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UV fluorescence, FTIR, and GC–MS analyses and resin fractionation procedures as indicators of the chemical treatability of olive mill wastewater

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ABSTRACT

The organic matter content in original, untreated (COD = 150,000 mg/L; TOC = 36,000 mg/L; oil–grease = 8,200 mg/L; total phenols = 3,800 mg/L), and chemically treated olive mill wastewater was examined in detail by instrumental and analytical (resin fractionation) methods. For this purpose, two approaches were followed: (i) UV-fluorescence, FTIR, and GC–MS analyses as well as (ii) structural fractionation assessment employing XAD-8, XAD-4, AG-MP-50, and Duolite A-7 exchange resins to obtain hydrophobic-acidic, basic, neutral, and hydrophilic-acidic, basic, neutral fractions, respectively. Acid cracking (pH 2.0; $T = 70^\circ C$), $FeCl_3$ coagulation (pH 3.0; $FeCl_3 = 2{,}500$ mg/L) electrocoagulation with stainless steel electrodes (pH 2.0; $T = 65^\circ C$; A = 50 mA/cm^2; $t = 120$ min), and the Fenton's reagent (pH 2.0; $T = 65^\circ C$; 20 mM Fe^{2+}: 200 mM H_2O_2; $t = 120$ min) were selected for convenient chemical treatment of the effluent. Resin fractionation analyses of the original wastewater sample indicated that the major organic content composed of hydrophobic moieties (75–95%). The percent relative inhibition of the original wastewater was concentrated in the hydrophobic neutral fraction (100%). By employing instrumental analyses, several organic compounds could be identified. Relatively low molecular weight chemicals were qualified via GC–MS analysis, whereas FTIR results revealed an appreciable loss of aliphatic moieties together with an increase in aromatic structures.

Keywords: Acute toxicity; Chemical treatment processes; Instrumental analyses; Olive mill wastewater; Structural resin fractionation

1. Introduction

In Mediterranean countries, large amounts of olive oil are produced by traditional and industrial olive mills over a limited time period, usually ranging from October to December. Simultaneously, huge amounts of so-called olive mill wastewater (OMW) are formed. OMW is characterized by its dark red to black color depending on the age and type of olive processed, slightly acidic pH (≈5), high salinity, and organic matter content bearing polyaromatic compounds, fatty acids, pectins, sugars, tannins, and in particular, polyphenols [1,2]. The presence of complex, heavily polluting, and toxic compounds renders OMW a

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problematic waste, making its treatment and disposal one of the main environmental concerns in all producing countries [3]. Although OMW treatment could be more or less solved technologically, it is still far from being solved in reality, owing to the small-scale and dispersed nature of olive farms, seasonality as well as lack of affordable efficient treatment solutions. Conventional chemical treatment and biological wastewater treatments inherently suffer from performance-related technical and economic drawbacks [4,5]. Therefore, it appears crucial to investigate alternative more efficient treatment strategies for OMW. For "difficult" effluents like OMW, appropriate indicators of degradation and removal are required to determine the effect and success of the suggested treatment techniques on the waste. Indeed, the environmental, physicochemical characterization-based assessment of OMW has been reported recently; however, without identifying any single or common definition [4–6]. In that case, only global (collective) parameters can be used to define the impact and efficiency of treatment methods on complex, difficult-to-treat industrial effluent.

Since the organic matter characteristics of OMW have significant influence on its treatability, it is of major importance to analyze and identify the major components of organic matter in this complex wastewater matrix. Until now, there has not been much work devoted to the structural properties of OMW due to the fact that it is rather difficult to define specific organic compounds in this complex wastewater by employing sophisticated analytical and/or instrumental techniques. More recently, a few studies have been reported employing GC–MS, FTIR, and fluorescence spectroscopy as analytical techniques to characterize OMW samples [7–9].

Resin fractionation procedures based on classifying the organic matter composition by its chemical characteristics has been offered as a useful tool for organic matter profiling and structural distribution analysis [10,11]. This approach is a promising means to categorize water or wastewater structurally into more specific fractions by retaining organic matter onto different resin types. Publications dealing with structural fractionation of real samples are very limited and mostly devoted to freshwater [12,13]. More recently, brewery wastewater [14] and landfill leachate [15] were also subjected to resin fractionation; however, no such study has been done for profiling resin fractionation of treated OMW.

As aforementioned, resin fractionation is an important and interesting tool for the detailed characterization of components found in real water and wastewater samples. For resin fractionation, four different resin types, namely XAD-8, XAD-4, Duolite A-7, and AG-MP-50 were most commonly employed to sequentially fractionate the sample into hydrophobic and hydrophilic structures. It should be noted here that the resins can be reused at least five times after regeneration and only small sample volumes (≈50 mL) are required for this procedure. Besides, serial resin fractionation of real effluent samples lasts not more than 8 h. Considering this practical information about the analytical method, it seems to be quite attractive to profile wastewater samples via serial resin fractionation.

