SLUDGE FROM THE WASTE WATER OF THE OLIVE PROCESSING INDUSTRY: A POTENTIAL SOIL FERTILIZER?

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SUMMARY

The humic acid fraction of sludge obtained from waste water of olive mills after disposal in isolated lagoons was chemically characterized by amino acid and sugar analyses and by flash pyrolysis-GC-MS. The sludge humic acid fraction consists of polysaccharides, proteins, lignins and relatively high amounts of C₁₆ and C₁₈ fatty acids. Although the composition of this material is different from soil humic acids it is concluded that the sludge has good soil fertilizer properties.

INTRODUCTION

The large amount of waste water (1-2 million cubic meters per season) generated during the production of olive oil is a major environmental problem in Spain. This waste water is mainly composed of particulate matter from olive pulp, sugars, pectins, tannins, mucilages, organic acids and polyphenols (ref. 1). Because of the extremely high BOD these waste water pollute the surface water seriously when spilled. Over the last five years, ponds and lagoons have been built for disposal of the waste water from which the water evaporates during the dry spring-summer period. The residual sludges are recovered in fall, before a new olive processing season starts. Some farmer used to irrigate olive tree orchards with this type of waste water to fertilize the soil. Therefore, it is of interest to study in detail the chemical composition of the sludge to evaluate its potential value as soil fertilizer. This preliminary study was focussed on the chemical characterization of the base-extractable and acid-precipitable humic acid fraction, because it is well-known that, at least in soils, humic acids are the most important substances related to fertility properties.

METHODS

The sludge sample was obtained from a lagoon located in Villanueva del Trabuco (province of Malaga, Spain). The humic acid fraction was prepared in the

usual way by solution in 0.5 N NaOH and subsequent precipitation with HCl (ref. 2). To investigate the possible changes in the humic acid fraction during the period of disposal as a consequence of microbial fermentations and transformations, a "fresh" waste water sample was also investigated. This sample was obtained from an experimental olive mill (Instituto de la Grasa, C.S.I.C., Sevilla). The waste water sample was taken immediately after formation and stored at -15°C until analysis. The humic acid fraction was obtained after centrifugation at 15.000 g, addition of 0.5 N NaOH to the solution and precipitation with HCl. The results of the sludge and the waste water humic acids can be compared since the olive oil production processes at both locations are very similar.

Elemental composition, sugar and amino acid analyses and pyrolysis technique have been described in detail elsewhere (ref. 2, 3).

RESULTS AND DISCUSSION

The sludge contained 40 % of humic acid and 28 % of acid soluble materials on a dry weight basis. Table 1 shows the elemental composition of the sludge (S-HA) and the waste water (W-HA) humic acids, before and after 6 N HCl hydrolysis.

TABLE 1

Elemental composition of sludge and waste water humic acid fractions (on a moisture- and ash-free basis)

%	W-HA	W-HA-H ¹	S-HA	S-HA-H ¹
C	62.5	66.4	61.3	61.2
H	8.5	7.9	7.0	7.0
N	3.4	1.2	2.6	1.9
S	0.3	0.3	0.3	n.d.
O	25.3	24.2	28.8	29.9
ash	10.8	0	8.0	0
weight loss after hydrolysis	46.9	-	27.8	-
N loss after hydrolysis	64.7	-	27.0	-
atomic H/C ratio	1.6	1.4	1.4	1.4

¹Acid hydrolysed samples

Hydrolysis was carried out to release polysaccharides, proteins, phenols and metals. In this way it was thought that the so-called core of the humic acid would remain, making a specific core investigation possible (ref. 4).

The elemental compositions of the W-HA and S-HA fractions were slightly different from those of soil humic acids (C and H are higher and O is lower) (ref. 5). As indicated by the weight loss and N loss the S-HA sample was much more resistant to hydrolysis than the W-HA sample, indicating that easily hydro-

lysolable substances are probably metabolized for the greater part by microbial attack during the disposal in the lagoon. The lower N-content of the S-HA sample compared to that of the W-HA sample also indicates biodegradation processes.

TABLE 2

Sugar distribution in 2 N H_2SO_4 hydrolysates of sludge and waste water humic acid fractions (mg/100 mg of sample)

sugar	W-HA	S-HA
glyceraldehyde	-	6.8
treose	-	0.3
rhamnose	1.6	1.3
ribose	0.2	0.3
arabinose	0.4	0.3
xylose	0.2	0.1
mannose	0.4	0.4
galactose	0.1	trace
glucose	2.8	2.0

The composition of the polysaccharides was investigated after hydrolysis with 2 N H_2SO_4 . Comparison of the S-HA and W-HA monosaccharides indicates that the pentose and hexose contents were slightly higher in W-HA. In the S-HA hydrolysate glyceraldehyde and treose were also present. The relatively high amount of rhamnose was not surprising in view of the abundant occurrence of this sugar as glucoside in olive anthocyanins (ref. 1).

