	7114 ± 222^{b}	7012 ± 142^{b}
Permeate	761 ± 70^{a}	699 ± 70^{a}	707 ± 87^{a}	4097 ± 140^{b}	3812 ± 177^{b}
Retentate	7194 ± 313^{a}	5932 ± 257^{b}	4457 ± 444^{c}	2041 ± 331^{d}	2189 ± 293^{d}
	% of introduced ^{14}C				
Inorganic	0.27 ± 0.15	0.57 ± 0.0	0.54 ± 0.3	19 ± 1.6	18 ± 0.8
Permeate	8.5 ± 1.0	8.2 ± 1.1	7.6 ± 0.6	46 ± 2.0	43 ± 1.6
Retentate	81 ± 6.1	69 ± 2.8	55 ± 1.2	23 ± 3.4	23 ± 0.2
Recovery	89 ± 6.7	78 ± 3.9	63 ± 2.1	89 ± 1.0	85 ± 2.3

[1] Unfiltered = ^{14}C-radioactivity in a sample before filtration through a 0.2 μm nitrocellulose filter to get permeate and retentate.

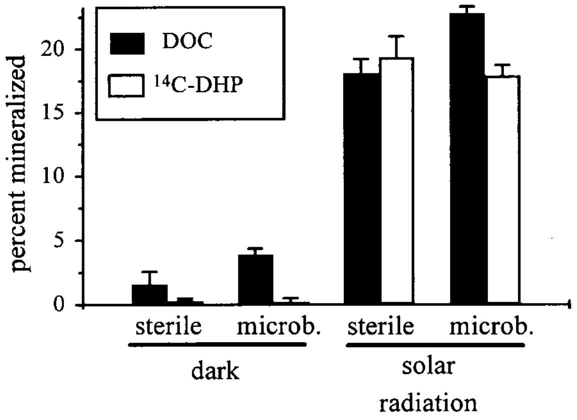

Figure 2. Relative mineralization of ^{14}C-DHP and DOC of Lake Valkea-Kotinen during 7 d of incubation in the sterile (<0.2 μm) and the microbial ($<$GF/D) filtrates of lake water in the dark or under solar radiation. The results are related to the introduced ^{14}C-DHP (54 DHP μg l^{-1}, 900 dpm of ^{14}C-label ml^{-1}) and to lake water DOC (1 mM C). Standard deviations (n = 3) are shown by narrow bars. The values for the DOC are from the tubes without DMF and ^{14}C-DHP (n = 1) with the relative SD found in the tubes containing DMF (n = 3, see Table 2).

dark the mineralization was <0.4% of the added ^{14}C-label (Table 1, Figure 2). Because only the aromatic rings were ^{14}C-labeled (Figure 1), the results indicate that under solar radiation the rings were cleaved and partly oxidized to inorganic carbon.

We did not use a pretrap for volatile organic compounds. In an earlier study (Hofrichter et al. 1999), the same batch of ^{14}C-DHP was used and the amount of ^{14}C-label of volatile organic compounds in the pretrap was 6.3 ± 3.3% (n = 16, and always <15%) of the inorganic ^{14}C-radioactivity. In that study the ^{14}C-DHP was degraded by manganese peroxidases of white-rot fungi (Hofrichter et al. 1999). Because both photochemical and fungal degradation of lignin are radical mediated oxidation processes (Gold et al. 1983), the proportion of produced volatile organic ^{14}C compared to the produced $^{14}CO_2$ was likely low also in the present study.

Under solar radiation lake water DOC was mineralized similarly as ^{14}C-labelled DHP (Figure 2). Eighteen and 23% of lake water DOC was converted to inorganic carbon in the sterile and microbial ($<$GF/D) filtrates of lake water, respectively (Figure 2). The difference between the sterile and microbial ($<$GF/D) filtrates was small suggesting that most of the mineralization was photochemical. In the dark 4% of lake water DOC was mineralized during the 7 d incubation in the microbial ($<$GF/D) filtrate (Figure 2). In the sterile filtrate 1.6% of DOC was mineralized indicating it was not fully sterile (Figure 2).

