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Microbial utilization and transformation of humic acid-like substances extracted from a mixture of municipal refuse and sewage sludge disposed of in a landfill

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"Capsule": *Humic acid-like structures do not play a role in long-term stabilization of landfill refuse.*

Abstract

The purpose of the research was to establish whether humic acid-like substances (HA) related to municipal refuse disposed of in a landfill can resist microbial degradation and if they contribute, in that way, to long-term stabilization of landfill refuse. Using a mixture of 0.1 M $Na_4P_2O_7$ + 0.1 M NaOH, we extracted HA from municipal refuse mixed with sewage sludge and disposed of for up to 12 months, in a 40-m^3 model landfill. In laboratory experiments under aerobic conditions, up to 50% of HA was utilized as a supplementary source of nutrients by an assemblage of soil microorganisms in only 21 days. The microbial utilization was enhanced to over 80%, and up to 98%, respectively, if HA served as the sole source of carbon or nitrogen. Remaining HA which could be re-isolated from microbial cultures were lower in carbon (<12%) and nitrogen (<2.3%). Spectroscopic analysis (UV, Vis, FTIR) indicated losses, especially in aliphatic structural units, and a relative enhancement in aromatic structures. It was postulated that for their high degree of degradability, HA indigenous to that anthropogenic environment would not play an important role in the long-term stabilization of landfill refuse.

Keywords: Municipal refuse; Sewage sludge; Landfilling; Humic acid-like substances; Microbial utilization

1. Introduction

Although the recycling of municipal refuse and sewage sludge should be prefered in a world with limited natural resources, their disposal in landfills remains widespread. In some countries, such as Finland, about one-third of sewage sludge produced annually is landfilled (Miikki and Hänninen, 1997). Municipal refuse is often mixed with a processed sewage sludge prior to landfilling. Often, landfills can create difficult environmental problems because of leakage water, odors and other nuisances, until biological and chemical stabilization occurs. Filip and Küster (1979) reported that the stabilization process in a sanitary landfill involves formation of humic-like substances (HAs) in the decomposing refuse. A considerable increase in the content of a HA fraction was found in the municipal refuse mixed with sewage sludge and disposed of in a landfill for 12 months (Filip, 1979). Using electron paramagnetic resonance spectroscopy, Filip et al. (1985) demonstrated the presence of Cu^{2+} and Fe^{3+} in different humic matter-related complexes from a landfill refuse. They concluded that the HA fraction originating from refuse could be of importance in retaining metal ions in the landfill. This, however, can apply only if that material is also stable enough under conditions of a highly microbially inhabited environment. In our recent review we cited numerous references about the microbial degradation of humic substances (Filip et al., 1998). The aim of our investigations was to establish whether municipal refuse mixed with sewage sludge and disposed of in a landfill would consist of HA which are more resistant against microbial degradation. For this reason we performed laboratory experiments, the results of which are reported in this paper.

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2. Materials and methods

2.1. Humic-like substances

HAs were extracted either from 'fresh' refuse collected in a domestic sector of the city of Braunschweig (Germany), or from the same refuse mixed 2:1 (w/w) with sewage sludge and disposed of for 2 or 12 months in a model landfill. The aerobically stabilized sludge also originated from a non-industrial community. In 2-m layers, the mixture of refuse and sludge was compacted in a 40-m^3 container and each layer was covered with about 10 cm soil. The construction details of the thermally insulated and bottom-drained model landfill was described by Spillmann and Collins (1986). Air-dried and finely milled sample material was extracted for 24 h under N_2 using a 1:1 mixture of 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH. The extraction was repeated up to three times until the extract was only pale yellow. A HA fraction was then precipitated with HCl (conc.) at pH 1.5, redissolved in 0.02 M NaOH, and purified by dialysis against distilled water, 0.3 M HF and water again. The yield was estimated gravimetrically. Dry HA preparations were finely ground in an agate mortar, and in portions of 100 mg added to 100 ml nutrient solution in 250-ml Erlenmeyer flasks which were closed with cotton plugs.

2.2. Nutrient solution

A Czapek-Dox nutrient broth containing 30 g D-glucose (water free), 3 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \times 7\ H_2O$, and 0.01 g $FeSO_4 \times 7\ H_2O$ in 1 l distilled water was prepared (pH 7). The broth was also prepared without either glucose or $NaNO_3$ to receive carbon- or nitrogen-deficient solution, respectively. Before HA was added, the broth was sterilized by autoclaving at 121°C for 20 min.