The present work was conducted within the scope of a comprehensive research project and focused on the effect of several chemical treatment processes (including chemical precipitation, coagulation, electrocoagulation (EC), and the Fenton's reagent) on the molecular size distribution and structural fractionation patterns of real OMW [16–18]. In this part of the study, it was aimed at supporting the already gathered experimental data by employing different instrumental techniques such as FTIR, UV-fluorescence as well as GC–MS. In this way, it was possible to apply instrumental analyses to OMW samples which were previously fractionated according to their structural features. This approach would enable a more in-depth and thorough evaluation of the structural changes brought about in OMW during its chemical treatment. Within this framework, untreated and chemically treated OMW samples were subjected to (i) instrumental analyses via UV-fluorescence, FTIR, and GC–MS as well as (ii) resin adsorption chromatography by employing XAD-8, XAD-4, AG-MP-50, and Duolite A-7 exchange resins. Changes in the major organic carbon components of OMW, namely COD, TOC, total phenols (TPh), and color (absorbance at 400 nm) together with the acute toxicity parameter (test organism: *V. fischeri*) were followed throughout the experiments.

2. Materials and methods

2.1. Characterization of the olive mill effluent

OMW was obtained from a three-phase olive mill extraction plant located in Bursa, Turkey. The OMW samples were stored in plastic carboys in a cool room at 4˚C prior to chemical treatment, environmental characterization, resin fractionation, and instrumental analysis. The environmental characteristics of the OMW sample before and after filtration through 450 nm cut-off membranes are presented in Table 1. Pretreatment of the OMW sample prior to resin fractionation was mandatory, since suspended solids and particulate matter hinder effective adsorption of

Table 1
Environmental characterization of the OMW sample

Parameter	Unit	Original OMW	Filtered OMW
COD	mg/L	140,000–155,000	70,000–72,000
TOC	mg/L	35,000–40,000	25,000–26,000
TPh	mg/L	3,800–4,200	2,900–3,000
Oil and grease	mg/L	12,000–16,000	8,000–9,000
Color[a]	cm^{-1}	130–145	40–50
ED_{50}[b]	% v/v	100	100
pH	–	4.8–5.0	4.8–5.0

[a] Absorbance was measured at 400 nm.

[b] ED_{50} refers to the "Effective Dilution" causing 50% inhibition of the test organism *V. fischeri*.

organic fractions onto the exchange resins by blocking their pores. Therefore, in order to apply this analytical procedure, the untreated OMW had to be prefiltered, and the prefiltered samples were used as reference samples to follow changes in the structural properties of the effluent after applying chemical treatment processes.

2.2. Chemical treatment processes

The experimental conditions of the studied chemical treatment processes were selected considering preliminary baseline experiments and previously published related work [16]. Acid cracking (ACr) was used as a pretreatment step to remove the oil–grease content of OMW. ACr was carried out by adjusting the pH to 2.0 with 0.26 N H_2SO_4 and heating the OMW samples at 70°C for 60 min. Thereafter, oil and grease was separated in a funnel for 30 min. The acid-cracked samples were subjected to chemical treatment processes. Coagulation was carried out with $FeCl_3$ with a dose of 2,500 mg/L, at pH 3.0.EC experiments were performed with stainless steel electrodes at pH 2.0 and a current density of 50 mA/cm^2 at 65°C for 120 min. Fenton treatment experiments were conducted at pH 2.0 with 20 mM Fe^{2+} and 200 mM H_2O_2 at 65°C and for 120 min.

2.3. Structural fractionation of the OMW sample

The fractionations of organic matter in the prefiltered, original (untreated) and chemically treated OMW samples were performed based on the serial resin adsorption procedure described by Leenheer [10], Marhaba et al. [11] as well as our former work carried out with OMW [17]. The organic matter content of original (untreated) and treated OMW was fractionated into the hydrophobics (HPO) and hydrophilics (HPI), such as hydrophobic acid (HPOA), hydrophobic base (HPOB), hydrophobic neutral (HPON), hydrophilic acid (HPIA), hydrophilic base (HPIB), and hydrophilic neutral (HPIN) fractions by the nonionic resins XAD-8 (SUPELCO) and XAD-4 (Amberlite); the cationic resin AG-MP-50 (BIO-RAD) and the weak anionic resin Duolite A-7 (Aldrich). The initial volumes of resin and OMW sample were 50 and 250 mL, respectively, and the filtration and desorption flow rates were adjusted to 3 and 1.5 mL/min, considering available procedures and recent publications [11,17,19]. Prefiltered OMW was used as the reference sample to compare results with chemically treated OMW. All steps involved in the experimental study are schematically shown in Fig. 1.

