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Chemical and spectroscopic analysis of olive mill waste water during a biological treatment

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Abstract

The treatment of olive mill waste water was studied on the laboratory scale. Physico-chemical analyses showed the final products had a mean pH of 5.4 without neutralisation and 5.7 when lime was added to the process. Raising the pH by adding lime had a positive outcome on the degradation of phenols, whose levels were reduced by over 76%. The lime also changed the structure of the organic matter, as seen in the infra-red spectra. Combining the FT-IR and ¹³C NMR data showed that with addition of lime, the density of aliphatic groups decreased to the benefit of aromatic groups, indicating that polymerisation of the organic matter occurred during the bioprocess. Under our experimental conditions, the biotransformation of olive mill waste water appears to favour the stabilisation of the organic matter through mechanisms analogous to those that lead to the formation of humus in the soil.

Keywords: Olive mill waste water; Aerobic bioprocess; Elemental analysis; FT-IR spectroscopy; ¹³C NMR spectroscopy

1. Introduction

Worldwide production of olive oil reaches about 2 million tonnes/year with almost 90% being concentrated in the countries of the Mediterranean basin (CAR/PP, 2000). The extraction of oil from the olives generates large quantities of liquid by-products (Sampedro et al., 2007). This effluent presents a high biological and chemical oxygen demand (BOD: 100 g/L and COD: 200 g/L, respectively) (Della Greca et al., 2004; Dias et al., 2004; Tardioli et al., 1997) and contains large quantities of aromatic compounds, which are responsible for the effluent's phytotoxic and anti-bacterial effects (D'Annibale et al., 2006; El Hajjouji et al., 2007; Gonzalez et al., 1990; Procida and Ceccon, 2006). Nevertheless, the effluent also contains useful levels of fertilisers such as nitrogen and potassium and can act as a

source of organic matter (Cereti et al., 2004; Zenjari et al., 1999, 2006), which could open the way for its use in agriculture.

Organic material is an essential component of soil, the very presence of organic matter is what defines a soil. It plays an essential role in various soil properties: holding water, buffering the pH, binding ions with varying degrees of reversibility, complexing ions, structuring the soil, and it is the combination of all these properties that finally determines the fertility of the soil. On a global scale, organic matter actually represents a phenomenally vast carbon reservoir (Kogel Knabner, 2005). Moreover, input of organic matter in the form of composted residues, to soil poor in organic matter can help stabilise the soil's structure and limit the risks of erosion. Compost also enhances the fertility of soil owing to the presence of humic substances (Ait Baddi et al., 2003; Senesi et al., 1996) and adding humus to soils stimulates plant growth and improves yields. Chen et al. (1994) reported that the simultaneous addition of

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humic substances and mineral elements leads to much greater increases in yield than adding the mineral elements alone. The study of the stability of the organic matter contained in waste is an important step in choosing the most appropriate way to deal with the waste. This can be approached from several directions: following the decrease in the level of organic matter and of organic carbon, the variation of the C/N ratio, the proportions of ash, and the levels of primary metabolites such as nitrate, ammonium, and volatile fatty acids (Smidt, 2001), the microbial activity (Scaglia et al., 2000) and the absence of phytotoxicity (Epstein, 1997). The degree of humification is also used as an index of stability and to determine the agricultural value of organic residues intended for use in the amendment of arable land (Ouatmane, 2000; Senesi et al., 1996). In addition, the degree of humification provides an index of the quality of the organic matter, which depends both on the composting process and on the organic matrix in question (Adani et al., 1997). The aerobic treatment of olive mill waste appears to be efficient at reducing the level of phenolics present (Di Gioia et al., 2001; Fakharedine et al., 2006; Zenjari et al., 2006). This reduction can be explained by the use of the phenols as an energy source by microorganisms (Paredes et al., 2002; Hafidi et al., 2004; Zenjari et al., 1999), and their incorporation during the neoformation of humic substances (Hafidi et al., 2004, 2005; Zenjari et al., 1999). Each step in the decomposition of organic matter is characterised by the production

of metabolites: IR spectroscopy can be used to provide useful information on the functional groups in these metabolites (Smidt and Meissl, 2006). Infra-red spectroscopy has been used by various authors to determine the maturity of composts (Ouatmane et al., 2000) and to characterise the humic substances occurring in the composts (Amir et al., 2004; Ouattmane et al., 2000; Sanchez Monedero et al., 2002). ^{13}C NMR spectroscopy has also been used to determine the chemical environment of the carbons (Ait Baddi et al., 2004; Amir et al., 2004; Castaldi et al., 2005; Hafidi et al., 2005).

