

A compact process for the treatment of olive mill wastewater by combining wet hydrogen peroxide catalytic oxidation and biological techniques

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ABSTRACT

A system based on combined actions of catalytic wet oxidation and microbial technologies for the treatment of highly polluted OMW containing polyphenols was studied. The wet hydrogen peroxide catalytic oxidation (WHPCO) process has been investigated in the semi-batch mode at atmospheric pressure, using aluminium–iron–pillared inter layer clay ((Al–Fe)PILC), under two different catalytic processes: ((Al–Fe)PILC/H₂O₂/ultraviolet radiations) at 25 °C and ((Al–Fe)PILC/H₂O₂) at 50 °C. The results show that raw OMW was resistant to the photocatalytic process. However ((Al–Fe)PILC/H₂O₂), system operating at 50 °C reduced considerably the COD, colour and total phenolic contents, and thus decreased the inhibition of the marine photobacteria *Vibrio fischeri* luminescence by 70%. This study also examined the feasibility of coupling WHPCO and anaerobic digestion treatment. Biomethanisation experiments performed with raw OMW or pre-treated OMW proved that pre-treatments with ((Al–Fe)PILC/H₂O₂) system, for more than 2 h, resulted in higher methane production. Both untreated OMW as well as 2-h pre-treated OMW revealed as toxic to anaerobic bacteria.

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1. Introduction

The major concern of olive oil industry is the treatment of important amounts of generated olive mill wastewaters (OMW). This effluent results into significant disposal problems in the Mediterranean countries [1] that require urgent solution to avoid serious environmental contamination, such as colour intensity and high chemical oxygen demand (COD). The COD of this wastewater is in the range of 80–200 g L⁻¹ which is about 200–400 times higher than those of a typical municipal sewage [2]. Phenols present in OMW at high concentrations (1–10 g L⁻¹) are considered as the major recalcitrant compounds which possessing antimicrobial properties, and are difficult to biologically degradation [3,4]. Due to the content of this effluent, the environmental problems and potential hazards caused by OMW had lead many countries to limit their discharge and to develop new technologies for reducing the polluting power such as chemical treatment [5–8] and biological treatment which have been lately investigated [4,9–11].

Since recalcitrant and toxic phenolic compounds cannot be directly treated in conventional wastewater treatment plants based on the activity of a microbiological consortium, the development of new technologies aimed at the easy degradation of such sub-

stances is of practical interest. Due to the combination of both environmental and economic advantages, the coupling between advanced oxidation processes (AOPs) and biological treatments can be a suitable solution for the removal of toxic compounds from water [12,13]. AOPs are based in the use of the highly and non-specific reactive hydroxyl radicals that make them potentially useful for degradation of a wide range of organic compounds. The main idea of coupling is to apply an AOP to a toxic and/or non-biodegradable effluent during a short time, optimizing chemicals and energy consumption, and generating an intermediate sample that is fully biodegradable, thus opening the possibility of a subsequent biological treatment for the complete removal of organic matter. Among these new technologies, the wet hydrogen peroxide catalytic oxidation (WHPCO) could appear to be very promising to achieve high conversion of organic pollutants and allows performing oxidation reaction at ambient or close to ambient conditions, limiting the investment costs [1,7,14–16]. The WHPCO process first adapted from the classical Fenton's reagent allowed high phenolic compounds oxidation efficiencies (up to 95%) under mild conditions, using hydrogen peroxide and a homogeneous Fe²⁺ catalyst [17]. Even though the systems show an effective elimination of organic pollutants, two main drawbacks limit its application: a narrow range of pH (usually around 3.0) and the difficulties of recovering the catalysts that may lead to a secondary pollution. The use of heterogeneous catalysts could be an alternative method of these problems. Recently, a great number of materials contain-

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ing iron and copper as precursors supported/intercalated on/in oxides, clays, zeolite and polymers as active catalysts for Fenton-type reactions have been proposed to remove organic compounds [14,17]. These catalysts exhibit the advantages of heterogeneously catalyzed process and got relatively higher oxidation efficiency as well as a lower sensitivity to pH compared with homogeneous catalysts at the same reaction conditions.

Pillared clays are complex microporous systems with an enormous potential of application in adsorption and catalysis processes. A large volume of research shows the interest in clays modified through pillaring processes and their application as catalysts in a wide range of reactions [18–20] such as total oxidation of organic compounds in an aqueous medium, oxidation of CO and volatile organic compounds, catalytic cracking of vegetable oils, etc. Previous studies carried out with clay based catalysts pillared by Fe hydroxo complexes [21] or mixed (Al–Cu or Al–Fe) complexes, have reported that the mixed pillared clays show the most promising results for organic compounds total oxidation in water, by using hydrogen peroxide as oxidant [22,23].