2.4. Analytical procedures

Treatment performance was evaluated in terms of the collective environmental parameters COD, TOC, TPh, and color (absorbance at 400 nm). With the exception of COD, all analytical procedures used for conventional wastewater characterization were accomplished according to Standard Methods [20], whereas the COD parameter was measured based on ISO 6060 [21]. TOC was monitored on a Shimadzu VCPN model carbon analyzer equipped with an autosampler. TPh in the original, pretreated, and chemically treated OMW were determined by the colorimetric reaction of the samples with the Folin-Ciocalteau reagent (Fluka) as described in Box [22]. Color was measured at the maximum visible absorption band of OMW at 400 nm on a Novespec II/Pharmacia LKB colorimeter in 1 cm optical path length glass cuvettes. Acute toxicity of the OMW samples was determined in accordance with the *V. fischeri* bioluminescence inhibition test protocol ISO 11348-3 [23] at an incubation period of 15 min. Firstly, the "ED_{50}" values (in v/v, %), i.e. the effective dilution causing 50% inhibition of the photobacteria were determined via serial dilutions in blank water. Based on the ED_{50} value, the OMW samples were 10-fold diluted prior to the inhibition tests.

FTIR spectra were recorded in ethyl acetate extracted samples, using a Perkin Elmer 1600 spectrophotometer over the 650–4,000 cm^{-1} range. UV–fluorescence spectroscopic analysis was carried out with 100-fold diluted original OMW sample. Fluorescence measurement was performed using a Perkin Elmer LS5 Luminescence Spectrophotometer. The spectra of the samples were recorded as emission and synchronous scan. Emission spectra were recorded over the range 400–600 nm at a constant excitation wavelength of 350 nm. Excitation spectra were

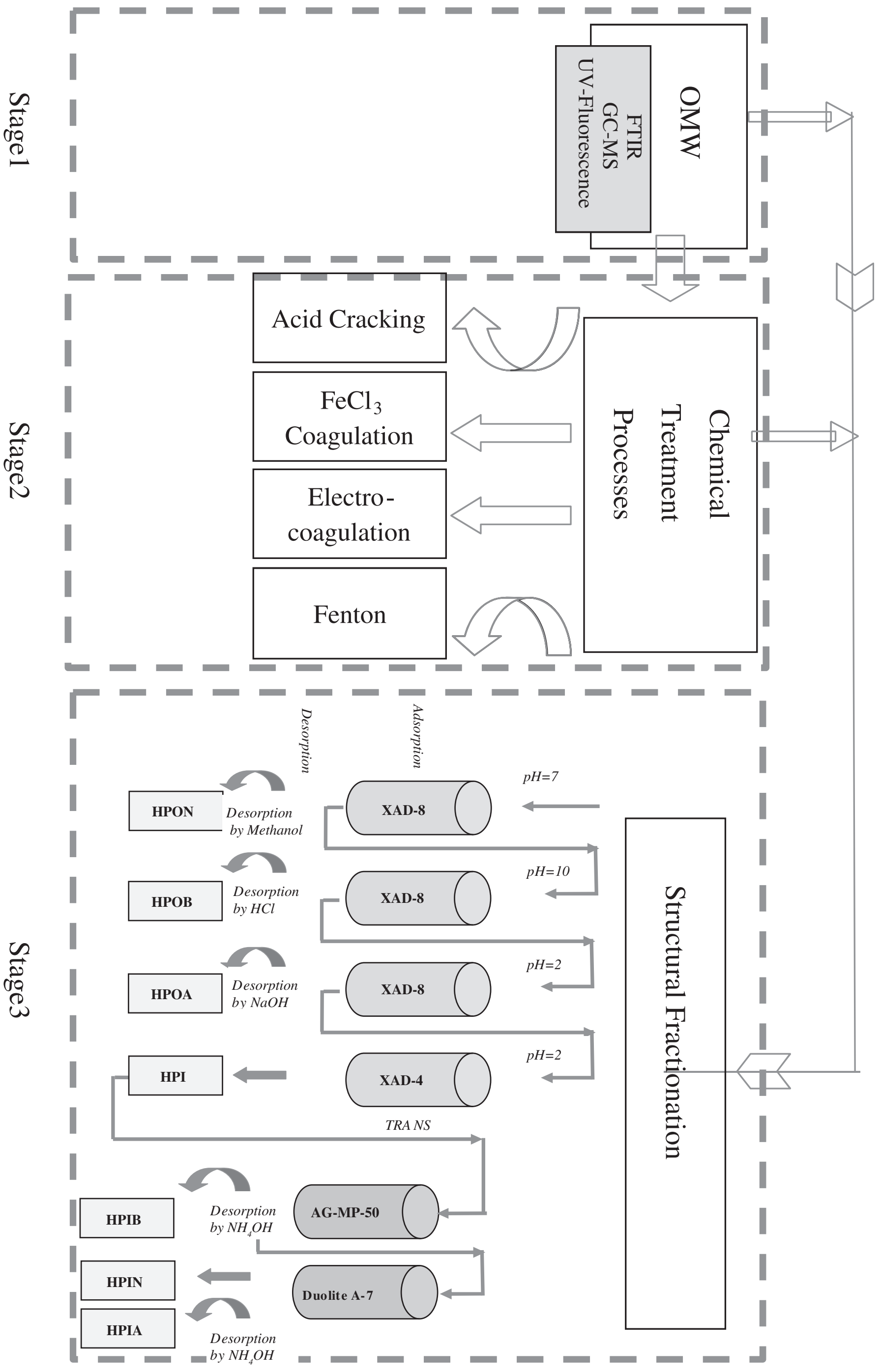

Fig. 1. Experimental approach of the present study.

