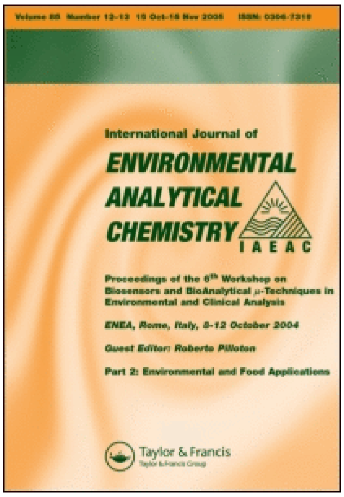

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LIGHT-INDUCED DEGRADATION OF AQUATIC HUMIC SUBSTANCES BY SIMULATED SUNLIGHT

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Filtered (0.22 μm) humic surface water and solutions of isolated fulvic and humic acids at pH 4 and 7 were treated with simulated sunlight. Silver nitrate was used as a bactericide in order to determine the extent of microbial degradation during exposure of the surface water sample. In most sets of samples the decrease in the dissolved organic carbon (DOC) content was found to be independent of the added silver. This reveals that the loss of carbon does not to any substantial extent result from growth of bacteria in the water. After prolonged irradiation part of the dissolved organic matter in the surface water sample was found to precipitate as iron complexes. Several aliphatic carboxylic acids of low molecular weight were quantified by gas chromatography/mass spectrometry after irradiation of the water samples for 48 h. These acids accounted for 10% of the remaining DOC in the surface water sample. The photo-induced degradation of the dissolved humic acid reference was slower than the corresponding degradation of the dissolved fulvic acid reference, and it was found to be further retarded at pH 4. Accordingly, the net concentration of the identified carboxylic acids was lowest in the humic acid solutions.

KEY WORDS: Light-induced degradation, simulated sunlight, humic substances, surface water, DOC, carboxylic acids.

INTRODUCTION

Coloured surface waters contain large amounts of dissolved organic matter (DOM), which can be quantified as dissolved organic carbon (DOC). According to Thurman[1], the DOC value is approx. 50% of the amount of DOM. The contribution of polydispersive, polyfunctional humic substances to the total content of aquatic DOC varies between 70% and 90% in wetland waters (DOC, 10–50 mg/l)[1] and is about 50% in uncoloured river waters (average DOC, 5 mg/l)[2].

Fractions of humic substances are defined operationally. Humic acid (HA) and fulvic acid (FA) are water-soluble fractions (at pH > 2), which are conveniently isolated from water by sorption onto XAD resins. The average molecular weight of the former is higher than that of the latter. Bog waters with high concentration of DOC are normally rich in humic acid (15–35% of the DOC)[2], whereas only small amounts of humic acid (≤ 5% of the DOC) can be isolated from colourless river waters[1]. In addition to the macromolecular acids trapped on the XAD resin, a pool of hydrophilic DOM will pass through the resin column.

The degradation of relatively resistant aquatic humic substances by microorganisms proceeds slowly[3]. However, since yellow-coloured, natural organic matter absorbs sunlight, solar radiation may accelerate the degradation processes, especially in surface waters, by cleaving the macromolecules to less stable components[4]. The low-molecular-weight (LMW) constituents of the DOM pool (simple organic acids, sugars, amino acids) are easily utilized by biota as a source of carbon and other elements[5,6].

Global solar radiation consists of ultraviolet (UV) radiation with wavelengths of approx. 290–400 nm, visible radiation of 400–700 nm, and infrared (IR) radiation > 700 nm. Shorter wavelengths imply higher energies and also a higher potential to destroy organic matter. Artificial UV radiation of 254 nm (UV_{254}) has been used to mineralize organic matter in water-treatment plants and to disinfect drinking water. Lately, the consequences of increased global UV radiation, which is caused by the decrease in the thickness of the ozone layer, have been of growing concern.

The effects of artificial UV irradiation on aqueous DOM has been the subject of many investigations (see, e.g., refs. 7–11), and the degradative power of this radiation is well documented. Various LMW carboxylic acids are known to be formed as degradation products[10,11]. It has also been shown experimentally that exposure of aquatic humic substances to *natural* radiation changes the absorbance and the fluorescence properties of the sample[4,5,9] and generates degradation products containing carbonyl and carboxyl groups[12–15]. The mechanisms involved in the sunlight-induced photochemistry of DOM has been discussed by e.g. Cooper *et al.*[16] and Hoigné *et al.*[17]

In a previous study[18] we demonstrated the use of an apparatus designed for testing lightfastness, to be used in the laboratory for studying photo-bleaching. Among other changes, we reported a decrease in both the UV absorbance (254 nm) and the DOC content of natural humic water as a result of exposure to *artificial* sunlight[18]. We also reported the presence of a brownish precipitate, which was formed during prolonged exposure[10,18]. The possibility of microbial degradation processes during long-term exposures of the filtered waters has, however, been of concern.