We measured mineralization of lake water DOC also *in situ* in Lake Valkea-Kotinen (Figure 3). Under solar radiation the DOC of lake water (1008±42,

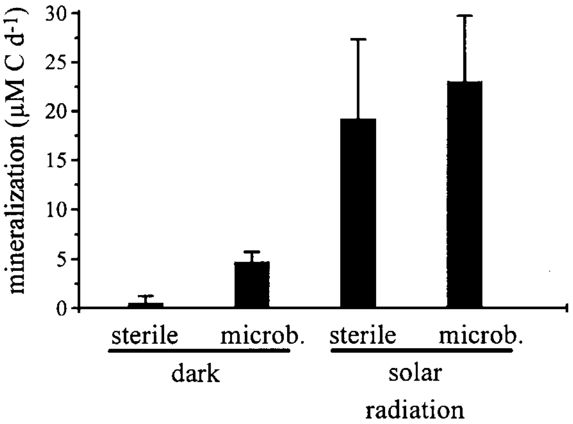

Figure 3. Mineralization of DOC (1008 ± 42, 933–1058 μM) from Lake Valkea-Kotinen during 1 d of incubation in the sterile (<0.2 μm) and the microbial (<1 μm) filtrates of lake water in the dark or under solar radiation. Samples were incubated *in situ* at depths of 0.8 to 2.0 cm. Narrow bars show standard deviation in weekly 1 d incubations from June 14 to August 16, 1994 (n = 10).

Table 2. Mineralization of DOC during 7 d of incubation in the dark and under exposure to solar radiation in the absence (n = 1) or in the presence DMF (0.28% v/v) and ^{14}C-DHP (54 μg l^{-1}, n = 3). The results express the mineralization in the microbial (<GF/D) and the sterile (<0.2 μm) filtrates of lake water.

Filtrate	Mineralization of DOC (μM inorganic C during 7 d, mean ± SD)			
	Under solar radiation		In the dark	
	No DMF	With DMF	No DMF	With DMF
Microbial	228	210 ± 4.8	39.5	23.5 ± 2.9
Sterile	181	230 ± 14	16.4	7.6 ± 4.7

933-1058 μM DOC) was mineralized at the rate 2.1 ± 0.85% d^{-1} in the sterile filtrate and 2.4 ± 0.67% d^{-1} in the microbial filtrate (<1 μm, Figure 3). In dark the mineralization of DOC was negligible in the sterile-filtrate (0.06 ± 0.07% d^{-1}, Figure 3), indicating low contamination of sterile-filtrate by heterotrophic micro-organisms during the 1 d experiments. Under solar radiation the small difference between sterile and microbial (<1 μm) filtrates (Figure 3) indicates that mineralization of DOC was mainly photochemical, similarly to that found in the 7 d experiment (Figure 2).

DMF was the solvent for ^{14}C-DHP. In the dark the presence of DMF reduced mineralization of DOC by 41–54% (Table 2) indicating its toxicity towards mineralization. If ^{14}C-DHP behaves similarly as DOC, the $^{14}CO_2$ yielding from ^{14}C-DHP in the dark (Table 1) may have been too low by a factor of two. Even then, the mineralization of ^{14}C-DHP under solar radiation would have been 27-fold higher than in the dark. The presence of DMF had no effect on the mineralization of DOC under solar radiation (Table 2). Therefore DMF probably did not affect the mineralization of ^{14}C-DHP found under solar radiation (Table 1, Figure 2).

Solubilization of ^{14}C-DHP under solar radiation

More ^{14}C-label permeated through 0.2 μm nitrocellulose filter in the lake water exposed to solar radiation compared to that kept in darkness (permeate, Table 1). To quantify solubilization, ^{14}C-radioactivity of reaction the mixture was measured before the 0.2 μm filtration (unfiltered, Table 1). The permeate contained 54–57% of the ^{14}C found in the unfiltered solar radiation exposed samples (Table 1). When kept in darkness the 0.2 μm permeate contained only 8–9% of ^{14}C found in the unfiltered tube content (Table 1). During 7 d of exposure to solar radiation the mean concentration of inorganic carbon increased from 34 ± 2.0 to 250 ± 19 μM of DIC (sterile and microbial-filtrates combined, Table 2, n = 8). This was an order of magnitude higher than the equilibrium concentration (21.5 μM of DIC, when bubbled with air) between the epilimnetic water and the atmospheric CO_2. The over-saturated DI^{14}C likely exchanged with atmospheric $^{12}CO_2$ before filtration. Thus, the ^{14}C-radioactivity in the permeate was mainly DO^{14}C.

