MICROBIAL ACTIVITY IN SANITARY LANDFILLS—A POSSIBLE SOURCE OF THE HUMIC SUBSTANCES IN GROUNDWATER?

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ABSTRACT

Humic substances account for the main part of the dissolved organic carbon in groundwater. Since groundwater aquifers located near to sanitary landfills usually contain higher concentrations of dissolved organic carbon, we made experiments in the laboratory to determine whether humic substances can be formed from simple non-humified organic substances by a complex microflora of municipal refuse. In liquid cultures incubated for two months humic substances were produced, especially when casein or starch was added. The highest amounts of humic acid-like substances were yielded from cultures inoculated with the indigenous microflora from a rotted (aerobic) landfill. Minor yields were obtained when the inoculum originated from a compacted (anaerobic) landfill or from a five years old landfill containing refuse and sewage sludge. Spectral characteristics indicated some similarities but also differences in the UV and visible regions between the newly formed humic acids and a humic acid from groundwater.

KEYWORDS

Humic substances; microorganisms; municipal refuse; groundwater.

INTRODUCTION

In colored groundwater humic substances account for 65% or more of the dissolved organic carbon (Thurman, 1985). Both beneficial and adverse effects of humic substances on the quality of groundwater have been reported (Alberts and Dickson, 1985, Carter and Suffet, 1985, Cooksey et al., 1985, Ishikawa et al., 1984, Jekel, 1983, Kölle, 1981). Less is known, however, about the origin of these substances in groundwater aquifers. Two hypotheses have been proposed by Thurman (1985). One is that humic material originates in overlying soils. Another hypothesis is that humic substances are leached from kerogen in the sediment of the aquifers. Since groundwater aquifers located near to sanitary landfills contain higher concentrations of dissolved organic carbon (Barber et al., 1981), investigations were made in our laboratory to estimate whether the indigenous microflora of municipal refuse is capable in forming humic substances from non-humified organic compounds such as casein and starch. These compounds may serve as models of proteinaceous and carbonaceous structural units of refuse components decomposing in a landfill.

MATERIALS AND METHODS

Refuse samples were taken from experimental tipping sites located at Wolfsburg and Braunschweig, F.R. of Germany, respectively. Two of them represented material from an aerobic non-compact ed (rotted) landfill, and from a compacted (anaerobic) one, both aged for about six months. The third sample represented an old tipping site, in which refuse was mixed with sewage sludge and
allowed to decompose for more than five years. In the following text these materials are designated as "rotted", "compacted", and "old". Microbiological characterizations of these samples were made using conservative cultivation techniques such as the "Most Probable Numbers Method", and plate counting (Filip and Kuster, 1979). Suspensions diluted up to $10^{-2}$ were prepared and 5 ml used as an inoculum in 2 l- Fernbach bottles containing 300 ml of the Thornton nutrient solution (0.5 g KNO$_3$; 1 g K$_2$HPO$_4$; 0.2 g MgSO$_4$·7 H$_2$O; 0.1 g CaCl$_2$; 0.1 g NaCl; 0.5 g asparagine; 1 g mannite; traces of FeCl$_3$; 1000 ml dist. water; pH 7.2). Alternatively, KNO$_3$ and mannite were replaced by 30 g l$^{-1}$ casein, or 26 g l$^{-1}$ starch plus 3 g l$^{-1}$ NH$_4$NO$_3$ or by 70 g l$^{-1}$ dry powdered refuse in the nutrient solution. At least three parallels of the inoculated cultures and control flasks were incubated for 60 days at 35°C in the dark. Humic substances from the liquid phase, i.e. humic acid (HA 1) and fulvic acid (FA 1), and those from the microbial biomass or a biomass-refuse mixture (HA 2, FA 2) were separated as described elsewhere (Filip et al., 1972).

Carbon contents of liquid and solid samples were estimated using the Astro TOC-TC Analyzer 1850, and a wet combustion after Alten et al. (1935), respectively. The total nitrogen was analyzed by the Kjeldahl method using a semi-automatic apparatus (Büchi Laboratoriums-Technik, Switzerland).

The optical density of humic acids was measured over the wavelength ranges of 200 - 400 nm, and 400 - 800 nm, respectively in the Spectracomp 601 (Carlo Erba, Italy) recording spectrophotometer. $E_4/E_6$ ratios were calculated from the extinction values at 465 nm and 665 nm.

The reference humic acid originated from a groundwater obtained from the Waterworks Fuhrberg near Hannover (F.R. of Germany). At that locality a colored groundwater had a DOC content of 6 mg l$^{-1}$ which was reduced to 2 - 3 mg l$^{-1}$ by the appropriate water treatment. During this treatment, humic substances were adsorbed on the anion-exchange resin Lewatit MP 500 A (Bayer AG, Leverkusen) and eluted with an alkali (Kölle, 1981). The final concentrate contained 20 g l$^{-1}$ total carbon, and 2.53 g l$^{-1}$ humic acid, respectively.

RESULTS AND DISCUSSION

Municipal refuse disposed of in a landfill is rich in microorganisms. Filip and Kuster (1979) reported counts of aerobic proteolytic bacteria and other groups of microorganisms in the range of $10^5$ to $10^{12}$ g$^{-1}$.

Similar concentrations of microorganisms were estimated in our samples of rotted, compacted and old refuse as shown in Table 1.