The aim of the present work was primarily to investigate further the light-induced degradation of DOM in surface water samples also treated with a biocide and in solutions of isolated humic substance references. The use of reference preparations eliminates variations in the starting material due to local and/or seasonal alterations in the DOM. In addition, such preparations contain less hydrophilic material. Furthermore, precipitation of material, which was observed earlier, was checked more carefully this time and examined by IR spectroscopy.

EXPERIMENTAL

Materials

Sample preparation. Surface water was collected from Lake Savojärvi (LS), which is located in a marsh region in southwestern Finland. Before use, the samples were filtered through 0.22 μm membrane filters (Millipore). The DOC content of the filtered water was 18.1 mg/l and the pH 5.9.

In some of the experiments a volume of 200 μl of aqueous silver nitrate ($AgNO_3$, p.a., Riedel & Haan) solution was added to 100 ml of filtered natural humic water as a bactericide. The final concentration of $AgNO_3$ in the water was 10 μg/l, an amount which has been reported to be sufficient to prevent bacterial growth in natural waters[19]. Bottled water, Aqua sterilisata (Kabi Pharmacia), was used as a solvent.

Nordic fulvic acid and humic acid references (NoFA and NoHA, respectively) were dissolved in water (40 mg/l) and stirred for 2 h in the dark, and were then used without additional filtration. The reference humic substances were isolated from humus-rich pond water (Hellerudmyra) in Norway according to the standard XAD-8 procedure described by Thurman and Malcolm[20]. The pH of the original solutions (3.7 and 3.4, respectively) was adjusted to 4 or to 7 with 0.01 M NaOH (Merck).

Model compounds and chemicals. Carboxylic acid model compounds were used for identification and determination of response factors in gas chromatography/mass spectrometry. Formic acid, acetic acid, propanoic acid, glycolic acid (2-hydroxyacetic acid), oxalic acid (ethanedioic acid), malonic acid (1,3-propanedioic acid), succinic acid (1,4-butanedioic acid), and benzyl bromide (z.S.) were obtained from Merck. Crotonic acid (2-butenoic acid), tetra-n-butylammonium hydroxide (TBAH) 1.5 M (40%), and strongly acidic cation exchange resin Dowex 50WX8 (50–100 mesh) were purchased from Fluka. Small volumes of acetone (99.8%), from Labscan, were used without further purification. Benzyl oxalate was a gift from the Department of Forest Products Chemistry, Åbo Akademi University.

Irradiation of water samples

Water volumes of 15 ml or 20 ml in ten test tubes (Duran, 18 mm OD × 16 cm, with screw-caps) were parallelly irradiated with intense radiation, simulating ‘outdoor sunlight’, in a Heraeus Xenotest 150 S apparatus at a temperature of 31 ± 1°C. The 1.3-kW xenon burner is surrounded both by a lantern composed of six infrared filters and one ultraviolet window, and an outer ultraviolet cylinder of special glass. The emitted radiation is thus filtered optically and a spectral distribution similar to that of natural sunlight is provided. The test tubes were placed at a distance of 2.5 cm from the filters on PTFE holders, which rotated around the burner and turned 180° during every revolution. The intensity of the filtered irradiance is reported to be approx. 0.1 W/m^2 at 290–300 nm, 146 W/m^2 at 300–400 nm and 1220 W/m^2 at 400–800 nm. According to the manufacturer, the glass walls of the test tubes absorb the radiation of wavelengths below 310 nm.

The filtered samples of natural water were irradiated for 24, 48, 72, 120, and 168 h. As dark controls, one set of samples was kept covered with non-transparent material in the irradiation chamber for 168 h. Solutions of humic references were exposed for 48 h, as was the solvent water blank containing NaOH. The samples were stored frozen before they were analyzed.

Analytical procedures

DOC determination. DOC and dissolved carbon dioxide were determined on a Shimadzu Total Organic Carbon 5050 Analyzer equipped with an automatic sample injector (Shimadzu ASI 5000).