2.3. Inoculum

Since the individual layers of the landfilled refuse were covered with soil, a soil sample from a top layer (0–10 cm) of a sandy brown earth was used as a source of microorganisms. The sample was suspended by vigorous shaking (150 rev./min) in sterile distilled water. Decimal dilutions up to 10^{-7} were prepared. The microbiological composition of the inoculum was tested by pouring in triplicate Czapek-Dox agar plates and examining the 10^{-2} to 10^{-7} dilutions. After 5 days at 25°C the average microbial counts were estimated in colony-forming units (CFU) per gram of soil (dry wt.) as 28.3×10^5 for bacteria, and 20.7×10^4 for microscopic fungi. Experimental samples containing HA and control flasks were inoculated using 1 ml of 10^{-3} diluted soil suspension. Triplicate samples were placed on a rotary shaker (100 rev./min) for 21 days in the dark at room temperature. The rotation was interrupted several times to avoid an excess of aeration. Since the triplicate samples did not differ from each other visually they were combined for analyses after the incubation was terminated. Table 1 shows the list of experimental variants.

2.4. Harvest of microbial biomass and re-isolation of HA

Cultures were centrifuged for 10 min at 14,344 ***g*** (15°C). The biomass pellets were washed several times with distilled water (pH 6.5) and centrifuged again. Thereafter the biomass which was only pale in color, and thus with no HA adsorbed, was dried in a Christ model Alpha I/12 freeze-drier. The yield was estimated gravimetrically. The supernatants were collected, unified with the individual washings, and filtered using a glass-fiber filter Schleicher & Schüll, No. 8 (pore-size ca. 1 μm). After that they were acidified with HCl to pH 1.5, and HA were allowed to precipitate over night. The precipitate was separated by centrifugation, dialyzed for 2 days against several changes of distilled water, freeze dried and weighed before analyses. The remaining solution was filtered before analyses using the glass fiber filter.

2.5. Analytical procedures

The pH was measured potentiometrically. Analyses for carbon, nitrogen and hydrogen were performed in a Carlo Erba elementar analyzer, model EA 1108. Oxygen was determined by difference. The total organic carbon in liquid samples was estimated using an Astro 1850 TOC-TC analyzer. Visible and UV spectra were collected using a Carlo Erba Spectrocomp 601 spectrophotometer. For this purpose, 200-ppm solutions in 0.05 M $NaHCO_3$ were used (Chen et al., 1977). Fourier transform infra-red spectra (FTIR) were obtained from 2% KBr discs which were pressed at 250 atm and examined in a Bruker IFS 48 FTIR spectrophotometer.

Table 1
List of experimental variants

Sample	Nutrient broth (Czapek-Dox)		
	Full strength	Less glucose	Less $NaNO_3$
Control (no HA)	+		
HA fresh[a]	+	+	+
HA 2 month[b]	+	+	+
HA 12 month[c]	+	+	+

[a] Humic acid-like substances (HA) from fresh municipal refuse.
[b] HA from a mixture of municipal refuse and sewage sludge disposed of for 2 months in a landfill.
[c] HA from a mixture of municipal refuse and sewage sludge disposed of for 12 months in a landfill.

Samples were heated at 500°C over night to determine ash contents. Depending on the amount of material available, the analyses were performed at maximum in triplicate and since the individual deviations were less than 25%, mean values were recorded. All spectroscopical data are based on single measurements.

3. Results

After 3 or 4 days of incubation the growth of microorganisms became visible as turbidity in the inoculated control flasks. The same visual features could not easily be observed in the flasks containing dark color HA, although some of these cultures yielded significantly higher amounts of microbial biomass (Table 2). This was true especially for experimental variants containing a full-strength broth with HA serving as an additional source of nutrients. Related to control with no HA added, the enhancement was 195.2% for experimental variants supplemented with HA fresh, 175.6% for those supplemented with HA 2 month and 152.6% for those containing HA 12 month. The addition of the HA preparation from fresh refuse also strongly supported microbial growth in the nitrogen-deficient nutrient broth. Here 328 mg biomass was yielded in 100 ml, i.e. 176.4% of the control yield. In all other nutrient-deficient experimental variants the microbial growth was strongly supressed, despite of the HA supplements. This applied especially for cultures containing nutrient broth without glucose, which yielded only between 31.3 and 36.6% of microbial biomass in comparison with the full-strength broth without HA added. On the other hand, the relative conversion of carbon into biomass was most effective in carbon-deficient but HA supplemented cultures (from 41 to 45%), while a maximum of 13.7% C was converted into biomass in cultures luxuriously furnished with C from both the added glucose and HA.