The main purpose of this work has been addressed to the investigation of heterogeneous catalytic pre-treatment of OMW that can be used to reduce the microtoxicity and increase the biodegradability of the OMW and thus to make possible the subsequent anaerobic biological treatment. In this work, the biorecalcitrant phenolic compounds present in OMW are oxidized by means of WHPCO over aluminium–iron-pillared montmorillonite ((Al–Fe)PILC). It should be noted that the (Al–Fe)PILC catalyst has been already characterized and tested with success in the photodegradation of aqueous solution of tyrosol [24] and both synthetic and real OMW solutions [25]. Anaerobic biological treatment was evaluated by biogas production of untreated and treated samples using methanogenic consortium provided by an OMW treating anaerobic filter reactor.

2. Materials and methods

2.1. OMW

The OMW used in this study was obtained from an evaporating pond located in the region of Sfax (Agareb), in the month of April. OMW was transported to our laboratory under refrigeration and in order to conduct the tests with the same wastewater, appropriate amounts of OMW were distributed in 1 L plastic bottles and stored at -20°C until use. At the time of the use, the OMW sample was thawed in a refrigerator and centrifuged 6000 rpm for 20 min to remove suspended solids and its properties are given in Table 1.

2.2. Catalyst synthesis and characterization

The clay used is a montmorillonite referenced KC₂ provided from CECA (France). Its general formula is $(\text{Si}_8\text{O}_{20}\text{Al}_{4-x}\text{M}_x)(\text{OH})_{24}\text{CE}_y \cdot n\text{H}_2\text{O}$, where CE are exchangeable cations. The composition of KC₂ is: SiO₂: 57.34% (w/w), Al₂O₃: 16.59% (w/w), Fe₂O₃: 2.72% (w/w), CaO: 1.59% (w/w), MgO: 2.65% (w/w), MnO: 0.03% (w/w), Na₂O: 2.46% (w/w), K₂O: 1.11% (w/w), TiO₂: 0.27% (w/w) and

Table 1
Physico-chemical characteristics of crude OMW.

Characteristic	Mean value
pH	5.2
Salinity (g L ⁻¹)	18.27
Soluble COD (g L ⁻¹)	64.42
Soluble BOD ₅ (g L ⁻¹)	13.75
COD/BOD ₅	4.68
Soluble total phenols (g L ⁻¹)	6.35
Total solids (g L ⁻¹)	61.87

Table 2
Characterization of the (Al–Fe)PILC.

Catalyst	d_{001} (Å)	S_{BET} (m ² g ⁻¹)	$V_{\text{microporous}}$ (cm ³ g ⁻¹)	Fe (wt.%)
(Al–Fe)PILC	16.89	190	0.0801	1.74

d_{001} : basal spacing; S_{BET} : Brunauer, Emmett and Teller surface area; $V_{\text{microporous}}$: Microporous volume.

P₂O₅: 0.14% (w/w). The intercalant solution was prepared by titration of an Al³⁺/Fe³⁺ cationic solution with 0.2 mol L⁻¹ NaOH. The cationic solution contained 0.18 and 0.02 mol L⁻¹ of AlCl₃ and FeCl₃, respectively. The NaOH solution was slowly added to the cationic solution at 70 °C until the OH/cation mole ratio was equal to 1.9. The intercalant solution was added to the clay suspension under stirring. The final (Al + Fe)/clay ratio was equal to 3.8 mol kg⁻¹ of dry clay. After aging for 24 h, the pillared clay precursor was washed until total elimination of chloride ions, dried at 60 °C and finally calcined at 500 °C for 5 h. Table 2 lists the main physico-chemical properties of the synthesized catalyst.

Catalyst morphology was examined by scanning electron microscopy (SEM) on a PHILIPS XL 30 microscope operating at acceleration voltages = 10–15 kV and magnification values up to 1400×. Samples were dusted on a double-sided sticky tape and mounted on microscope slides [26]. Energy dispersion X-ray (EDX) spectra for the fresh and used catalyst samples were recorded using an instrument consisting of a scanning electron microscope and X-ray microanalyzer EDAX 9100/60 (PHILIPS). The recovery of catalyst was made by settling, washing three times with ultrapure water to remove residual OMW organic matter and then filtering through Whatman filter paper number 42. The filter paper with catalyst particles was dried in an oven for 2 h at 60 °C. The recovered catalyst was used for after reaction characterization.

2.3. Wet hydrogen peroxide catalytic oxidation

OMW oxidation was carried out in the semi-batch mode using a stirred and thermostated Pyrex well mixed slurry reactor with a valid volume of 500 mL. With the purpose of enhancing the decomposition of hydrogen peroxide towards hydroxyl radicals and minimizing their decomposition to water, either photochemical or temperature activations were tested.

Ultraviolet (UV) experiments were performed using 200 mL of OMW placed under UV light. The lamp (APLEX UV-lamp, 30 W) radiated predominantly at 254 nm with an incident intensity of 3.83×10^{-5} Einstein s⁻¹ determined by potassium ferrioxalate actinometry according to the method of Hatchard and Parker [27]. The distance between solution and UV source was constant, 15 cm in all experiments. The solid catalyst was introduced into the above solution under continuous stirring (0.5 g L⁻¹). H₂O₂ (30%, w/w) constantly added throughout the reaction at a rate of 0.5 mL h⁻¹ was started (zero time reaction). Control experiments were run in the dark. Blank experiments using no catalyst, but exposed to UV light were also performed.