The present study consists in following the changes that occur in a liquid effluent in aerobic conditions after improving the supply of nitrogen available to the microorganisms and bringing the pH up to values more favourable to their growth. In these experimental conditions, the changes in the organic matter were followed by a combination of complementary techniques (elemental analysis, infra-red spectroscopy, and ^{13}C nuclear magnetic resonance) to determine the degree of stability of the organic matter in the effluent.

2. Methods

2.1. The samples and the treatments

Liquid effluent was taken from a modern 3-phase centrifugation olive oil production unit in the Marrakech area

Fig. 1. Experiment scheme.

(Morocco). It was treated by aerobic digestion (volume: 2 l) for 45 days (Fig. 1). It was submitted to different treatments (T1 and T2) with three replicates for each treatment.

For T1, ammonium nitrate (NH_4NO_3) was added to bring the C/N ratio to 50.5, the pH was not adjusted (initial value 4.85). Treatment T2 involved bringing the C/N ratio to 48.08 with NH_4NO_3 and bringing the pH to 6.18 by addition of lime (CaO). For both treatments, run at ambient temperature ($\sim 25^\circ\text{C}$), aeration was achieved by bubbling compressed air through the liquids. After homogenisation samples of the initial effluent were taken at time 0, then after 15, 30 and finally 45 days. Before studying the organic matter, samples were dialysed (Spectra Por membrane MWCO 1000 Da) to eliminate the excess salts, then freeze dried.

2.2. Methods of analysis

2.2.1. Standard chemical analyses

All chemical analyses were performed in triplicate and the values reported here are the means. The pH was measured at ambient temperature according to the method of Rodier (1971). The total organic carbon (TOC) was determined using Anne's method based on potassium dichromate, as described by Aubert (1978). Total Kjeldahl nitrogen (TKN) was determined using the standard French procedure AFNOR T90-110 (1975). Chemical oxygen demand (COD) was assayed by the method of Rodier (1984). Total phenols were extracted and purified with ethyl acetate, as reported in Macheix et al. (1990) and were assayed according to Folin-Ciocalteu (Vasquez Roncero et al., 1974).

2.2.2. Elemental analysis

The instruments used for C, H and N were a Fison Carlo Erba EA 1110, and a Carlo Erba 1106 for O. About 1 mg (0.8–1.2 mg) of the sample in a capsule made of tin (assay of C, H and N) or of silver (for assaying oxygen) was burned at 920–1000 $^\circ\text{C}$. The quantity of each element is expressed in percent mass. Each determination was performed in duplicate.

2.2.3. Fourier transform infra-red (FTIR) spectroscopy

A quantity of 1.5 mg of sample was compressed under vacuum with 250 mg of KBr. The pellets obtained were analysed with a Perkin-Elmer series 1600 FTIR spectrophotometer covering a frequency range of 4000–400 cm^{-1} .

2.2.4. ^{13}C nuclear magnetic resonance (^{13}C NMR)

^{13}C NMR spectra were obtained from ~ 150 mg of the freeze-dried olive mill waste water resuspended in 3 ml 0.5 M Na OD. The spectra were recorded on a Bruker WB-AM 300 spectrometer at 75.4 MHz with a 10 mm probe head. Chemical shifts (δ) are reported in ppm, relative to 3-(trimethylsilyl)-propane sulfonic acid sodium salt as external reference. To suppress nuclear Overhauser enhancement, a pulse program with inverse gated proton decoupling was used. Spectra were acquired with a repetition time between impulsions of 2.8 s, a sweep width of 16,700 Hz and a pulse width of 5 μs (35°). (50,000–60,000) FID files were accumulated. The FIDs were treated with a 50 Hz line-broadening function. Integration of the spectra was performed with Bruker Win NMR software.

3. Results and discussion

3.1. Physico-chemical characterisation of the olive mill waste water

The proportion of water present in the effluent decreased strongly during the two treatments (Table 1), but remained higher in T1 (53.76%) than in T2 (20.51%). As the surface area and the temperature were the same for both treatments, the difference can be attributed to higher microbial activity: aerobic microbes developed preferentially around the air outlets, which become partly blocked, restricting the flow of air and hence the degree of evaporation.