The quantitative utilization of the HA preparations is documented in Table 3. When added as a supplemental nutrient source, 32.3% of HA fresh was utilized, and the percentage increased up to 50% for HA 12 month from refuse mixed with sewage sludge. The rate of utilization rose over 80% in microbial cultures containing HA preparations as sole sources of carbon. The percentage of the HA utilization was even higher (from 92 to 98%) if the HA preparations were used as the sole nitrogen sources for microorganisms. Thus, both the carbon and nitrogen of the HA preparations appeared easily utilizable by soil microorganisms.

As far as the amounts of HA preparations remaining in the individual cultures allowed, we performed analyses to elucidate some possible elemental and structural alterations of the HA. The light absorption in the visible wave range (400–800 nm) for the HA obtained from municipal refuse with sewage sludge added and aged for 12 months is shown in Fig. 1. The individual extinction curves appear to be featureless. Their slopes, however, show distinct differences. In comparison with the HA fresh (Fig. 1E), an enhancement for the preparations re-isolated from the microbial cultures obviously occurred (Fig. 1A–D). Also, in the UV range of light, featureless extinction curves were obtained (not shown in the figure). Different changes in the elemental composition of HA exposed to the activities of soil microorganisms could be also established. As shown in Table 4 a strong depletion has been found in carbon: up to 8.8% for HA fresh, 6.7% for HA 2 month, and 12.1% for HA 12 month. The nitrogen content was differently altered. It

Table 2
Carbon content in microbial cultures, yield of biomass and carbon in biomass as influenced by humic acid-like substances (HA)

Culture	Carbon in cultures (mg/100 ml)	Yield of biomass		Carbon in biomass		
		mg/100 ml	% of control	%	Total (mg/100 ml)	Conversion (%)
Control (full broth) (FB)	1200.0	185.9	100	52.7	98.0	8.2
FB + HA fresh[a]	1263.2	362.9	195.2	47.7	173.1	13.7
FB-C + HA fresh	63.2	60.6	32.6	47.0	28.5	45.1
FB-N + HA fresh	1263.2	328.0	176.4	55.8	183.0	14.5
FB + HA 2 month[b]	1259.1	326.4	175.6	48.3	157.6	12.5
FB-C + HA 2 month	59.1	58.2	31.3	44.3	25.8	40.8
FB-N + HA 2 month	1259.1	128.8	69.3	55.0	70.8	5.6
FB + HA 12 month[c]	1259.2	283.7	152.6	49.3	139.9	11.0
FB-C + HA 12 month	59.2	68.0	36.6	38.8	26.4	44.6
FB-N + HA 12 month	1259.2	128.8	69.3	49.8	64.1	5.1

[a] Humic acid-like substances (HA) from fresh municipal refuse.
[b] HA from a mixture of municipal refuse and sewage sludge dispo-sed of for 2 months in a landfill.
[c] HA from a mixture of municipal refuse and sewage sludge disposed of for 12 months in a landfill.

rose in HA prepartions which served as nutrient supplements to the full-strength broth, and on the other hand, in HA 12 month serving as a sole source of nitrogen, the nitrogen content was diminished by 2.3% in comparison to the original sample.The content of hydrogen was generally diminished and that of oxygen strongly enhanced, e.g. up to 17.5% in HA 12 month re-isolated from the culture deficient in nitrogen.

Table 3
Recovery of humic acid-like substances (HA) from cultures of a soil microbial community and percentage of their microbial utilization after 21 days incubation

Sample	HA added (mg/100 ml, dry wt.)	HA recovered (mg/100 ml, dry wt.)	HA utilized (%)
Control (full broth) (FB)	None	None	None
FB + HA fresh[a]	100	67.7	32.3
FB-C + HA fresh	100	12.7	87.3
FB-N + HA fresh	100	6.1	93.9
FB + HA 2 month[b]	100	52.7	47.3
FB-C + HA 2 month	100	16.1	83.9
FB-N + HA 2 month	100	8.2	91.8
FB + HA 12 month[c]	100	49.9	50.1
FB-C + HA 12 month	100	13.2	86.8
FB-N + HA 12 month	100	2.4	97.6

[a] Humic acid-like substances (HA) from fresh municipal refuse.
[b] HA from a mixture of municipal refuse and sewage sludge disposed of for 2 months in a landfill.
[c] HA from a mixture of municipal refuse and sewage sludge disposed of for 12 months in a landfill.