Three mild temperatures (25, 50 and 70 °C) were investigated during the WHPCO of 200 mL of OMW in the presence of 0.5 g L⁻¹ of (Al–Fe)PILC. The continuous feed of H₂O₂ (30%, w/w) at a rate of 0.5 mL h⁻¹ was started (zero time reaction), periodically up to a reaction time of 8 h, to achieve a final H₂O₂ concentration of 2×10^{-2} M.

All catalytic experiments were performed in duplicate, at the natural OMW pH (5.2) since it was found to be in the optimum range close to 5 of catalytic oxidation reaction [24]. Samples were taken throughout of each run to measure COD, phenols and colour conversion after removing solid catalyst by centrifugation.

2.4. Anaerobic digestion

The fermentations were carried out in 500 mL glass bottles containing 250 mL working volume as already described [28]. They were filled with the sample (OMW or pre-treated OMW) at same desired concentration and glucose-COD (1000 mg L^{-1}) was used as co-substrate providing reducing equivalents with electron fission in order to reduce biogas production in the lag-phase giving that OMW natural sugars were already fermented during their stay in evaporation ponds.

Inoculum was provided by an OMW treating anaerobic filter reactor. A total of 8 g COD of OMW (raw or pre-treated) was filled discontinuously per anaerobic batch as soon as the biogas production is almost lacking. Catalytically pre-treated OMW Samples were stored at least 48 h before starting fermentation experiments to minimize the presence of short life sub-products and residual hydrogen peroxide [29]. The pH of different OMW samples was buffered to 7 with adding appropriate amounts of anhydrous NaHCO_3 to avoid methanogens inhibition by acidic pH. The anaerobic conditions in filling operation were maintained by flushing with nitrogen gas since the fermentations were conducted in batch culture. The bottles were then incubated at the temperature of 37°C and magnetically stirred. The volume of biogas (CH_4 and CO_2) produced was measured by water displacement method.

2.5. Analytical methods

The analysis of monomeric phenolic compounds was performed by HPLC (high performance liquid chromatography) using a Shimadzu 10AVP chromatograph equipped with a Shimadzu 10AVP UV detector. The column used to analyze phenols was C-18 ($4.6 \text{ mm} \times 250 \text{ mm}$) Shim-pack VP-ODS. Eluates were detected at 280 nm. The temperature was maintained at 40°C . The mobile phase used was 0.1% phosphoric acid (Prolabo, France) in water versus 70% acetonitrile (Dharmadrug GmbH, Germany) in water for a total running time of 50 min. The flow rate was 0.6 mL min^{-1} , and the injection volume was $50 \mu\text{L}$.

Phenolic compounds in OMW were identified using the gas chromatography coupled to a mass spectrometer (GC–MS), which was performed with an HP model 5975B inert MSD, equipped with a capillary DB-5MS column (30 m length; 0.25 mm i.d.; $0.25 \mu\text{m}$ film thickness, Agilent Technologies, J&W Scientific Products, USA). The carrier gas was He used with a 1 mL min^{-1} flow rate. The oven temperature program was as follows: 1 min at 100°C , ramped from 100 to 260°C at 4°C min^{-1} and 10 min at 260°C . The chromatograph was equipped with a split/splitless injector used in the split mode. The split ratio was 100:1. $100 \mu\text{L}$ of bis(trimethylsilyl)acetamide (BSTFA) was added to $100 \mu\text{L}$ of the ethyl acetate extract of OMW sample. The obtained solution was incubated for 60 min at 80°C .

The microtoxicity test, according to ISO 11348-2 [30], consists of the inhibition of the bioluminescence of *Vibrio fischeri* LCK480 using the LUMISTox system (Dr Lange GmbH, Düsseldorf, Germany) after removing the residual H_2O_2 with addition of Na_2SO_3 [31]. Percentage inhibition of the bioluminescence was achieved by mixing 0.5 mL of OMW and 0.5 mL luminescent bacterial suspension. After a 15 min exposure at 15°C , the decrease in light emission was measured. The toxicity of the sample is expressed as the percent of the inhibition of bioluminescence ($\%I_B$) relative to a non-contaminated reference. A positive control (7.5% NaCl) was included for each test.

pH, salinity, total solids and 5-day biochemical oxygen demand (BOD_5) were determined according to Standard Methods [32] for the examination of water and wastewater. The soluble COD amount was determined spectrophotometrically according to Knechtel [33], after centrifugation of OMW during 20 min at 4000 rpm. Total phenolic concentrations were quantified by means of Folin–Ciocalteu colorimetric method using gallic acid as stan-

dard [34]. The absorbance was determined at $\lambda = 725 \text{ nm}$. The colour is measured at the two characteristic wavelengths of OMW 395 and 495 nm. The colour is given by the spectral absorption coefficient (SAC) [35]. SAC can be measured by the absorption E in a cuvette with a layer x at the mentioned wavelength by

$$\text{SAC} = \frac{E}{x} \quad [\text{SAC}] = \frac{1}{m}$$

For practical handling Dopkens et al. [36] defines the colour number CN which is characterized by the weighted average of the SAC. It can be calculated by

$$\text{CN} = \frac{\text{SAC}_{395}^2 + \text{SAC}_{495}^2}{\text{SAC}_{395} + \text{SAC}_{495}}$$

with the spectral absorption coefficients SAC_{395} and SAC_{495} at the two characteristic wavelengths.