UV_{254} absorbance. Absorbances were recorded using a Shimadzu UV-160 A UV-Vis spectrophotometer.

Sample preparation for IR recordings, IR spectra. A precipitate formed during irradiation was collected (after 168 h) from ten parallel silver-free samples.

The supernatant was decanted and the residue, about 0.4 mg, was freeze-dried and analysed by IR spectroscopy. Filtered Lake Savojärvi water was concentrated by rotary evaporation, the residual water was frozen out, and an IR spectrum of the unexposed humic material was recorded for comparison.

IR-spectrum scans were accumulated on a Perkin-Elmer Model 1710 FT-IR spectrometer, resolution 4.0 cm^{-1}, using the KBr-pellet technique.

Enrichment and derivatization of carboxylic acids. The carboxylic acids formed during the irradiation experiments were analysed as their benzyl esters[21,22].

A volume of 100 ml of the sample water (i.e. the content from five test tubes), containing 100 µg of crotonic acid as internal standard, was passed through the cation exchange resin (H^+-form, about 5 ml). The test tubes were washed with 10 ml of water and the resin column finally with an additional 5 ml of water.

The water sample, now containing free acids (pH ca. 3.5) was titrated to pH 8 with a 0.08 M or 0.008 M TBAH solution using a pH meter to indicate the end-point, thus avoiding harmful excess of alkali. The consumption of 0.08 M TBAH was between 0.4 and 1.4 ml. Instead of TBAH, tetraethylammonium hydroxide could have been used as the base[23]. Water was removed from the sample by rotary evaporation (35°C), 200 µl of acetone were added and the solution was evaporated to dryness. The carboxylic acids salts formed are soluble in organic solvents such as acetone, and the TBA anion acts as a good leaving group in the nucleophilic displacement reaction with benzyl bromide[21]. Thus, a molar excess of benzyl bromide in acetone (1:20 dilution), usually 500 µl, was added and the esterification was allowed to proceed at room temperature for 2 h, while the flasks were shaken.

In order to avoid any decomposition of the derivatives[21], the samples were analysed by gas chromatography/mass spectrometry (GC/MS) shortly after preparation. This method of derivatization was preferred because the benzyl esters of volatile carboxylic acids such as formic and acetic acids, elute sufficiently slowly on a GC column compared to extremely volatile methyl and trimethylsilyl esters. In addition, the peaks originating from the benzylation by-products did not overlap any of the peaks of interest.

For the determination of retention times and response factors, 100 µg of the acids in question were dissolved in water, and the solution was worked up as described above.

GC/MS. The samples were analysed on a Hewlett-Packard gas chromatograph (HP 5890 Series II) combined with a mass selective detector (HP 5971A) working in scan mode. The GC system was equipped with a 25 m × 0.2 mm i.d. fused silica capillary column coated with a 0.33 µm film of cross-linked methylsilicone gum (HP-1) as a stationary phase. The injector temperature was 250°C, and about 1 µl of the sample was injected at a split ratio of 1:30. A small piece of silanized glass wool was inserted into the injector liner as a trap for non-volatile components. A column head pressure of 11 psi (ca. 76 kPa) provided a linear carrier gas (helium) velocity of 27 cm/s at 100°C. Oven temperature programme: 80°C for 2 min, then to 300°C at 10°C/min.

Response factors were calculated on the basis of known concentrations, and the results of the analysis were corrected for the response.

RESULTS AND DISCUSSION

Degradation of DOM in surface water samples. In accordance with a previous study[18], the DOC content of the samples was found to decrease during irradiation (Figure 1),