TABLE 3

Amino acids distribution in hydrolysates of sludge and waste water humic acid fractions (mg/100 mg sample)

amino acid	W-HA	S-HA
aspartic acid	1.43	0.80
threonine	0.65	0.26
serine	0.76	0.39
glutamic acid	1.81	0.98
proline	0.97	0.77
glycine	0.70	0.43
alanine	0.68	0.39
valine	0.79	0.36
methionine	0.10	0.03
isoleucine	0.46	0.24
leucine	0.96	0.46
tyrosine	0.56	0.29
phenylalanine	0.72	0.27
glucosamine	0.15	0.08
histidine	0.32	0.13
lysine	0.12	0.04
arginine	0.74	0.27
NH_4	0.26	0.13

The amino acid distribution analysed after 6 N HCl hydrolysis was similar to that reported for pulp olives as far as the most abundant components are concerned (ref. 2). The W-HA fraction yielded about twice as much amino acids compared to S-HA fraction. The compositions of W-HA and S-HA were somewhat different: proline was relatively more abundant in W-HA and phenylalanine and arginine were relatively increased in S-HA.

Apart from the amino acids, three phenols were released after 6 N HCl hydrolysis. They were identified by HPLC and TLC as protocatechuic acid, caffeic acid and *p*-coumaric acid, protocatechuic acid being the most abundant. These compounds have previously been found as free phenols in the waste water (ref.1).

Curie point evaporation/pyrolysis-gas chromatography-mass spectrometric analysis of the humic acid fractions was applied to characterize other components than proteins and sugars. A complex mixture of evaporated components and pyrolysis products from high molecular weight substances resulted. The identifications of the major products are listed in Table 4. The peak numbers in Figure 1 correspond to the numbers indicated in Table 4.

TABLE 4

Pyrolysis/evaporation products of sludge and waste water humic acid fractions

1 Carbon monoxide	20 Pyrrol
Carbon dioxide	21 Toluene
Methane	22 Dihydropyran
Ethene	23 Oct-1-ene
Ethane	Ethylpyrrol
Propene	24 Maleic anhydride
Propane	25 Octane
But-1-ene	3-Furaldehyde
Butane	26 2-Furaldehyde
2 Acetone	Benzyl alcohol
3 Pent-1-ene	27 Ethylbenzene
4 Ethanethiol	28 Methylpyridine
5 2-Methylpropanal	3-Methylcyclopent-2-en-1-one
6 Butan-2,3-dione	29 m- and/or p-Xylene
7 2-Methylpentane	30 Styrene
8 2-Methylfuran	31 o-Xylene
9 3-Methylfuran	32 C ₃ -alkylfuran
10 Crotonaldehyde	33 α-Angelicalactone
11 Hexadiene	34 Non-1-ene
Hexatriene	35 Nonane
12 Hexatriene	36 Ethylpyridine
3-Methylbutanal	37 Benzaldehyde
13 2-Methylbutanal	38 Dimethylpyridine
Benzene	39 Vinylpyridine
Acetic acid	40 4-hydroxy-5,6-dihydro-2H-pyran-2-one
14 Hept-1-ene	41 Dimethylpyridine
15 Heptane	42 Phenol
16 2,5-Dimethylfuran	43 Methylethylpyridine
17 2,4-Dimethylfuran	44 2-Hydroxy-3-methyl-2-cyclopenten-1-one
18 N-Methylpyrrol	45 4-Hydroxy-6-methyl-5,6-dihydro-2H-pyran-2-one
19 Pyridine	