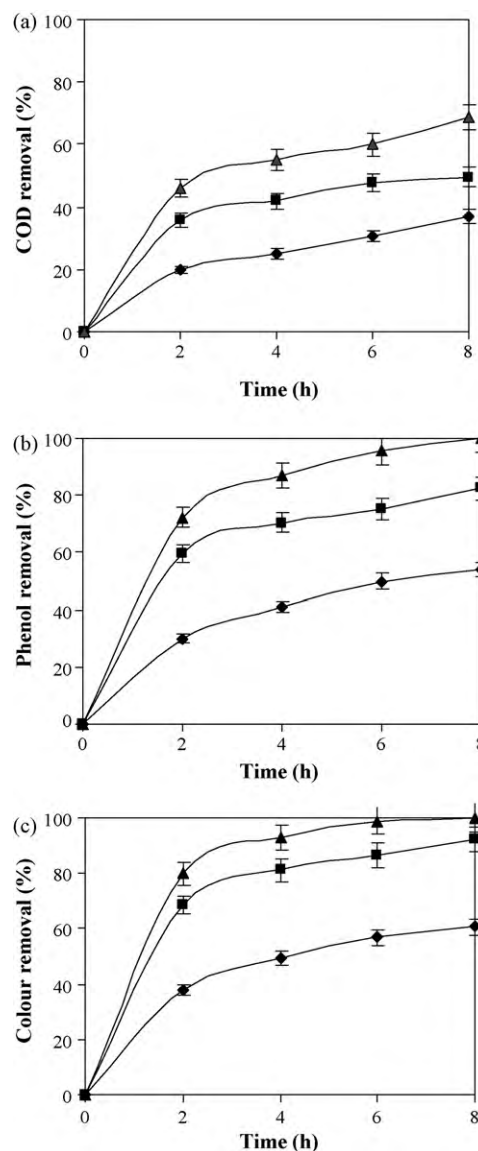


Fig. 1. COD (a), total phenols (b) and colour (c) removals (%) during the WHPCO treatment of OMW at different reaction temperatures: (◆) 25°C , (■) 50°C , (▲) 70°C . Experimental conditions: 1.25 g L^{-1} initial phenol concentration, 0.5 g L^{-1} (Al–Fe)PILC and $2 \times 10^{-2} \text{ M H}_2\text{O}_2$.