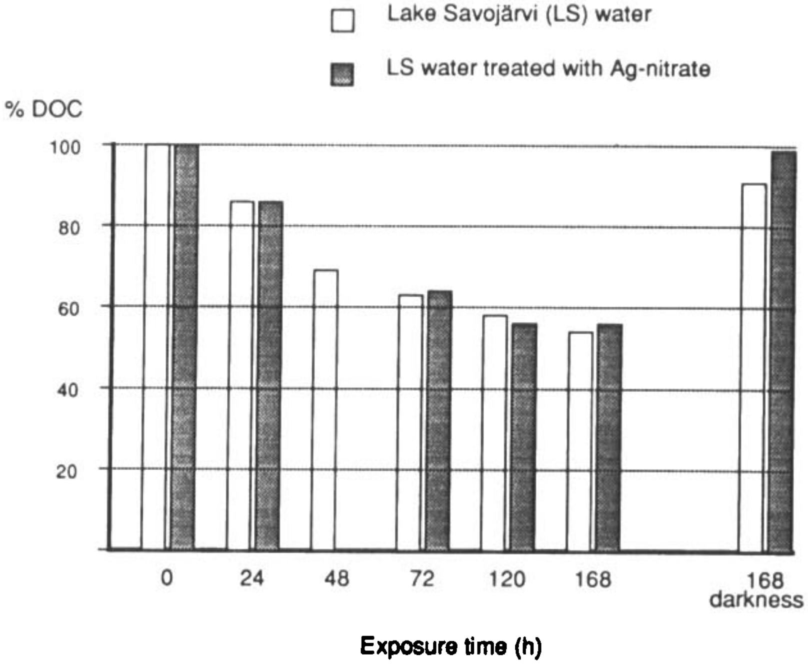

Figure 1 Dissolved organic carbon remaining in Lake Savojärvi water after irradiation with simulated sunlight for different lengths of time.

while photo-induced degradation led to partial mineralization of the carbon compounds to carbon dioxide. The artificial light also had a tendency to lower the pH slightly, to bleach the colour of the water markedly, and to decrease the absorbance of UV light at 254 nm (A_{254}) of water (results not shown).

The DOC content was 7.5% higher in the dark control sample which had been treated with silver nitrate than in the silver-free dark control (Figure 1). This reveals that some mineralization in the silver-free samples may be due to the presence of micro-organisms. A comparable loss of carbon, ca. 5% after 120 h, was observed in a dark control in our earlier study[18]. The filter membranes used were of fine pore size, 0.22 μm, but the other conditions for sample preparation may not have been strictly aseptic. The very even descending trend in the DOC values of both series of irradiated samples (Figure 1) shows, however, that the bacterial growth did not to any notable degree interfere with the light-induced processes.

The DOC content of the natural water samples decreased about 45% during the 168-h irradiation. In the previous study[18] as much as 60% of the carbon compounds was decomposed during the same period of time. The differences are probably due to seasonal variations in the composition of the DOM.

Analysis of precipitated matter. During extensive irradiation (≥ 120 h), part of the DOM is sedimented as water-insoluble macromolecules, which further lowers the DOC values. Similar precipitation has also been observed at high UV_{254} doses[10]. Using size-exclusion chromatography, it was possible to record the beginning of the aggregation into larger molecular units until the insoluble matter was formed[10,18]. Sedimentation of the colloidal precipitate was found to be enhanced by aerobic conditions; if the test tubes were completely filled with water, no detectable amount of solid matter was formed.

The IR spectrum of the isolated precipitate is shown in Figure 2a, together with the IR spectrum of the isolated and dried starting material (Figure 2b). The similarities in the spectra are obvious; the spectra are both composed of bands that normally occur in spectra of humic substances in salt/complexed form[24,25]. The absorption in the IR region is caused by different rotational and vibrational movements of the structural units.

The driving force for this precipitation was revealed in another context. The supernatant layer was analysed by atomic absorption spectrometry after exposure of the surface water to UV_{254} radiation for 9 h.* The data obtained indicated that the amount of dissolved iron, Fe_{tot}, had decreased during the experiment from 0.76 μg/ml to less than 0.13 μg/ml. That is, the rest of the iron was concentrated in the precipitate. Presumably, iron flocks were built up during the photoprocesses (e.g. $Fe^{2+} \rightarrow Fe^{3+}$) when the metal hydroxides formed were complexed with organic matter (OM). This led to coagulation and sedimentation of insoluble Fe-OM aggregates.

Mineralization of aquatic humic substance references. Figure 3 shows the photo-induced mineralization of the DOM of Nordic humic substance references during 48 h at two different pH values. The decomposition of fulvic acid was found to be independent