TABLE 1 Numbers of Some Microorganisms in Refuse Samples (n x g$^{-1}$ dry matter)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Rotted</th>
<th>Refuse Sample</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Compacted</td>
<td></td>
</tr>
<tr>
<td>Aerobic Proteolytic Bacteria</td>
<td>1.8 x 10$^8$</td>
<td>1.2 x 10$^6$</td>
<td>4.2 x 10$^6$</td>
</tr>
<tr>
<td>Anaerobic Proteolytic Bacteria</td>
<td>2.0 x 10$^9$</td>
<td>2.5 x 10$^{10}$</td>
<td>4.4 x 10$^6$</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>7.3 x 10$^6$</td>
<td>2.6 x 10$^6$</td>
<td>1.9 x 10$^6$</td>
</tr>
<tr>
<td>Fungi</td>
<td>5.8 x 10$^6$</td>
<td>1.5 x 10$^6$</td>
<td>1.2 x 10$^5$</td>
</tr>
</tbody>
</table>

Compacted refuse contained the highest counts of anaerobic bacteria. In other groups of microorganisms the concentrations declined from rotted to compacted, and old refuse.

Casein and starch represent easily utilizable substrates which can be converted to humic substances by complex populations of soil microorganisms (Filip, 1968; Novak, 1970).

After about 20 days of incubation, the liquid cultures gradually turned yellow-brown and then almost dark brown as the humic acid-type polymers formed. The addition of casein accelerated the color change and the intensity of the dark color. The actual amounts of humic acids recovered from the medium and the cells, and also the yield of biomass are presented in Table 2. The cultures contained casein produced higher amounts of humic acid than other cultures, and the inoculum originated from the rotted refuse was most effective. The amounts of HA 2, e.g., yielded from biomass were in a relation of 100:21:1:7.4 when compared the inoculum from the rotted, compacted, and old refuse. This indicates a higher humification activity of microorganisms from the rotted refuse. When compared the values of HA from variants enriched with either casein or starch one can recognize up to ten times higher concentration of humic acids in the
<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Yield</th>
<th>Nutrient Solution</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Alone</td>
<td>Casein Added</td>
<td>Starch Added</td>
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<tr>
<td>Rotted</td>
<td>Biomass</td>
<td>33</td>
<td>1800</td>
<td>3792</td>
<td>112867</td>
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<tr>
<td></td>
<td>HA 1</td>
<td>6</td>
<td>356</td>
<td>44</td>
<td>106</td>
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<tr>
<td></td>
<td>HA 2</td>
<td>143</td>
<td>474</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Σ HA</td>
<td>149</td>
<td>830</td>
<td>74</td>
<td>110</td>
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<tr>
<td>Compacted</td>
<td>Biomass</td>
<td>433</td>
<td>4700</td>
<td>4211</td>
<td>104487</td>
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<tr>
<td></td>
<td>HA 1</td>
<td>12</td>
<td>306</td>
<td>12</td>
<td>178</td>
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<tr>
<td></td>
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<td>18</td>
<td>100</td>
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<td>5</td>
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<tr>
<td></td>
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<td>406</td>
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<td>Old</td>
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<td>3064</td>
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</tr>
<tr>
<td></td>
<td>Σ HA</td>
<td>157</td>
<td>376</td>
<td>103</td>
<td>184</td>
</tr>
</tbody>
</table>

*a) A mixture of biomass and refuse added.

Fig. 1 Visible spectra of selected humic acids (HA). A = HA from groundwater; B = HA from a sample of fresh rotted refuse; C = HA from the liquid phase of cultures enriched in casein and inoculated with an infusion of rotted refuse; D = same as C but inoculated with an infusion of compacted refuse; E = same as C but inoculated with an infusion of old refuse.
The curves of visible light absorption observed for humic acids are given in Figure 1. According to the slopes of the curves the optical density is greatest for the groundwater humic acid and lowest for humic acids recovered from cultures inoculated with the indigenous microflora from compacted and old refuse. The UV-spectra given in Figure 2 also show some differences between the individual preparations. Whereas the groundwater humic acid and also humic acid recovered from a fresh sample of rotted refuse show featureless spectra between 240 and 400 nm, there are some absorption shoulders at 281 nm and 330 nm in the preparations of humic acids from those cultures which received starch or casein as substrates. These indicate the presence of some phenolic and chinolic chromophores (Williams and Fleming, 1971). More distinct absorption maxima at 214 nm, 224 nm, 230 nm, and 235 nm can be attributed to low saturated carbonic acids, esters, and also to substituted benzene rings. Similar absorption maxima have been observed Filip et al. (1976) in humic acids produced by different soil fungi.

According to Chen et al. (1977), a correlation exists between the values of the \( E_4/E_6 \) ratios and a particle size (or molecular weight) of humic acids. A high value indicates a smaller molecule. The \( E_4/E_6 \) value was 5.9 for the groundwater humic acid but for several preparations of newly formed humic acid this value was higher than 7. This was true for all humic acids from cultures where a finely milled refuse served as substrate, e.g.

In conclusion, the indigenous microflora of deposited municipal refuse is capable of forming humic substances from simple non-humified organic materials such as casein and starch. The newly formed humic substances partly resemble to those recovered from groundwater. It shall be further tested if these humic substances of microbial origin may contribute to the enhancement of dissolved organic carbon in groundwater aquifers located near to landfills or tipping sites.
Microbial activity in sanitary landfills

REFERENCES