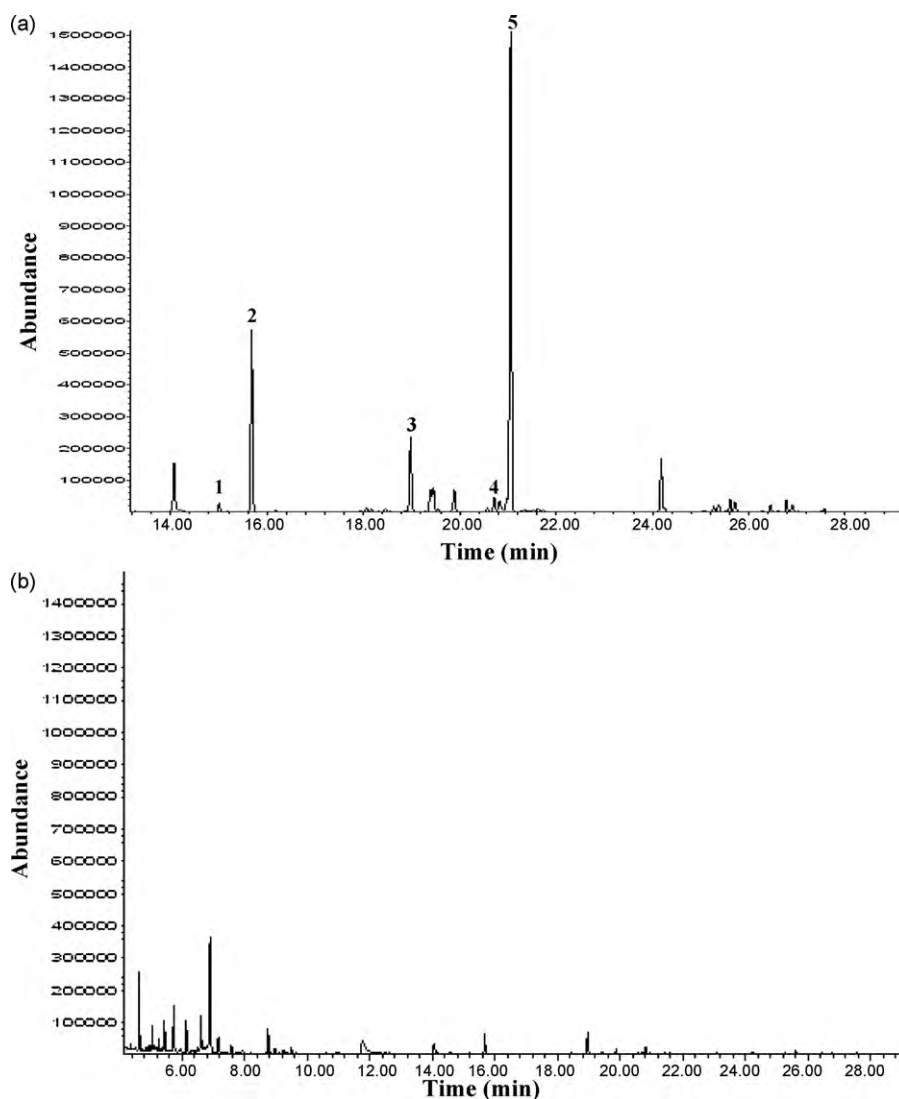


Fig. 2. GC–MS chromatograms of samples taken before and after WHPCO treatment of OMW: (a) original sample; (b) after 8 h WHPCO treatment: (1) *m*-Tyrosol, (2) *p*-tyrosol, (3) homovanillyl alcohol, (4) hydroxycinnamic acid and (5) hydroxytyrosol.

3. Results and discussion

3.1. WHPCO of OMW over (Al–Fe)PILC catalyst

The performance of pillared clay catalyst was mainly studied with success in our previous works in the photodegradation of model and real OMW phenolic compounds with a 0.5 g L^{-1} as the best catalyst loading [24,25].

To evaluate the efficiency and the benefit of the catalytic process for raw OMW detoxification, a preliminary photodegradation experiments were carried out, at room temperature ($\approx 25^\circ\text{C}$), under the following systems: (1) UV/ H_2O_2 , (2) $\text{H}_2\text{O}_2/(\text{Al–Fe})\text{PILC}$ and (3) UV/ $\text{H}_2\text{O}_2/(\text{Al–Fe})\text{PILC}$ in the presence of 200 mL of raw OMW ($64.42 \text{ g COD L}^{-1}$ and 6350 mg L^{-1} total phenols). Results showed that raw OMW effluent used without any prior dilution is resistant to the oxidation over UV/ H_2O_2 system. Moreover, this effluent is unwilling to the catalytic photo-oxidation since both $\text{H}_2\text{O}_2/(\text{Al–Fe})\text{PILC}$ and UV/ $\text{H}_2\text{O}_2/(\text{Al–Fe})\text{PILC}$ systems have led to the same removal yields even after 24 h reaction time (41% and 25% phenol and COD removal yields, respectively) which means that UV irradiation did not enhance OMW catalytic oxidation. A plausible explanation of this finding could be the clouding and penetration limitation for ultraviolet rays in solutions [37] espe-

cially black coloured ones such as OMW besides to the low molar extinction coefficient of H_2O_2 at 254 nm ($19.6 \text{ M}^{-1} \text{ s}^{-1}$) when compared with $3300 \text{ M}^{-1} \text{ s}^{-1}$ for ozone for example [38]. Therefore, the weak removal yields (<10%) obtained in the absence of catalyst are undoubtedly due to the single effect of H_2O_2 . It should be noted that further dilutions (up to 5-fold) and H_2O_2 addition (up to 10^{-2} M) did not significantly improve the photocatalytic oxidation performance, since the dark colour of OMW still persists (data not shown).

In the following section, the influence of temperature on OMW catalytic oxidation will be studied. Since raw OMW was resistant to the catalytic oxidation, we have opted for using 5-fold diluted OMW ($12.5 \text{ g COD L}^{-1}$ and 1250 mg L^{-1} total phenols) for the current catalytic tests which are performed under atmospheric pressure and over (Al–Fe)PILC at the temperatures of 25, 50 and 70°C , maintaining all the other parameters constant. Blank experiments conducted for the effect of increasing the temperature in the presence and absence of H_2O_2 , without catalyst addition, were performed and obtained results showed that removal yields for both COD and phenols were not significant (less than 10% after 8-h treatment). As reported in the literature, temperature affects the wet hydrogen peroxide oxidation process performances by enhancing hydroxyl radicals generation and accelerating Fe^{2+} production [39].