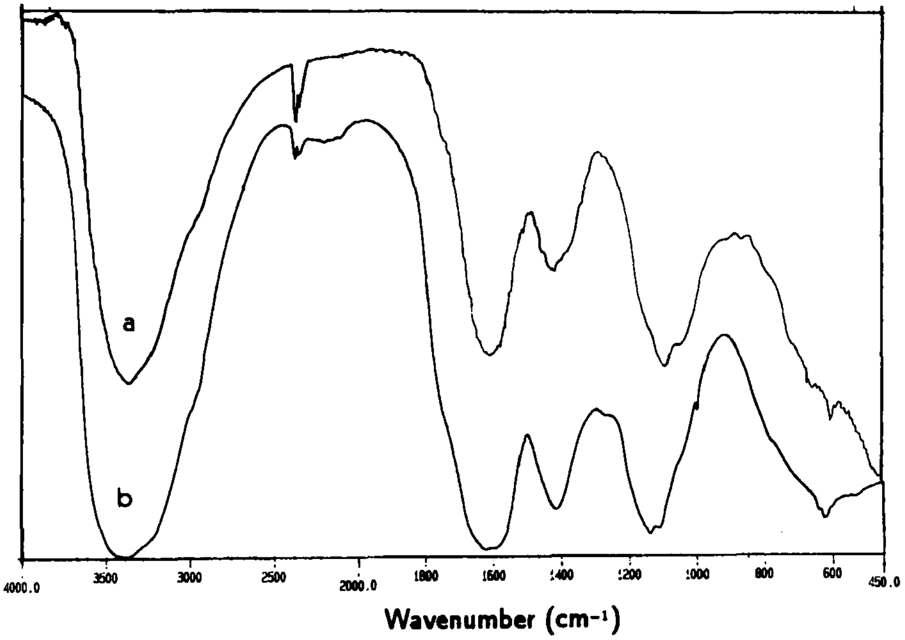

Figure 2 Infrared spectra of (a) a precipitate formed during prolonged irradiation of Lake Savojärvi water with simulated sunlight, and (b) freeze-dried, unexposed Lake Savojärvi water.

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* P. Backlund, Åbo Akademi University, unpublished results.

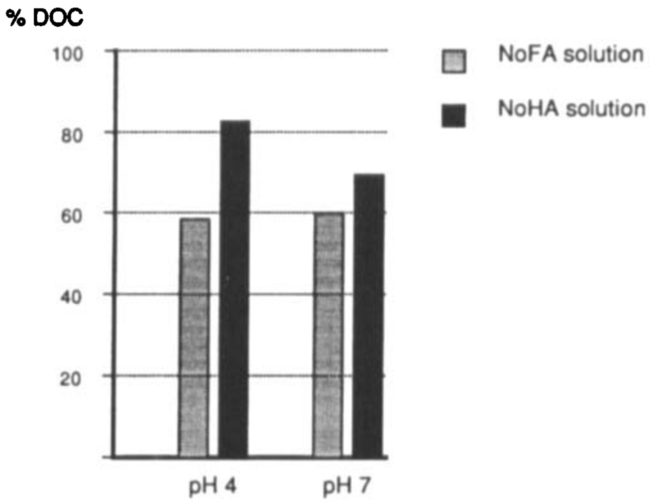

Figure 3 Dissolved organic carbon remaining in aquatic solutions of Nordic fulvic acid (NoFA) and humic acid (NoHA) references after irradiation with simulated sunlight for 48 h.

of the pH of the sample, whereas at lower pH the humic acid decomposition was retarded (measured values at pH 4: 12.4% and 21.6% decrease in the carbon content). At both pH values the degree of humic acid mineralization was less than the loss of carbon in the corresponding solutions of fulvic acid. A higher rate of degradation in an aquatic solution of fulvic acid at pH 7 compared to a humic acid solution has also been observed in other studies[11]. In addition, results have been published which indicate that dissolved humic material of lower apparent molecular weight is more susceptible to photodegradation[5]. Without further studies, however, it is not possible to explain why the DOM of NoHA in less ionized/polarized form at pH 4 was more stable than the same DOM at pH 7.

Analysis of carboxylic acids. Carboxylic acids which were identified and the concentrations of which were found to increase during irradiation for 48 h, were formic, acetic, propanoic, glycolic, oxalic, malonic, and succinic acids. Figure 4 shows total ion chromatograms (TIC) of the acids as benzyl esters, enriched from Lake Savojärvi (LS) water.

For positive matching the retention times of derivatized standard carboxylic acids were needed, because in most cases the mass spectra of the benzyl esters showed no molecular ions. In general, all the fragments in the mass spectra that were heavier than m/z 91 or 107 (tropylium ion and benzyloxy ion, respectively) were present in relatively small amounts. The sum of the net concentrations of the carboxylic acids after irradiation of the LS water is 57.8 ± 3.9 μM (n = 3) and the sum of the carbon content of these acids is 1.3 mg/l, which accounts for 10.1% of the DOC remaining in the water.