recorded over the range 300–500 nm at a fixed emission wavelength of 520 nm. Synchronous-scan excitation spectra were measured by simultaneously scanning both the excitation and the emission wavelengths (from 200 to 700 nm), while maintaining a constant, optimized wavelength difference $\Delta\lambda$ of 18 nm. GC–MS analyses were performed with an Agilent model-6890N GC, 5975C MSD equipped with a DB-5MS capillary column (60 m × 0.25 mm × 0.25 µm, Agilent technologies, US) was used. The ethyl acetate extracted samples were subjected to GC–MS analyses. OMW components were identified in the extracts according to their retention

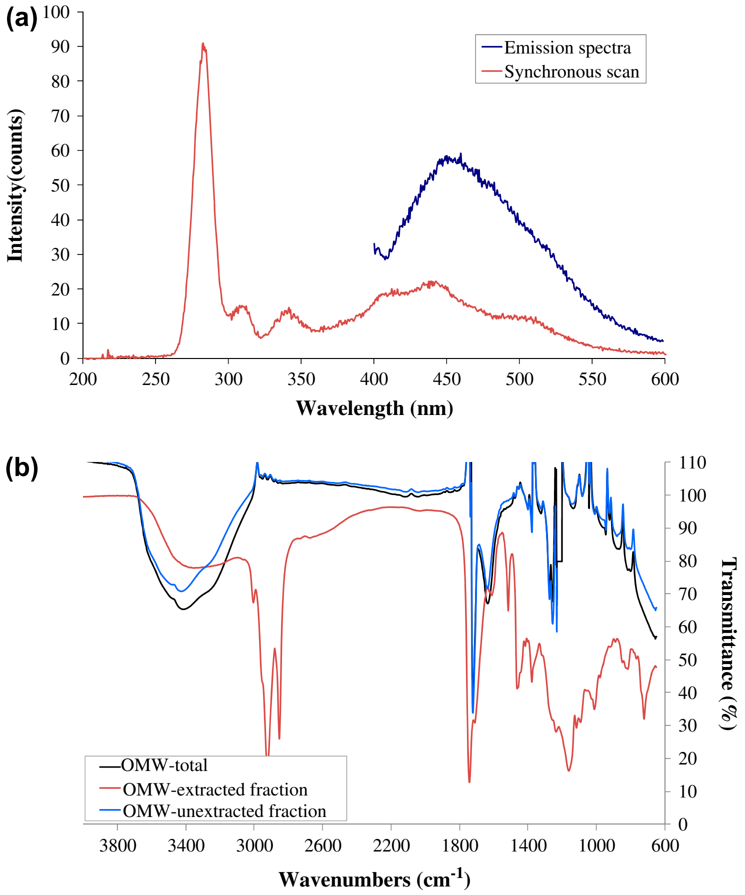

Fig. 2. UV Fluorescence (a) and FTIR (b) profiles of the original OMW sample.

time and molecular weight by referring to a previous study [9].

3. Results and discussion

3.1. Characterization of untreated OMW

The environmental and ecotoxicological problems associated with OMW are mainly due to the presence of polyphenolic compounds, which are responsible for its dark color and high toxicity [1]. The phenolic content arises from simple phenolics and polymeric polyphenols of higher molecular mass; i.e. >60 kDa [24]. In order to analyze OMW by instrumental methods, it was necessary to pretreat the original sample by filtration, extraction, or evaporation. After exploring different solvent and extraction procedures, liquid–liquid extraction by ethyl acetate was found to be most suitable for the sample. However, it should be noted here that the extracted fraction was not characterizing the entire effluent sample. Only relatively low-molecular mass fractions could be extracted by ethyl acetate, the rest was defined as higher molecular weight compounds [24]. For the original OMW sample, 20% of the TOC could be identified as relatively low molecular weight organics (6,000 mg/L). According to GC–MS and FTIR analyses, this fraction mainly comprised of carboxylic acids and alcohols after solvent extraction. Similarly, TPh (4,000 mg/L) could be subdivided in to relatively low molecular weight phenols (800–900 mg/L) and higher molecular weight polyphenols such as lignin-like (2,900–3,000 mg/L) in this sample fraction.

Fig. 2 displays UV Fluorescence (a) and FTIR (b) analyses of the untreated OMW samples. In the synchronous scan spectra, two main bands at 280 and 450 nm were obtained as shown in Fig. 2(a). The band centered around 280 nm revealed the presence of polyaromatic hydrocarbons, phenolic aromatics, benzoic acid, and aniline derivatives. According to the emission spectra, the peak obtained around 460 nm was identified as chromophore groups [25].