With treatment 1, the pH increased with time. This could, of course, result from the release of ammonium from protein degradation but considering the behaviour of the C/N ratio this is not very likely. It is more probable that a decrease occurs in the levels of $-\text{COO}^-$ and/or $-\text{OH}$ due to both the microbial degradation of certain compounds (Hamdi et al., 1992) and the condensation-polymerisation reactions involving these moieties.

With treatment 2, the reverse occurred. Ouabbi (1999) explained this pattern by the stronger activity of the microorganisms producing metabolites such as organic acids, which contribute to a general acidification. Among these metabolites, since the reactions occur in aerobic conditions, are carbonates and bicarbonates which, like $-\text{COOH}$, can bind the Ca^{2+} contained in the added lime.

The increase of the lower pH (T1) and the decrease of the higher pH (T2) suggests convergence to a final value of between 5.39 and 5.69, which was arrived at after 45

Table 1

Physico-chemical characteristics of the olive mill waste water at the initial (Ti) and final stages of treatments (Tf)

Stage	pH	Moisture (%)	TOC (%)	TKN (%)	C/N by weight	COD (%)	Drop in COD (%)	Drop in phenols (%)
T1i	4.85 $\pm$ 0.01	82.18 $\pm$ 0.05	50	0.99 $\pm$ 0.17	50.50	137.84	–	–
T1f	5.39 $\pm$ 0.01	38.63 $\pm$ 1.21	45	1.26 $\pm$ 0.06	35.86	107.81	21.8	50.6
T2i	6.18 $\pm$ 0.01	80.93 $\pm$ 0.20	50	1.04 $\pm$ 0.05	48.08	104.95	–	–
T2f	5.69 $\pm$ 0.03	64.33 $\pm$ 0.20	35	1.30 $\pm$ 0.01	27.03	99.87	4.8	75.8

TOC = total organic carbon, TKN = total Kjeldahl nitrogen, COD = chemical oxygen demand.

days and which corresponds to the “stabilisation” that is reached at the end of the treatments (El Hajjouji et al., 2007). The C/N ratio, which fell to 28.9% for T1 and 43.8% for T2 confirms this pattern (decrease of carbon and conservation of nitrogen), indicating that the effluent is approaching the “stabilisation” state, especially with treatment T2.

For the chemical oxygen demand (COD), comparing the initial values T1i and T2i shows a clear effect of the addition of lime on the initial material. The simple act of adding lime lowered the COD by 23.9%. This decrease was pursued during the treatment, particularly for T1 with a drop of 21.8% while the drop in T2 only reached 4.8%. The action of the lime at the initial stage degrades or modifies numerous carbon structures. Moreover, the parallel between the slight decrease in the COD during T2, the value of C/N and of the TOC suggests neoformation of compounds that are stable and thus not readily degradable (Casa et al., 2003).

Treatment 2 significantly reduced the pollutant load of the effluent in terms of phenols, with a drop that reached 75.8%. In conditions of neutral pH, phenols become transformed into phenates, lose part of their antimicrobial activity and become suitable for use as a carbon source for microbial activity (Borja et al., 1994). For treatment T1, we recorded a decrease in the C/N ratio, indicating degradation of the organic compounds. A decrease occurred in the COD and in the level of phenols: decreased by 21.8% and 50.6%, respectively. The drop in C/N, COD and phenols resulted from the degradation of organic matter by the microorganisms which use the carbon as an energy source (Borja et al., 1994; Hafidi et al., 2005). The addition of a readily bioavailable form of nitrogen, NH_4NO_3 , favoured microbial activity and hence the degradation of phenols and also of other compounds.

3.2. Elemental analysis

The elemental composition of the organic material in the effluent at various stages of treatment is presented in Table 2 (parts a and b). By far the main components were carbon, hydrogen and oxygen. At the end of the treatment it was found that the proportion of carbon had increased slightly for T1 and decreased for T2. The decrease can be explained by the greater microbial activity during T2, the organisms using the organic material available in the medium as a

source of energy (Borja et al., 1994). This resulted in a decrease in the C/N ratio, which fell from 24.06 to 21.01 for treatment 1 and 25.90 to 21.76 for treatment 2. The drop can be explained by the release of CO_2 and the humification process (Hargitai, 1994).