A more detailed insight into the structural alteration of the HA preparations could be obtained from the FTIR spectra. Table 5 shows an overview of the individual IR absorptions and gives their proposed assignments. In Fig. 2 the FTIR spectra of HA preparations are presented. Obviously the spectra of HA from refuse alone or from that mixed with sewage sludge demonstrate a high proportion of aliphatic structures as indicated by the C–H stretching vibrations at 2920–2850 cm^{-1}. In the latter preparations, the C=O stretching of aldehydes and ketones at 1711 cm^{-1} was almost completely overlapped by amide I stretch at 1654 cm^{-1}. At 1033 cm^{-1} C–O stretching in carbohydrates dominated. These typical features remained non-influenced by an aging of refuse for 2 or 12 months in the landfill (Fig. 2B, D). The microbial utilization of the HA in cultures deficient in nitrogen, however, resulted in a strong decrease in the absorbance intensity of the methyl and methylene groups (2920–2850, 1457 cm^{-1}) and that of amide I and II bands at 1653 and 1540 cm^{-1} (Fig. 2C). In microbial cultures deficient in carbon, the HA

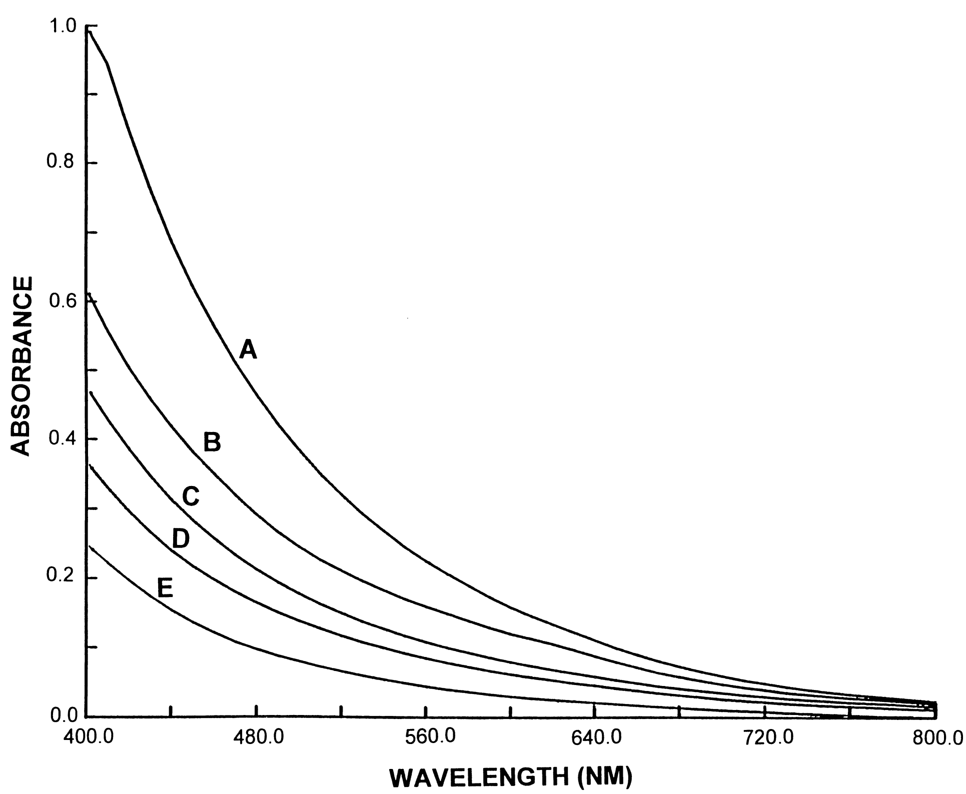

Fig. 1. Visible spectra of humic acid-like substances (HA) from a mixture of municipal refuse and sewage sludge: (A) HA 12 month re-isolated from a microbial culture deficient in carbon; (B) HA 12 month re-isolated from a microbial culture deficient in nitrogen; (C) HA 12 month re-isolated from a full-strength microbial culture; (D) HA 12 month (extracted from refuse disposed of for 12 months in a landfill); (E) HA fresh (extracted from a fresh refuse).