FTIR spectra were monitored to analyze the functional groups of the organic content in each fraction. Fig. 2(b) presents the FTIR spectra of the original fractionated OMW samples. The OMW samples were analyzed after evaporation. For measuring low molecular weight fractions, OMW was extracted with ethyl acetate, whereas the unextracted sample was analyzed as the high molecular weight fraction. According to the FTIR spectra of the original OMW sample, strong peaks were observed at 3,277–3,381 cm^{-1} corresponding to the phenolic compounds. In previous studies investigating the organic matter profiles of OMW by FTIR, an intense, broad band that was centered in the range of 3,000–4000 cm^{-1} was identified as H–O bond vibrations belonging to carboxyl, hydroxyl, or phenolic groups [7]. The peak at 2,975–2,855 cm^{-1} being observed in extracted OMW corresponded to aliphatic moieties defined as long-chain lipids. The peak around 1,712 cm^{-1} could be attributed to free carboxyl groups of fatty acids or simple sugars according to previous related work [7]. The appearance of peaks at 1,575–1,624 cm^{-1} supported the presence of aromatic double bond structures. While the functional groups of OMW were defined by FTIR analyses, the organic components present in OMW extracts were qualified by GC–MS analyses (data not shown). GC–MS results indicated the presence of aromatic hydrocarbons, such as benzoic acid, caprinic acid, capronic acid, coumaric acid, oleic acid, valeric acid, catechol, phenol, hydroquinone, glycerol, 4-vinyl phenol, tyrosol, and hydroxytyrosol. However, it should be noted here that the compounds identified by GC–MS analysis corresponded to the lower molecular weights components present in OMW, to <350 kDa [26].

The resin fractionation method based on classifying organic matter composition by its chemical characteristics was employed to profile organic composition of OMW as has been reported elsewhere [17]. The resin fractionation patterns of the prefiltered, untreated OMW in terms of the followed collective environmental parameters COD, TOC, TPh, color, and acute toxicity were presented in Table 2. Structural fractionation demonstrated that over 70% of the OMW's COD and TOC content was hydrophobic, whereas the remaining organic content was distributed among the hydrophilic fractions. The TPh parameter exhibited different characteristics from COD and TOC; over 90% of the TPh content was hydrophobic and only 9% was hydrophilic according to the structural fractionation results. For this parameter, especially the HPON fraction was rather significant, corresponding to 56% of the TPh of the untreated OMW sample. In a previous study, it was concluded that high molecular weight polyphenolic compounds are typically categorized as hydrophobic due to their polyaromatic nature [27]. Fifty-six percent of the TPh that accumulated in HPON fraction coincided with the acute toxicity of the wastewater. The inhibitory effect toward *V. fischeri* was measured as 75% (dilution ratio) on volumetric basis. The acute toxicity of the OMW sample was concentrated in the HPON fraction which could be associated with the polyphenolic content of OMW. Regarding the color of the OMW sample which varied from dark red to brownish black, a major fraction appeared to be hydrophobic.

The GC–MS results obtained after structural fractionation of the original OMW sample arepresented by their main components with their relative abundance (%) in Table 3. By GC–MS analyses, a rather qualitative assessment of the sample extracts for low molecular weight compounds was carried out speaking for the presence of catechol, hydroquinone (31%), vanillin (23%), and homovanillic alcohol (16%) in the HPOA fraction; 4-vinyl phenol (49%) and vanillic acid (20%) in the HPOB fraction; tyrosol (40%) and monosaccharides (20%; galactitol, glucitol, and mannitol) in the

Table 2
Structural fractionation of COD, TOC, TPh, color (absorbance), and percent relative inhibition (acute toxicity) parameters for the original OMW sample

Parameter fraction	COD (mg/L)	TOC (mg/L)	TPh (mg/L)	Color[a] (cm^{-1})	Relative Inhibition[b] (%)
HPOA	19,000	7,500	800	9	0
HPOB	18,000	7,000	400	8	0
HPON	17,000	5,000	1,600	11	75
HPIA	5,000	3,000	200	1	0
HPIB	6,000	3,000	70	0.2	0
HPIN	5,000	1,500	0	0	0
Total	70,000	27,000	3,070	29	75

[a] Absorbance was measured at 400 nm.

[b] ED_{50} refers to the "Effective Dilution" causing 50% inhibition of the test organism *V. fischeri*.