In contrast, the C/H ratio remained stable during both treatments. Similar results have been reported by Ait Baddi et al. (2004) for fulvic acids extracted from various organic sources. According to Ait Baddi et al. (2004) the stability of the C/H ratio is independent of the degree of maturity of the compost.

The percentage of nitrogen increased at the end of the treatments, it rose from 2.73% to 3.16% for treatment 1 and 2.39% to 2.63% for treatment 2. Piccolo et al. (1992) report that high levels of nitrogen indicate high levels of non-humified biomolecules (polypeptides). Likewise, Ait Baddi et al. (2004) attributed the high levels to the incomplete hydrolysis of the protein fraction. In the present situation, the high levels of organic nitrogen also indicate assimilation of the added nitrogen.

The O/C ratio remained practically constant, indicating that the proportion of functional groups was almost unchanged during the treatments.

Table 2b shows that treatment 2 led to a higher level of ash than treatment 1; this is due to the initial addition of lime. At the end of treatment 2, the level of ash rose from 6.47% to 13.90%, the result of the degradation of organic matter. In contrast, after treatment 1 ash decreased following the release of ammonia or the uptake of calcium by the organic structure.

The method of Fictitious Atomic-Group Separation (FAS) described by Ndira (2006) and Tardy et al. (2000, 2005) was applied to the above results. The ratios between the FAS ($\text{CH}_2\text{O}/C_{\text{total}}$, $\text{CH}_2/C_{\text{total}}$ and $C_{\text{dry}}/C_{\text{total}}$) are reported in Fig. 2.

The addition of lime at the start of treatment 2 acted directly on the initial material: it increased the level of CH_2O and decreased that of CH_2 . This cannot result from the release of small molecules because the samples were dialysed. It probably results from the opening of peripheral aromatic rings on large molecules through oxidation.

For T1, during the first 15 days, the proportion of CH_2O increased while that of CH_2 decreased. Microbial attack required about 15 days to reach almost the same result as that obtained by the immediate action of lime. After 15 days, in T1, the proportion of CH_2O decreased while that

Table 2a

Elemental composition of the dry organic matter contained in the olive mill waste water and the atomic ratios at the various stages of treatment 1 (day 0, day 15, day 30 and day 45)

	Percent weight						Atomic ratio		
	C	H	O	N	CHON	Ash	C/N	C/H	O/C
T1-D0	56.21 ± 0.28	7.71 ± 0.18	27.74 ± 0.09	2.73 ± 0.04	94.39	5.61	24.06	0.61	0.37
T1-D15	55.26 ± 0.09	7.22 ± 0.01	30.26 ± 0.19	2.79 ± 0.02	95.53	4.47	23.12	0.64	0.41
T1-D30	56.41 ± 0.06	7.44 ± 0.04	28.82 ± 0.19	3.15 ± 0.01	95.82	4.18	20.89	0.63	0.38
T1-D45	57.03 ± 0.21	7.54 ± 0.03	27.29 ± 0.19	3.16 ± 0.07	95.02	4.98	21.02	0.63	0.36

Fig. 2. Ratios between fictitious chemical groups (FAS) during aerobic treatments. (a) $\text{CH}_2\text{O}/\text{C}_{\text{total}}$ for treatments 1 (T1) and 2 (T2). (b) $\text{CH}_2/\text{C}_{\text{total}}$ for treatments 1 (T1) and 2 (T2). (c) $\text{C}_{\text{dry}}/\text{C}_{\text{total}}$ for treatments 1 (T1) and 2 (T2).

of CH_2 rose, indicating the disappearance of oxygen. This can correspond either to the elimination of oxygen groups or more likely to condensation reactions in between oxygen-containing molecules.

For T2, there were fewer relative variations but the proportion of CH_2O rose slightly after the 30th day, indicating oxidation of macromolecules during this period.

The ratio $\text{C}_{\text{dry}}/\text{C}_{\text{total}}$ rose significantly during the first 15 days, which could indicate an increase in the aromatics during this period. After 15 days, the variations were too slight to be significant.

Overall, the addition of lime acted not only on the initial state of the waste water but also on its evolution with time.

3.3. Infrared spectroscopy (FTIR) of the organic matter

Table 3 reports the infrared bands of the treated olive mill waste water, with reference to the works of Ait Baddi et al. (2004), El Hajjouji et al. (2007), Hafidi et al. (2005), Lguirati et al. (2005), Ouattmane (2000) and Smidt and Meissl (2006).