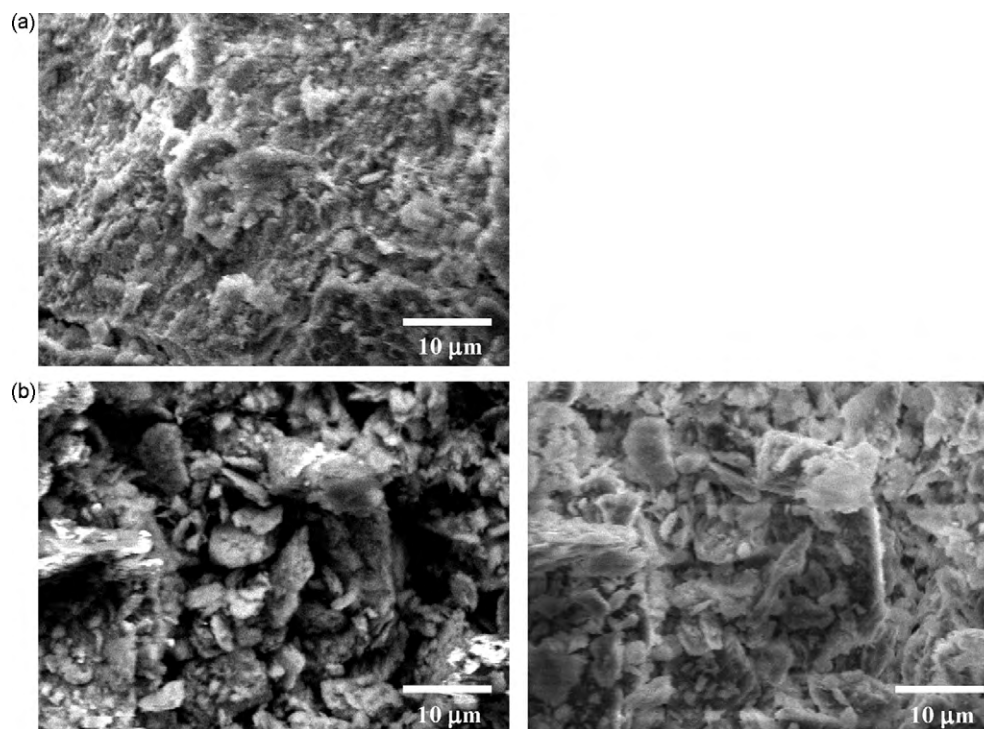


Fig. 3. SEM image of the fresh (a) and used (b) (Fe–Al)PILC catalyst collected after 8 h WHPCO operated at 50 °C.

Fig. 1 shows the behaviour of (Al–Fe)PILC catalyst in the conversion of OMW pollutants versus reaction time for experiments performed at different temperatures. As expected, an increase in the temperature significantly enhanced the OMW pollutants conversion (phenols, COD and colour). It must be noteworthy that a fast removal stage, followed by a slower second step, where phenols, COD and colour removals are levelled off, was observed. After 8 h, the initial OMW COD reduction is about 37%, 50% and 69% at 25, 50 and 70 °C, respectively (Fig. 1(a)). After 8 h catalytic pre-treatment, 54%, 83% and 100% phenol reduction were obtained at 25, 50 and 70 °C of operating temperatures, respectively (Fig. 1(b)). Therefore the rate of phenolic conversion is higher than the rate of COD abatement indicating a preferential reduction of these toxic compounds with respect to COD abatement. This behaviour suggests that the chemical pre-treatment enhanced by increasing reaction temperature, strongly affects the degradation of phenols and the cyclic intermediates produced during the reaction. When all these compounds are eliminated, the oxidation of remaining aliphatic compounds (mainly carboxylic acids) occurs much slower and the overall COD removal slows down [40]. This result is in agreement with the objective to reduce the toxicity, to improve biodegradability and to minimize as much as possible the total organic carbon reduction, because the OMW after WHPCO should be converted into biogas during the anaerobic biological treatment.

As seen in Fig. 1(c), temperature affects also colour removal efficiency. In fact, OMW colour is due to the presence of phenolic acids and mainly polyphenols and tannins co-pigments [41]. OMW decolourization could be explained by breaking of phenols as probe

molecules by hydroxyl radicals generated during this oxidation process assured a rapid decolourization of OMW samples without appreciable mineralization. In the other hand, OMW decolourization was found to be enhance with reaction temperature increase that causes dissociation of the co-pigmentation complexes giving colourless compounds, thus resulting in a loss of colour [42].

Samples drawn prior to and after chemical pre-treatment were analyzed by means of GC–MS and representative chromatograms are shown in Fig. 2. Different phenolic compounds are present in the original sample (Table 3), namely *meta*-tyrosol, *para*-tyrosol, homovanillyl alcohol, hydroxycinnamic acid and hydroxytyrosol. In the other hand, many OMW phenolic acids such as syringic acid, protocatechuic acid, caffeic acid and ferulic acid are not recorded in the present GC–MS chromatogram which may be due to their rapid oxidation with atmosphere's oxygen and light in the evaporating ponds. All phenolic compounds originally present in the sample do not appear at detectable concentrations after 8 h of catalytic pre-treatment and this is consistent with the reduced concentration of total phenols recorded (Fig. 1(b)).

3.2. Catalyst characterization in terms of leaching, morphology and carbon adsorption

In order to evaluate the leaching of active species in the liquid phase that include a secondary pollution, the iron metal content was measured, at the end of the catalytic reaction, in terms of leaching for the catalytic experiment operated at 50 °C and 0.5 g L⁻¹ initial catalyst concentration. In general, extend of iron leaching

Table 3
Abbreviated mass spectra of major phenolic monomers identified in OMW used in this study.