46 Indene	76 Levomannosane
47 Limonene	77 Methyl-2,6-dimethoxyphenol
48 Methylvinylpyridine	78 Levogluconase
49 p-Cresol	79 trans-Isoeugenol
50 Toluolaldehyde	80 Vanillic acid
51 C ₄ -alkylbenzene	81 Ethylquinoline
52 3-Hydroxy-6-methyl-3,4-dihydro-2H-pyran-2-one	82 Pentadec-1-ene
53 Methylvinylpyridine	83 Pentadecane
54 Guaiacol	84 Vinyl-2,6-dimethoxyphenol
55 Methyllethylpyridine	85 C _{14:0} fatty acid
56 o-Cresol	86 Dialkyl phthalate
57 Benzyl cyanide	87 trans-Propenyl-2,6-dimethoxyphenol
58 C ₃ -alkylhydroxypyridine	88 Heptadecadiene
59 Undec-1-ene	89 Heptadec-2-ene
60 Undecane	90 Heptadec-1-ene
61 Ethylphenol	91 Heptadecane
62 Xylenol	92 Octadec-1-ene
63 Methylguaiacol	93 C _{16:1} fatty acid methyl ester
64 1,4:3,6-Dianhydro- α -D-glucopyranose	94 Dialkyl phthalate
65 Catechol	95 C _{16:0} fatty acid methyl ester
66 Vinylphenol	96 Dialkyl phthalate
67 Dodec-1-ene	97 C _{16:0} fatty acid
68 C ₆ -Alkylbenzene	98 C _{16:0} fatty acid ethyl ester
69 Ethylguaiacol	99 Eicosane
70 Indole	100 C _{18:2} fatty acid methyl ester
71 Vinylguaiacol	101 C _{18:1} fatty acid methyl ester
72 Methylcatechol	102 C _{18:0} fatty acid methyl ester
73 Levogalactosane	103 C _{18:2} fatty acid ethyl ester
74 2,6-Dimethoxyphenol	104 C _{18:1} fatty acid
75 Eugenol	105 C _{18:1} fatty acid ethyl ester
76 Methylindole	106 C _{18:1} fatty acid propyl ester
77 Methylquinoline	107 C _{18:1} fatty acid butyl ester
78 Ethylcatechol	108 Dialkyl phthalate

The chromatograms of both humic acids were relatively similar, although distinct qualitative and quantitative differences were observed. The major compounds were free and esterified n -C₁₆ and n -C₁₈ fatty acids. It can be assumed that these acids are present as such in the samples and just evaporate since they are the major lipids present in olive oil. These fatty acids and their esters probably adsorb to particulate matter due to incomplete removal of the oil during the production process. The occurrence of fatty acid methyl esters is in agreement with the findings of such esters in the oil extracted from microbial degraded olives (ref. 6) and indicates a biological activity of microorganisms during the olive storage and/or disposal in the lagoon. The presence of ethyl-, propyl-, and butyl-C₁₈ esters is noteworthy since these esters have not yet been reported in microbially altered olives.

Interestingly, C_{18:2} fatty acids were only present in the W-HA sample, which means that these acids are removed after disposal in the lagoon, either biologically or photochemically, since an oily layer was present at the water-air interface.

Figure 1. TIC traces of waste water (W-HA) and sludge (S-HA) humic acid fractions. Peak numbers refer to the compounds listed in Table 4. Compounds A_1 and A_2 are unidentified nitrogen-containing products. Compounds B_1 - B_3 are probably cyclic sesquiterpenoids.

All three types of lignin unit pyrolysis products were present and they were relatively more abundant in the S-HA sample. This suggests that lignin, which is reported to be resistant to biodegradation in anaerobic environments (ref. 7), accumulate in the lagoon. Although typical pyrolysis products from the *p*-coumaryl lignin unit such as phenol and vinylphenol were present, an origin of these phenolic compounds from other substances present in the waste water can not be precluded. Catechol might originate from protocatechuic acid, which was present in the hydrolysates after HCl treatment. It is known that the carboxyl group in these type of molecules is easily removed upon pyrolysis (ref. 8).

Sugar pyrolysis products were somewhat more abundant in the pyrolysate of the W-HA sample. Relatively high amounts of pyridine and alkylpyridines were encountered in both the S-HA and W-HA samples. These compounds are only present in minor amounts in pyrolysates of soil humic acids. Although one might speculate about a protein origin of the alkylpyridine-type pyrolysis compounds they have not yet been reported in pyrolysis studies of proteins (ref. 9).

By comparing S-HA and W-HA with soil humic acids the differences are evident. Although both types of materials contain polysaccharides, proteins, lignins and lipids, their relative amounts differ. Thus, the C_{16} and C_{18} fatty acid were very high in S-HA and W-HA and the abundantly present alkylpyridines are virtually absent in pyrolysates of soil humic acids (ref. 3).

In spite of these differences the sludge can be considered as a good fertilizer on a chemical basis. Proteins and sugars are still present in the sludge and the C_{16} and C_{18} fatty acids are high quality substrates for micro-organisms and plants. On the other hand, the high concentrations of the fatty acids might cause some problems since they give the sludge a hydrophobic nature.

Two additional aspects are important in the utilization of the sludge. From an environmental point of view, the disposal of waste water in isolated lagoons protects the surface waters against pollution. The second aspect is of economical nature. The evaporation of water results in a more concentrated product which has not lost much of its nutrient value and it can be distributed much cheaper. It is further demonstrated that the olive processing industry is losing relatively high amounts of valuable oil. A more efficient removal of C_{16} and C_{18} fatty acids before waste water disposal would improve both the economy of the olive oil industry and the physico-chemical properties of the soil to which the sludge has been added.

Long-term experiments are required to further test the sludge as a soil fertilizer.

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