The following total net concentrations were found for the humic substance references in aquatic solutions: NoFA at pH 4, 59.4 μM and at pH 7, 53.1 μM; NoHA at pH 4, 38.2 μM and at pH 7, 41.7 μM. However, the various amounts of organic material in the sample waters should be taken into consideration, and thus the concentrations must be given per unit of initial DOC. The following values, expressed as μM × l/mg C, are obtained: LS, 3.2; NoFA pH 4, 3.9 and pH 7, 3.5; NoHA pH 4, 2.0 and pH 7, 2.2.

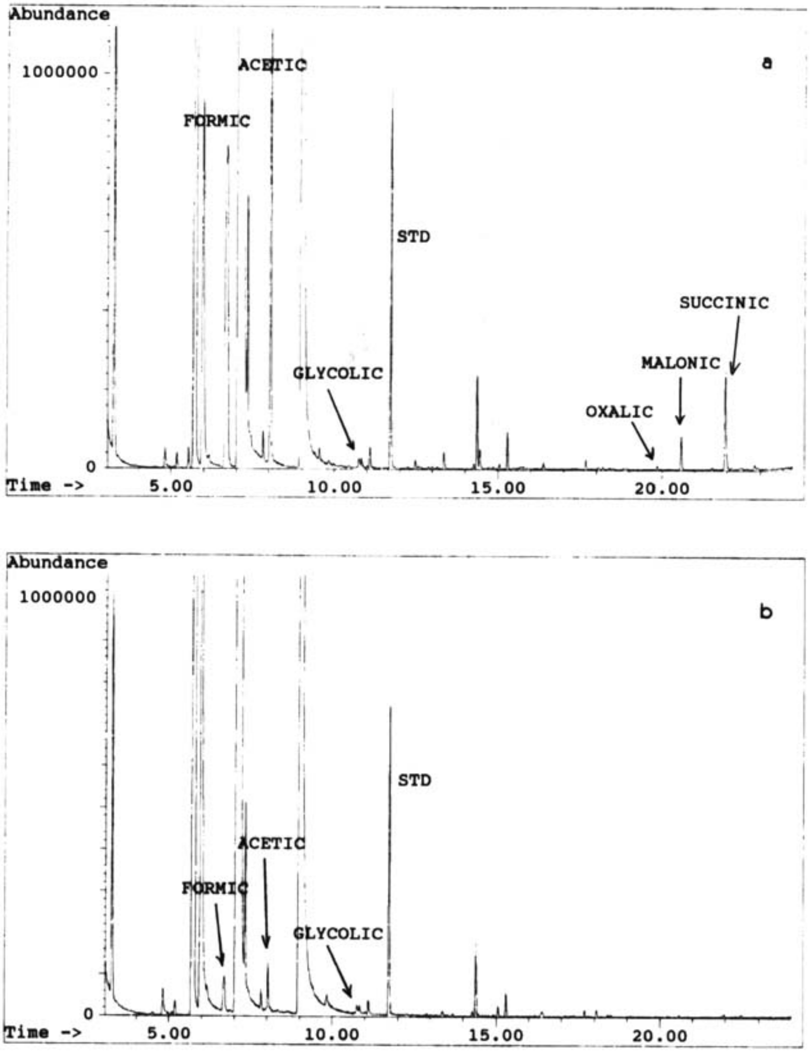

Figure 4 Total ion chromatograms (TIC) of carboxylic acids as benzyl esters, enriched from Lake Savojärvi water. STD, internal standard, crotonic acid, 1 mg/l. (a) Sample irradiated with simulated sunlight for 48 h, (b) unexposed sample.

Determined on the basis of both presentations of the data, during irradiation the largest quantities of carboxylic acids were produced in the LS sample and in the NoFA solutions whereas less of the carboxylic acids were produced in the NoHA solutions. The DOM in the pH 4 NoHA solution was again found to be most resistant to light-initiated decomposition measured in this way.