The spectra for the two treatments were clearly different from the very start. Compared to T1i, the spectra of T2i show, in relative values, a decrease in the $-\text{CH}$ groups at around 2925 cm^{-1} , another decrease at 1744 cm^{-1} and an increase in the peak at 1072 cm^{-1} . The addition of lime, raising the pH, obviously modified the habitat for the microorganisms, but also the ionic environment of the organic matter and thus certain properties of the organic matter, leading to an increase in the signal of the $-\text{OH}$ groups (3400 and 1072 cm^{-1}).

With increasing duration of the treatment time, the pattern of variation of the $-\text{OH}/-\text{CH}$ ratio (region $3400/\text{region } 2925$) during T1 (proportionally fewer $-\text{CH}$ groups at the end of treatment) was the opposite of that observed during T2. Moreover, the maximum of the $-\text{OH}$ peak (region 3400 cm^{-1}) drifted between the initial and the final states: for T1 the drift was too slight to be significant (11 cm^{-1}) but for T2 it was sufficient (34 cm^{-1}) to be taken into account. At the end of treatment T2 it appears that bound $-\text{OH}$ groups make up slightly longer chains which should be carried by larger molecules.

For T2, the $-\text{CH}$ groups (around 2925 cm^{-1}) also showed a slight shift (22 cm^{-1}) between the initial (T2i) and final (T2f) states. Here again, it would appear that the lime, by modifying the macromolecular structure, is at the origin of the shift. At the end of the treatment period, as the pH returns to more acid values, this peak in T2f occupies the same position as on T1i and T1f.

During treatment T1, the peak at 1744 cm^{-1} disappeared. It could correspond to ester carboxyls which can usually be attacked by microorganisms. There was also an increase in the relative intensity of the peak at 1638 cm^{-1} . This can be attributed to conjugated ketone $\text{C}=\text{O}$ groups, $\text{C}=\text{C}$ or conjugated $\text{C}=\text{C}$. This attribution seems to fit best with the phenomena observed, in particular, with the FAS variations between 0 and 45 days.

The action of the lime also brings out the peak at 1577 cm^{-1} , which then diminished over time. This peak

Table 2b

Elemental composition of the dry organic matter contained in the olive mill waste water and the atomic ratios at the various stages of treatment 2 (day 0, day 15, day 30 and day 45)

	Percent weight						Atomic ratio		
	C	H	O	N	CHON	Ash	C/N	C/H	O/C
T2-D0	53.16 ± 0.14	7.41 ± 0.12	30.57 ± 0.05	2.39 ± 0.18	93.53	6.47	25.90	0.60	0.43
T2-D15	53.44 ± 0.19	6.97 ± 0.19	29.75 ± 0.17	2.83 ± 0.07	92.99	7.01	22.00	0.64	0.42
T2-D30	53.10 ± 0.23	6.95 ± 0.04	28.98 ± 0.19	3.03 ± 0.01	92.06	7.64	20.45	0.64	0.41
T2-D45	48.98 ± 0.06	6.50 ± 0.01	27.99 ± 0.28	2.63 ± 0.04	86.10	13.90	21.76	0.63	0.43

can be attributed to amine and/or carboxylate groups but the absence of specific absorption at around 3200 cm^{-1} supports COO^- , especially since there is slight absorbance around 1400 cm^{-1} .

3.4. ^{13}C NMR spectroscopy

The ^{13}C NMR spectra showed the presence of heterogeneous structures. Our interpretation of the spectra was particularly based on the works of Amir et al. (2004), Castaldi et al. (2005), Hafidi et al. (2004), Jouraiphy et al. (2007) and Lguirati et al. (2005).

The signals at 16.7 ppm, 25.4 ppm, 32.5 ppm and 39.5 ppm can be attributed to the carbons of the alkyl chains. The major signal at 32.5 ppm is characteristic of $(\text{CH}_2)_n$ in the fatty acid chains and of ramified aliphatic structures and/or long chains in humic acids (Ouatmane, 2000).