Table 4
Elemental composition and some atomic ratios of humic acid-like substances (HA) from a mixture of municipal refuse and sewage sludge, and residual preparations from 21-day microbial cultures[a]

Sample	C	N	H	O	C:N	C:H	H:C	O:C
HA fresh								
Original sample	62.2	5.3	9.5	23.0	13.6	0.5	1.8	0.3
From FB[b]	53.4	6.9	8.3	31.4	9.1	0.5	1.9	0.4
From FB-C	54.0	5.5	6.2	34.3	11.5	0.7	1.4	0.5
From FB-N	nd	nd	nd	nd	–	–	–	–
HA 2 month								
Original sample	59.1	6.3	8.6	26.0	10.9	0.6	1.7	0.3
From FB	52.4	7.1	8.2	32.3	8.6	0.5	1.9	0.5
From FB-C	53.1	5.8	7.0	34.1	10.8	0.6	1.6	0.5
From FB-N	52.8	nd	nd	nd	–	–	–	–
HA 12 month								
Original sample	59.2	6.3	8.4	26.1	11.0	0.6	1.7	0.3
From FB	52.8	7.5	7.9	31.8	8.3	0.6	1.8	0.4
From FB-C	51.3	4.8	5.6	38.3	12.6	0.8	1.3	0.6
From FB-N	47.1	4.0	5.3	43.6	13.5	0.7	1.4	0.7

[a] Elements in ash-free %.
[b] FB, full strength broth; nd, not determined.

Table 5
Observed infra-red absorption bands in humic acid-like substances (HA) from a mixture of municipal refuse and sewage sludge, and in residual preparations extracted from microbial cultures

Band (cm^{-1})	Proposed assignment[a]
3696–3620	OH stretch
3416–3318	OH stretch, H-bonded OH groups and partly NH groups
2928–2919	C–H stretching vibrations in CH_3 groups of aliphatics
2852	C–H stretching vibrations in CH_2 groups of aliphatics
2362–2338	Si–H groups
1734–1711	C=O stretching in COOH, aldehydes and ketones
1654	Amide I (hydrogen-bonded–C=O stretch)
1560–1540	Amide II (NH_2 or NH bending)
1457	C–H deformation of CH_2 or CH_3 groups
1263–1230	C–O stretching and OH deformation of COOH, C–N of amide III
1127–1102	C–O stretching of carbohydrates, Si–O of silicate impurities
1033	C–O stretching (polysaccharides, aromatics)
537–472	C–O–C bonding and deformation (aromatics) Si–O, Me–OH groups

[a] After Bellamy (1975), Mac Carthy and Rice (1985), Orlov et al. (1972), Silverstein and Bassler (1964).

apparently lost the carbohydrate structural units, for the respective C–O stretch (in polysaccharides) was eliminated from the FTIR spectrum (Fig. 1E). Also, due to microbial activities, the IR absorbances at 537–472 cm^{-1} became rather undetectable (Fig. 1C, E).

4. Discussion

When composting or landfilling, municipal refuse is often mixed with sewage sludge, and this may result in an increased content of HA. Miikki and Hänninen (1997) reported such an increase from 80 to 143 mg g^{-1} within a 4-year period. This is because sewage sludge itself can serve as a source of HA. In different kinds of sewage sludge their amount varied between 2 and 5% and the ratio of humic to fulvic acids oscillated between 0.3 and 3.0 (Riffaldi et al., 1982; Aiwa and Tabatabai, 1994). Iakimenko and Velichenko (1997) found more than 7% of sewage sludge carbon in humic substances, but again, a fulvic acid fraction predominated. According to Flis-Bujak et al. (1997) fulvic acids predominated also in humic substances extracted from municipal refuse composted with sewage sludge (C_{HA}:C_{FA} = 0.69). Different carbonaceous components of sewage sludge could be secondarily incorporated into humic substances in the course of landfilling or composting. In our experiments, reported in a comprehensive paper recently, the yield of a humic acid fraction (in mg/100 g) was 275 at month 0, 488 after 2 months, 988 after 6 months, but only 251 after 12 months and 220 after 20 months in a 2:1 mixture of municipal refuse and sewage sludge disposed of in a landfill (Filip, 1997). These numbers indicate that both humification and mineralization processes apparently take place in a landfill.