In Figure 5 the obtained net concentrations of the carboxylic acids are compared in another normalized form. In addition to the carbon content of the initial solution, the 'quality' of the DOM is also taken into account, measured as light absorbance, A_{254}. The molar concentration is thus divided by a DOC-normalized absorbance, and given per

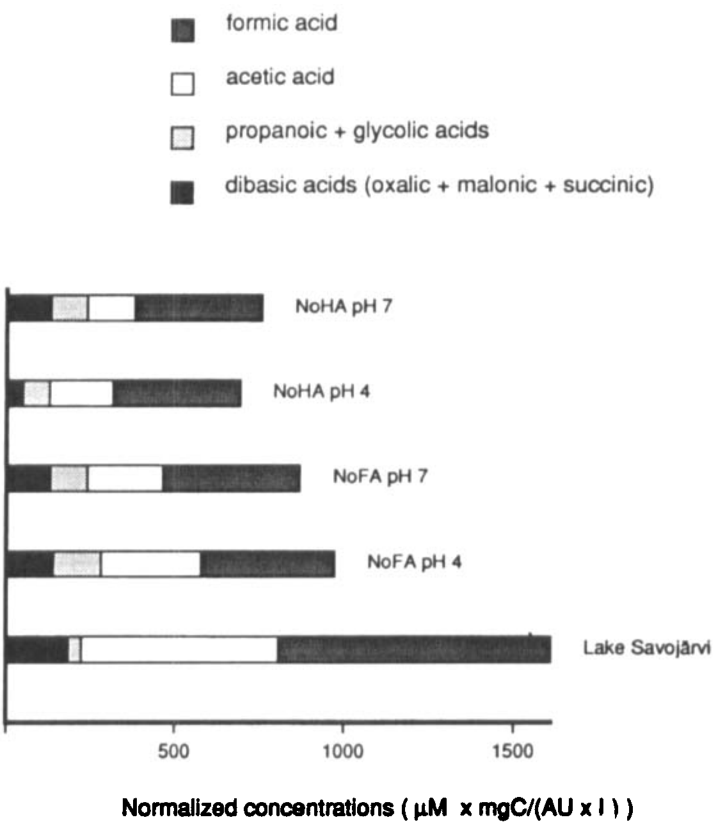

Figure 5 Normalized concentrations (concentration/(A_{254} × DOC^{-1})) of identified carboxylic acids formed in different humic waters during irradiations for 48 h with simulated sunlight.

absorbance units (AU) per DOC unit, i.e. in units of µM × mg C/(AU × l). The DOC-normalized initial absorbances were: LS, 0.036; NoFA, 0.061, and NoHA, 0.055 AU × l/mg C. According to this presentation (Figure 5), the sum of the normalized concentrations of identified carboxylic acids in the LS water is clearly largest, while the total acid concentration in the NoHA pH 4 solution is still the smallest.

The differences in the results of the above comparison may be partly caused by the numerous hydrophilic components which are present in natural waters, like in the LS sample, but which are separated from the isolated humic substances. Some of these compounds may be of lower molecular weight and less light-absorbing than humic substances, but at the same time they may easily be decomposed and give rise to large quantities of organic acids, especially formic and acetic acids, during irradiation. The fact that more degradation products were found in irradiated fulvic acid solutions than in humic acid solutions, agrees with the above finding (Figure 3) that isolated humic acid was more stable than fulvic acid towards light-initiated mineralization.

Several studies have focused on carbonyl compounds and in particular on α-keto carboxylic acids formed during light-initiated degradations of DOM[12–15]. In the present study, however, no production of either glyoxylic (oxoacetic acid) or pyruvic acid (2-

oxopropanoic acid) could be detected. The former is excluded simply because it is not derivatizable with the chemicals used, probably due to hydrate formation. Pyruvic acid, on the other hand, is readily derivatizable by this method, but its concentrations may have been one or more orders of magnitude lower than the concentrations of the acids that were quantified. Alternatively, with the intense artificial radiation used here, the pyruvic acid formed may have undergone a further photochemical decarboxylation[12,13] during the 48-h experiment.

Determination of oxalic acid by the present method is complicated for two reasons. Firstly, the response factor measured from the TIC peaks is unfavourably small. This was controlled by mixing synthesized oxalic acid dibenzyl ester with the derivatized standard. Secondly, oxalic acid does not react quantitatively with the reagents, which further lowers the response. The low reactivity may well be caused by steric factors. In the present work the estimated net concentration of oxalic acid in LS water was 1.9 ± 0.4 μM (n = 3).

Natural DOM is a complex mixture, the nature of aquatic humic substances is heterogeneous, and the light-initiated reactions are also complex in humic surface waters. Very little is, for example, known about the possible biologically mediated degradation pathways during light-exposure[16]. Further studies are needed to get a better knowledge of these processes.

Acknowledgements

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