The area from 50 to 105 ppm corresponds to carbons in polysaccharides e.g. oleuropein β -D-glucopyranose or other glucolipids. The signal around 65.5 ppm mainly arises from the methoxyl groups of aromatic ethers. Amino acids generally give a signal around 61.5 ppm. Carbohydrates appear at 73 ppm, often around 70–90 ppm and at 102 ppm, 106 ppm and 115 ppm which corresponds to anomeric carbons. Unsubstituted aromatic carbons show signals at 118.9 ppm and 128 ppm. Unsaturated carbons ($\text{C}=\text{C}$) can also be found in this region. Substituted aromatic carbons appear around 134 ppm, 137.5 ppm and 139.7 ppm. The signal at 151.9 ppm is mainly due to phenyl carbons, aromatic ethers and/or *N*-substituted aromatic carbons. The strong resonances at 173.8 ppm, 177.5 ppm and 184 ppm can come from phenolates or carboxyl and amide moieties.

Comparing the areas under the peaks – aliphatic, aromatic, and carboxylic (Table 4) – reveals a high proportion of aliphatic carbons compared to aromatic or carboxylic carbon. For treatment 1, there was an increase, near the end of the treatment, in aliphatic and aromatic carbons. There was also a slight decrease in carboxylic carbon,

Table 4

Distribution of carbon among various chemical structures in the olive mill waste water as a percentage of the areas under the peaks in the initial (Ti) and final (Tf) stages for treatments 1 and 2

Stage	C-aliphatic	C-aromatic	C-carboxylic
T1i	37.82	17.98	13.33
T1f	47.73	18.45	12.62
T2i	40.53	12.30	22.54
T2f	32.60	14.54	18.17

attributed to the degradation of lipid. In contrast, for treatment 2, the process led to a decrease in aliphatic and carboxylic carbons, and an increase in aromatic carbon.

During the treatments, the microbial population generally used easily degradable aliphatic compounds, lipids and peptides as energy sources. There was also production of volatile fatty acids such as acetic acid, which disappeared during dialysis and which accounted for the drop in pH noted to occur during treatment 2. In parallel, the aromatic structures became substituted with phenol, methoxyl and carboxyl groups, the latter decreasing with time (Amir et al., 2004). The results obtained indicate that the degradable compounds such as the aliphatic chains were modified to generate condensed aromatic structures containing large proportions of hydroxyl, methoxyl, carboxyl and carbonyl groups. These resistant structures are preserved and contribute to the neoformation of humic acids.

4. Conclusion

Aerobic biodegradation of olive mill waste water led to a final product with a pH of 5.4 after treatment 1 and 5.7 after treatment 2 and highly significant decreases in the levels of phenols. The addition of ammonium nitrate favoured the microbial activity, and hence the degradation of the organic matter. Neutralising the pH by the addition of lime (treatment 2) had a positive effect on the degradation of phenols, lowering their levels by about 76%. It also changed the actual structure of the organic matter, as is clearly apparent from the infra-red spectra. Pooling the results of

Table 3

Absorbance bands from the IR spectra of the treated olive mill waste water

Wavelength(cm^{-1})	Attribution
3400 cm^{-1}	–OH (phenols, alcohols and carboxylic groups)
2925 cm^{-1}	C–H stretching vibration in aliphatic structures (fatty acids, waxes and various aliphatics)
2840 cm^{-1}	Symmetrical CH stretching vibrations in $-\text{CH}_2$ (fatty acids and alkanes)
1716–1740 cm^{-1}	C=O stretching vibrations in esters, carboxyl COOH and ketone groups
1620–1660 cm^{-1}	C=O stretching vibrations in primary amides
	C=O stretching vibrations from conjugated ketones, acids and/or quinones. Aromatic C=C stretching vibrations
1550–1650 cm^{-1}	C=O stretching vibrations in COO^- (with a weaker bond around 1400 cm^{-1})
1540–1570 cm^{-1}	N–H vibrations in secondary amides
1525 cm^{-1}	Aromatic C=C stretching vibrations
1450–1460 cm^{-1}	Aliphatic C–H stretching vibrations
1384 cm^{-1}	Symmetrical $-\text{CH}_3$ stretching vibrations
900–1300 cm^{-1}	Vibrations of C–H and deformation of OH functions, carboxyls, C–O of ethers on aromatic rings and N–H of secondary amides
700 and 900 cm^{-1}	Aromatic groups

elemental analysis, FTIR, and ^{13}C NMR shows that during treatment 2, there was neoformation of humic substances resistant to chemical degradation, implying polymerisation of the organic matter. The polymerisation leads to the stabilisation of the olive mill waste water, lowering the risks of toxicity upon use as an agricultural amendment.

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