The results presented in this study confirm that HA from a landfilled mixture of municipal refuse and sewage sludge can be readily utilized by a mixed microbial community under aerobic conditions. About 50% was utilized in a full-strength nutrient solution which may correspond with conditions existing in a young landfill copiously supplied with easily metabolizable nutrient sources. After the nutrients are spent by microorganisms, i.e. under rather carbon and/or nitrogen-deficient conditions, the utilization of secondary nutrient sources

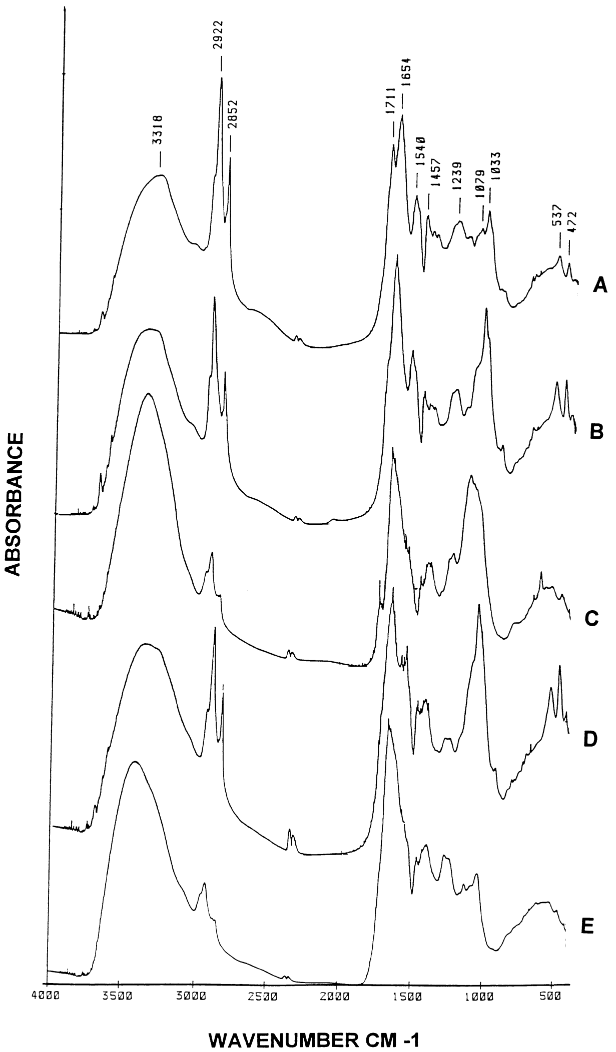

Fig. 2. Fourier transform infra-red spectra (FTIR) spectra of humic acid-like substances (HA) from a mixture of municipal refuse and sewage sludge: (A) HA fresh (see subheadings of Fig. 1); (B) HA 2 month (extracted from refuse disposed of for 2 months in a landfill); (C) HA 2 month re-isolated from a microbial culture deficient in nitrogen; (D) HA 12 month (see subheadings of Fig. 1); (E) HA 12 month re-isolated from a microbial culture deficient in carbon.

may start. In our experimental variants deficient in carbon more than 80% of HA were utilized as a sole source of carbon. The utilization rate was enhanced if carbon was available, and the HA preparations served as sole sources of nitrogen (up to 98% of HA utilized). This high degree of utilization evidenced indirectly rather simple and easily degradable structures in the HA preparations. Newman et al. (1987) demonstrated the presence of carbon in proteins and peptides, carboxylic acids, ketones, carbohydrates, lipids and also in aromatic rings in the HA preparations similar to those used in our experiments. The relative participation of aromatic structures rose from months 0 to 20 of refuse disposal. Correspondingly, the FTIR spectra collected from the HA preparations re-isolated from microbial cultures demonstrate a decrease in carbonaceous and nitrogenous aliphatic structural units as a result of microbial degradation (Fig. 2), and an increase of optical density (Fig. 1). The latter issue may stand for more strongly polymerized HA (Kononova, 1966; Chen et al., 1977). Slightly decreasing values of H:C ratios and simultaneously increasing values of O:C ratios also brings some evidence on the improving quality, i.e. higher aromaticity, of the HA preparations re-isolated in small amounts from microbial cultures (Steeling, 1985; Thurman, 1985).

Although we were able to demonstrate earlier that HA isolated from municipal refuse are capable of complexing appreciable amounts of metallic ions (Filip et al., 1985) this capacity should not be generally attributed to a desirable retention of metals in such environments according to results presented here. This is because HA appear highly susceptible to microbial degradation and a release of the complexed metals can be a consequence.

5. Conclusions

Municipal refuse mixed with sewage sludge and disposed of in a landfill contains HA which cannot sufficiently resist microbial degradation. We were able to demonstrate this in aerobic microbial cultures supplemented with nutrients and also in such deficient in C or N. For this reason refuse-related HA apparently may not earn much importance as agents supporting the stabilization process in the landfilled refuse.

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