Discussion

Biological degradation of HMW-HM with non-hydrolysable bonds (like lignin) requires extracellular, nonspecific and nonhydrolytic system (Kirk & Farrell 1987). Such systems have low activity in the plankton of natural waters (Münster et al. 1998). The high activities of bacterioplankton related to HMW-HM (Tulonen et al. 1992; Amon & Benner 1996) may base on hydrolyzable constituents of HMW-HM. The non-hydrolytic core parts of HMW-HM can be expected to be recalcitrant against biological degradation. In this study this expectation was verified using ^{14}C-labelled DHP as substrate. Planktonic micro-organisms degraded ^{14}C-labelled DHP negligibly (Table 1, Figure 2).

Photochemical reactions increase the number of hydroxyl, carboxyl and keto groups in lignin but decrease the number of methoxyl groups (Heitner &

Scaiano 1993; Argyropoulos & Sun 1996). These chemical changes increase water solubility of lignin. The water solubility of humic acid also increases after photochemical formation of carboxyl and carbonyl functional groups (Kulovaara et al. 1996). Photo-oxidation also depolymerizes lignin to phenolic monomers (e.g., Argyropoulos & Sun 1996; Crestini & D'Auria 1997). These monomers may be further photo-oxidized to aliphatic compounds (Sun et al. 1998). Similarly to lignin, photochemical reactions decrease the molecular weight of HMW-HM generating low molecular weight organic compounds (Moran & Zepp 1997). The photochemically produced hydrophilic functional groups may explain the observed increased water solubility of ^{14}C-labelled DHP (Table 1). However, it is more likely that low molecular weight hydrophilic monomers contributed to the increased the water solubility of ^{14}C-label (Table 1) as was found by Hofrichter et al. (1999).

Unmodified lignin is photochemically inactive under radiation $>$350 nm, but the presence of other absorbing components (sensitizers) enhances photochemical degradation (Bonini et al. 1998). In natural waters chromophoric dissolved organic matter (CDOM) is the most important sensitizer of photochemical reactions (Zafiriou et al. 1984; Cooper et al. 1989; Mopper & Zhou 1990). In this study the high concentration of CDOM in the water of Valkea-Kotinen (absorption at 350 nm = 23 m^{-1}) may have stimulated the photochemical degradation of ^{14}C-DHP (Table 1, Figure 2). Photo-oxidation increases the absorption of lignin at the UV and the visible range of the spectrum (Castellan et al. 1987; Bonini et al. 1998). Therefore the absorption of ^{14}C-labelled DHP likely increased, also above 350 nm, after the initial photochemical modification by CDOM sensitizers. The photochemically modified ^{14}C-labelled DHP was thus not only degraded by indirect sensitized photochemical reactions, but also by direct photochemical reactions.

Photochemical reactions modify and mineralize particulate lignin (Opshal & Benner 1993; Argyropoulus & Sun 1996) and its dissolved derivatives (Opshal & Benner 1998; Sun et al. 1998). The same holds also for unspecified HMW-HM both in particulate (Vähätalo et al. 1998) and in dissolved forms (Kieber et al. 1990; Wetzel et al. 1995; Bertilsson & Tranvik 1998). Amador (1989) demonstrated that the photochemical reactions increased degradation of glycine bound peripherally to HMW-HM. Here we show that solar radiation degraded the aromatic cores of ^{14}C-DHP to $^{14}CO_2$ and to water soluble organic photoproducts (Table 1). It is likely that CO_2 and biologically labile organic photoproducts from natural dissolved organic matter (Figures 2–3; Moran & Zepp 1997) may originate also from HMW-HM with non-hydrolysable bonds. Because this material is biologically recalcitrant (Table 1, Figure 2), our results suggest that solar radiation remarkably enhances degradation of polyphenolic HMW-HM in surface waters.

Acknowledgments

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