Table 3
Lower molecular weight components and their percent relative abundancies in the original OMW sample identified by GC–MS analysis

Fraction	Relative abundance (%)	Molecular weight (Da)	Retention time (min)
HPOA			
Catechol, hydroquinone	31	124	20.33
Vanillin, benzoic acid	23	152	20.44
Homovanillic alcohol, vanillic acid	16	168	20.11
Tyrosol	5	138	17.18
Butenoic acid	5	86	9.80; 16.01
HPOB			
4-vinyl phenol	49	120	16.03
Vanillic acid	20	168	17.58; 18.63; 22.15
Ferulic acid	4	194	26.76
Galactitol, glucitol, mannitol	4	182	19.08
HPON			
Tyrosol	40	138	16.02; 16.13; 16.31
Galactitol, glucitol, mannitol	20	182	15.93; 18.62
Homovanillic alcohol, vanillic acid	8	168	17.45
Coumaric acid	8	164	26.77
Linoleic acid	3	278	20.93
HPIs			
Linoleic acid	20	278	17.80; 20.09; 20.93; 21.91; 26.87
Galactitol, glucitol, mannitol	21	182	18.62; 19.08
Coumaric acid	17	164	26.76
Benzoic acid	15	122	26.65
Homovanillic alcohol, vanillic acid	4	168	23.82

HPON fraction. On the other hand, linoleic acid (20%) and monosaccharides (21%), coumaric acid (17%) and benzoic acid (15%) were observed in the hydrophilic fractions. In a study investigating the structural profiles of raw surface water, the presence of fulvic acids in the HPOA fraction; several hydrocarbon and tannin compounds in the HPON fraction; a multitude of aromatic amines in the HPOB fraction; several polyuronic acids in the HPIA fraction; sugar compounds in the HPIN fraction as well as peptides and amino acids in the HPIN fraction were reported [28]. In a previous related work, OMW was categorized into different molecular mass fractions such as low (F1 < 8 kDa), medium (8 kDa < F2 < 60 kDa), and high molecular mass (>60 kDa) by ultrafiltration [24]. These fractions were characterized by GC–MS and gel filtration analyses. In the low molecular mass fraction, three types of aromatic compounds corresponding to simple phenolic, o-diphenols, and monomeric flavinoids in a first group, hydrolysable tannins in a second group, and some condensed tannins and antocyanins in the higher volumes were identified. After ethyl acetate extraction, this fraction was analyzed by GC–MS. The identified compounds were tyrosol, hydroxytyrosol, prototechuic acid, syringic acid, gallic acid, p-coumaric acid, cafeic acid, ferulicacid, vanillic acid, vanillin, fatty acids, and reducing sugars. The F2 fraction being monomer-free was composed of polyphenolic compounds, whereas the F3 fraction indicated a group of aromatics.

3.2. Chemical treatment of OMW

Speaking of the chemical treatment of OMW, significant amounts of oil–grease (95%) and particulate matter (96%), corresponding to 58, 43, 39, 80% COD, TOC, TPh, and color removals, respectively, were obtained by ACr. Acid cracked samples were subjected to $FeCl_3$ coagulation, EC with stainless steel electrodes, and the Fenton's reagent. The COD, TOC, TPh, color, and percent relative inhibition (acute toxicity) profiles obtained after application of different chemical treatment processes are summarized in

Table 4
Environmental characterization of the original (untreated) and chemically treated OMW

OMW and treatment process	COD (mg/L)	TOC (mg/L)	Oil–Grease (mg/L)	TPh (mg/L)	Color[a] (cm^{-1})	Inhibition[b] (%)
Original OMW	150,000	40,000	8,200	3,800	131	75
ACr	63,000	23,000	400	2,300	25	70
ACr + $FeCl_3$	57,500	21,000	350	2,000	25	82
ACr + EC	51,000	18,500	325	1,065	25	91
ACr + Fenton	56,000	20,000	320	1,480	25	77

[a] Absorbance at 400 nm.

[b] Inhibition tests were conducted with 10-fold diluted OMW samples for an incubation period of 15 min.

Table 4. For the COD and TOC parameters, major removals occurred during ACr pretreatment (58 and 43%, respectively), which was related to the removal of oil–grease and particulate organic matter present in the OMW. On the other hand, none of the chemical treatment processes exerted an additional positive effect on COD and TOC removals after application of ACr. The additional COD and TOC removals were improved by only 10%. Eighty percent of the original color being mostly in the particulate form was removed via ACr and no further increase in removal efficiency was obtained during chemical treatment processes. However, ACr combined with EC and the Fenton's reagent was found to be more efficient in terms of the TPh parameter; TPh removal rates increased from 39 to 72 and 70%, respectively, whereas no significant additional COD and TOC removals were obtained after ACr. This rather poor improvement in removal rates was attributable to the fact that the remaining effluent was mostly composed of soluble organic matter being resistant to further chemical treatment. The major difference observed for the TPh parameter could be its colloidal character and concentration in the HPON fraction being affected by the application of both EC and the Fenton processes. On the other hand, the *V. fischeri* toxicity appreciably