TMS derivatives of	Retention time (min)	Mass spectra (<i>m/z</i> and % of the base peak)
<i>meta</i> -Tyrosol	15.0	267 (M ⁺ , 28); 193 (7); 126 (4); 103 (65); 73 (100).
<i>para</i> -Tyrosol	15.6	282 (M ⁺ , 19); 179 (100); 149 (3); 126 (3); 103 (8); 73 (30).
Homovanillyl alcohol	19.4	312 (M ⁺ , 30); 282 (4); 209 (100); 179 (17); 133 (3); 103 (3); 73 (25).
Hydroxycinnamic acid	20.7	310 (M ⁺ , 21); 179 (100); 73 (66).
Hydroxytyrosol	21.0	370 (M ⁺ , 42); 267 (100); 193 (19); 147 (2); 73 (35).

increased with decreasing pH and increasing temperature. In our experimental conditions the leaching of iron is still low and negligible (<4 ppm).

To further understand the morphology of catalyst samples and visualize the surface reaction process, the fresh and used catalysts were characterized by scanning electron microscopy in conjunction with EDX. Fig. 3(a) shows scanning electron micrograph of fresh (Al–Fe)PILC catalyst, revealing a highly porous and rough surface. The fresh catalyst appeared to have an abundance of crystals on the external surface of the supports. EDX elemental analysis over a number of selected crystals showed that they are basically composed of Si, Al, O, Fe, Ca, Na, Mg and K (Fig. 4(a)). Si is a component of the clays, which is supposed to stay in the clay layers. However, the existence of a large amount of Si in the clay crystals implies that Si may migrate to the surface in the intercalation process. Obtained results are in good agreement with that found by Guo and Al-Dahhan [26]. In this work, the EDX analysis of the fresh catalysts exhibited a nearly uniform distribution of iron throughout the external surface area. This indicates that the preparation procedure is efficient for obtaining highly dispersed metal-active species.

Fig. 3(b) shows several scanning electron micrographs of used catalyst. With a 1400 \times magnification one can observe different morphology crystals from those of fresh catalyst with the formation of some life-like material on the used catalyst crystals surface as seen in comparing fresh and used catalyst. EDX analyses of these crystals reported the presence of elements C, Al, Si, Fe, Ca, Na, Mg,

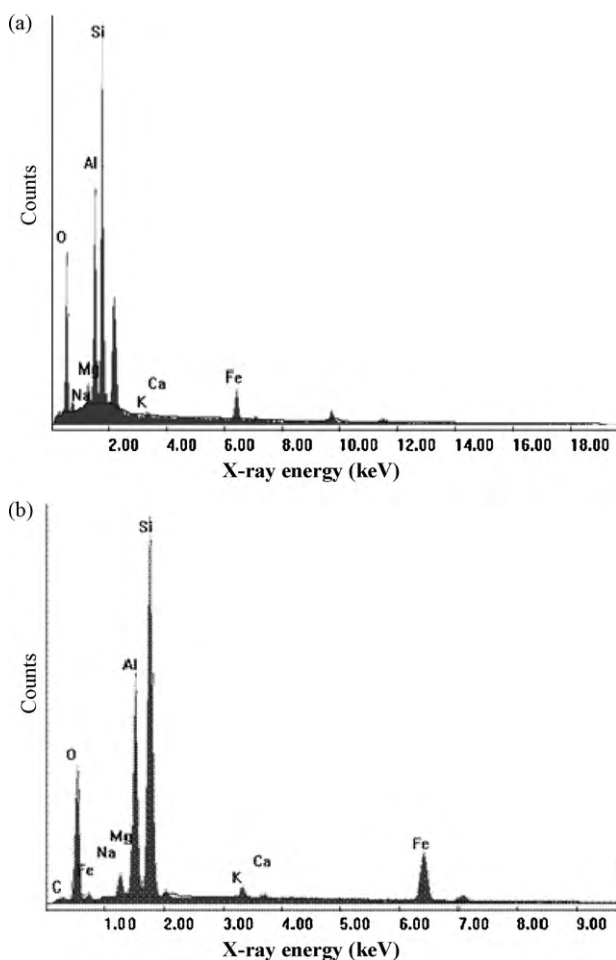


Fig. 4. The EDX qualitative analysis of the fresh (a) and used (b) (Fe–Al)PILC catalyst collected after 8 h WHPCO operated at 50 °C. The horizontal axis represents X-ray energy (0–18 keV) and the vertical axis shows X-ray fluorescence intensity.