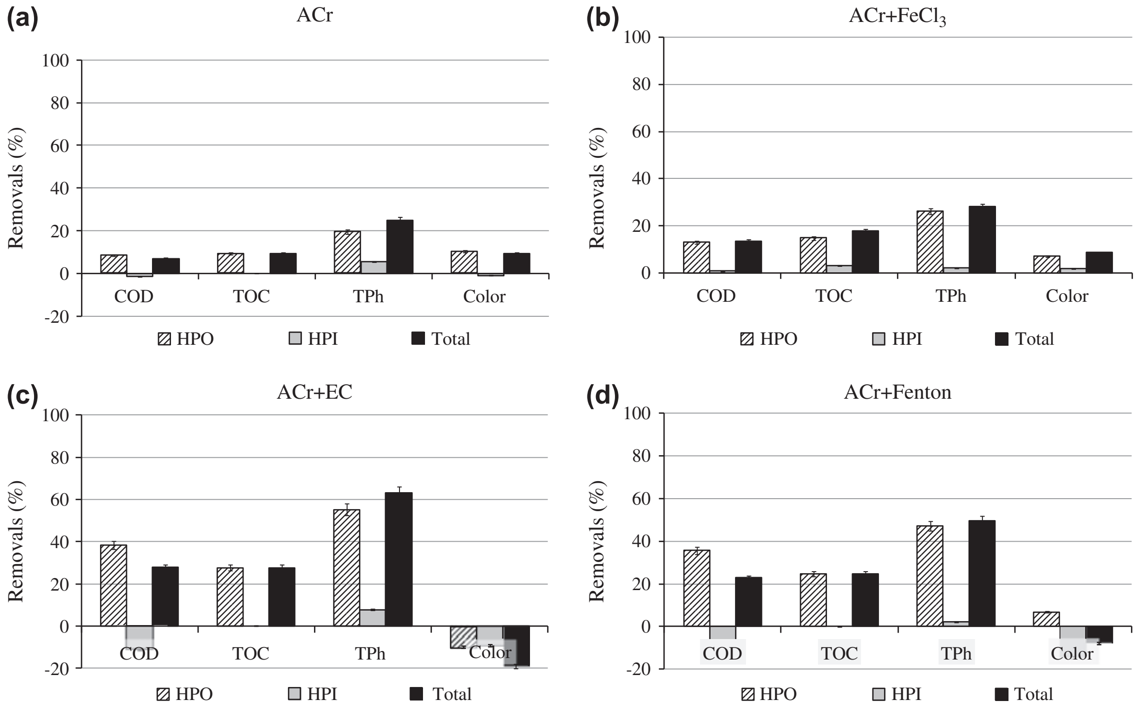

Fig. 3. Removal efficiencies obtained in the structural fractions after ACr (a), $FeCl_3$ coagulation following ACr (b), EC following ACr (c), and Fenton's reagent following ACr (d).

increased from 75 to 91% after chemical treatment that could be due to the evolution of degradation products being more toxic than the original compounds present in OMW.

The removal efficiencies obtained in the structural fractions of OMW after application of chemical treatment processes are shown in Fig. 3. When comparing prefiltered original and chemically treated OMW it can be deduced that different removal rates were observed since the filtration step resulted in the elimination of the mainly organic particulate content. For the COD and TOC parameters, the highest removals (25–28%) were obtained by EC and the Fenton's reagent applied after ACr pretreatment. Mostly hydrophobic fractions were removed during these two chemical treatment processes. Depending on the polarity of the specific organic constituent, a shift in the structural distribution pattern was observed for the COD parameter from mainly hydrophobic to more hydrophilic fractions (from 29 to 38%).This result can be explained by the oxidation of hydrophobic substances resulting in the formation of more polar and hydrophilic degradation products. In a previous study investigating ozonation and H_2O_2/UV-C treatment of municipal wastewater, the polarity and hydrophilic nature of the sewage sample increased considerably (from 35 to 70–80%) during advanced oxidation [19]. In this work, TPh removal was mainly observed in the hydrophobic (HPOA and HPON) fractions; however, it remained dominant in the HPON fraction. The highest TPh removals were observed after ACr pretreatment combined with EC and the Fenton's reagent as 63 and 50%, respectively, where the contributing effect of ACr was 20%. For the color parameter, removal efficiencies were in the range of 10–20% for each treatment processes relative to the filtered and original OMW. The inhibitory effect that increased from 75 to 91% in the 10-fold diluted samples after chemical treatment mainly did not shift and appeared in the HPON fraction.

Results of FTIR analyses carried out with extracted OMW samples before and after EC are given in Fig. 4. As is evident in the Fig. 4, two main peaks at 3,450–3,200 and 1,720–1,701 cm^{-1} were obtained, of which 3,450–3,200 cm^{-1} corresponded to phenolic compounds, whereas those at 1,720–1,701 cm^{-1} were related to the presence of carboxylic groups. A phenolic compound was evident and appeared to be within the HPOA, HPOB, HPON, and HPI (total) fractions. According to Fig. 4, after EC, the peak at 1,722 cm^{-1} disappeared that corresponded to less condensed products with free –OH and –COOH groups. The changes observed after chemical treatment could be explained by the degradation of less condensed structures in the form of readily biodegradable compounds, being transformed to more recalcitrant and toxic ones [29]. When comparing acute toxicity test results of the original and chemically treated OMW, it could be deduced that the disappearance of the peak at 1,722 cm^{-1} after treatment was compatible with the increase in the inhibitory effect observed in the chemically treated samples. On the other hand, considering the inhibitory effect which was observed in the HPON fraction, it is not possible to correlate a direct relation exist between FTIR results and inhibition in the HPON phase.

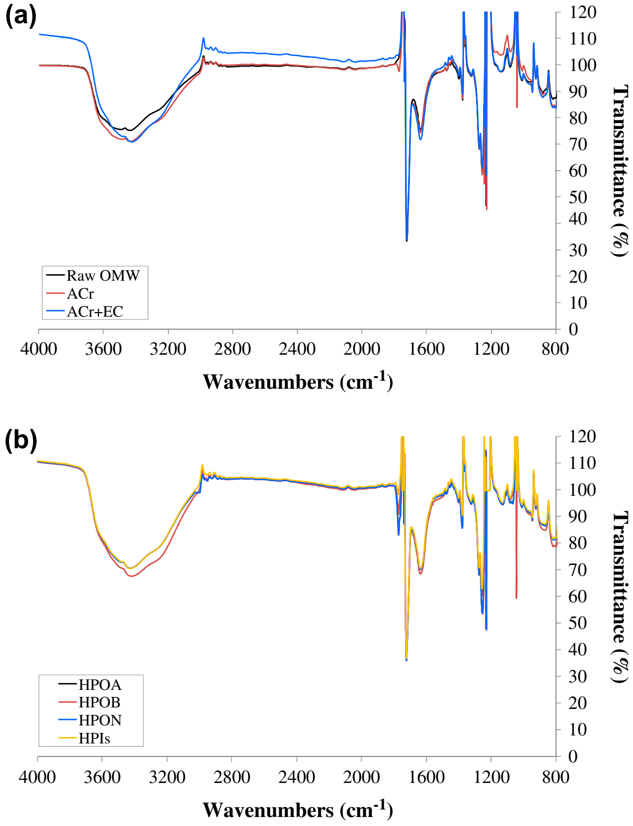

Fig. 4. FTIR analysis of OMW samples obtained after ACr and EC (a); structural fractions obtained after EC following ACr (b).

4. Conclusions and recommendations

In the present study, real olive mill effluent was subjected to environmental characterization, acute toxicity testing, instrumental analyses, and structural fractionation before and after ACr, filtration as well as application of different chemical treatment processes. The following conclusions could be drawn from this work:

(1) According to resin fractionation results, all environmental parameters characterizing the organic matter were of mainly hydrophobic nature (75–95%). Among the structural fractions, HPON was of most environmental concern since the acute toxicity (100%) and TPh (56%) parameters mainly comprised this fraction.
(2) Organic matter removals were mainly obtained in the hydrophobic fractions after ACr and application of chemical treatments. During EC and Fentosn processes, a shift in the structural distribution pattern from dominantly hydrophobic to rather hydrophilic fractions was evident for the COD parameters revealing that the degradation of organics resulted in more polar and hydrophilic products.
(3) Acute toxicity and hydrophilic fractions generally increased during treatment applications as a consequence of incomplete degradation of OMW's organic carbon content. The appreciable increase in the effluent's toxicity after chemical treatment was supported by FTIR analysis results confirming the decrease of band intensities in carboxylic groupings (absorbance peaks at 1,701–1,720 cm^{-1}) after chemical treatment.
(4) The Fenton's reagent and EC which were applied after thermal ACr were not effective on COD and TOC removal rates. On the other hand, the above-mentioned treatment processes enhanced TPh removal efficiencies considerably.
(5) However, none of the investigated processes was effective enough to be used as a standalone treatment option for organic carbon removal from OMW, and it was concluded that an integrated treatment scheme would be needed for efficient treatment of OMW.
(6) According to GC–MS analyses, 20–25% of the TOC and TPh content of the original OMW sample were identified as relatively lower molecular weight compounds, while the remaining content was mainly composed of relatively higher molecular weight compounds.
(7) On the other hand, FTIR and UV-Florescence analyses provided more information about the high molecular weight compounds present in the OMW sample. It was possible to at least partially identify the complex organic compounds present in OMW by employing instrumental analyses.
(8) Among the instrumental methods applied, FTIR was found to be more convenient to characterize the total, low, and high molecular weight fractions in OMW samples.

Since, by directly employing instrumental analysis it is rather difficult to characterize "complex" effluents like OMW; structural fractionation after pretreatment seems to be rather promising and supportive to gain more detailed information about the wastewater characteristics. Additionally, resin fractionation helps to elucidate the effective removal mechanisms during treatment applications as well as to understand which effluent components are toxic, inert and thus, very critical factors for their efficient treatment.

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