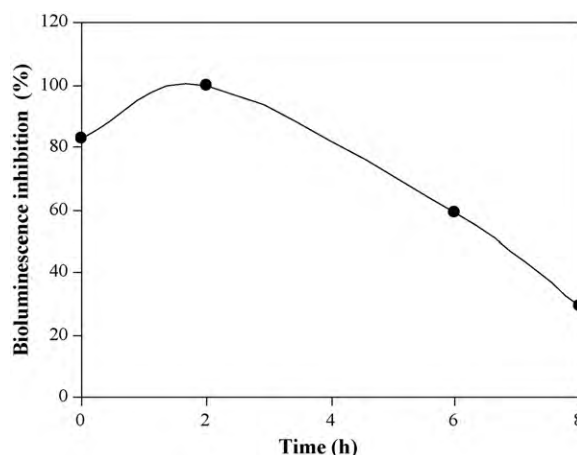


Fig. 5. Toxicity curve of OMW throughout the WHPCO pre-treatment. Experimental conditions: 1.25 g L⁻¹ initial phenol concentration, 0.5 g L⁻¹ (Al–Fe)PILC, 2×10^{-2} M H₂O₂, reaction temperature: 50 °C.

K and O (Fig. 4(b)). In contrast, all elements excluding C exist in fresh catalyst. The addition of element C to the spent catalyst suggests the formation of congregated carbon over the active site of Fe in the crystal through the Fe–O–C bindings. Parenthetically, elemental analysis was used to detect carbon adsorption in catalysts with EDAX instrument, since deactivation has been also attributed to the formation of carbonaceous deposits in the crystal surface irreversibly adsorbed on active sites [43]. However, in our study low values of carbon were detected in the recovered (Al–Fe)PILC catalyst (<1% C, w/w).

3.3. Effect of WHPCO pre-treatment on anaerobic digestion

The high COD value of OMW suggests the best perspectives of anaerobic biological treatment that can remove efficiently the high organic load and producing valuable energy [44]. However, difficulties in the anaerobic treatment of OMW are mainly connected with high organic concentrations and, first and foremost, the presence of biorecalcitrant and/or inhibiting substances, essentially phenolic compounds which can be removed by a mild oxidation in the first step into biodegradable intermediates, which can then be subsequently treated biologically. Since the WHPCO of OMW reduced considerably the total phenolic compounds concentration, catalyst-free aliquots from the reaction mixture operated at 50 °C were sampled at regular time intervals in order to evaluate their acute toxicity towards the bioluminescent bacteria *V. fischeri*.

Fig. 5 reports the toxicity curve of OMW throughout chemical pre-treatment reaction time. The acute toxicity to marine bacteria *V. fischeri* increased during the first 2 h of the catalytic reaction, probably due to the accumulation of toxic intermediates [24]. However, as the reaction proceeded, a significant reduction in the toxicity of pre-treated OMW was obtained ($I_B = 30\%$ after 8 h catalytic pre-treatment); pointing out that the WHPCO resulted in the transformation of toxic phenolic compounds into non-toxic and biodegradable intermediates such as organic acids, which is confirmed by the decrease of pH during the reaction test from 5.2 to 4.5 (data not shown).

Afterwards, this effluent was also treated by a combined WHPCO–anaerobic process. The anaerobic digestion was carried out using untreated OMW and OMW pre-treated in the presence of 0.5 g L⁻¹ catalyst and at 50 °C. Fig. 6 shows the variation of the volume of cumulated biogas as a function of time in the anaerobic digestion of the untreated OMW and pre-treated OMW withdrawn at regular time intervals (2, 4, 6 and 8 h reaction time), respectively. Except for OMW sample withdrawn after 2 h reaction time,

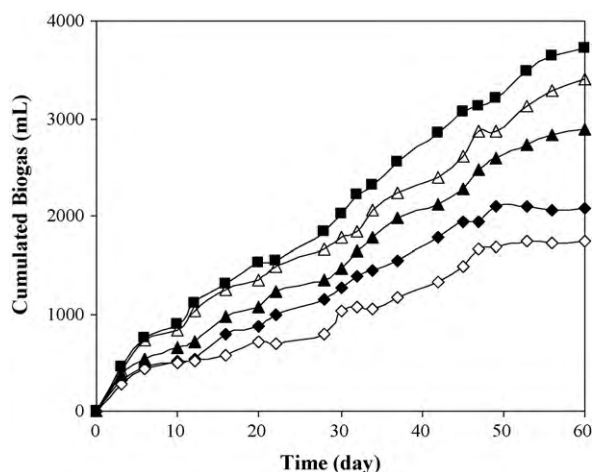


Fig. 6. Cumulated Biogas versus fermentation time in methanogenic tests on OMW after different WHPCO pre-treatments intervals: (◆) 0 h, (◇) 2 h, (▲) 4 h, (△) 6 h, (■) 8 h; WHPCO experimental conditions: 1.25 g L^{-1} initial phenol concentration, 0.5 g L^{-1} (Al-Fe)PILC, $2 \times 10^{-2} \text{ M H}_2\text{O}_2$, reaction temperature: 50°C .

these tests confirm the effectiveness of the pre-treatment. Indeed, the biogas production from untreated OMW showed a saturation profile, while the pre-treated OMW maintained satisfactory biogas production without remarkable inhibition effects. The biogas production was found to decrease for 2 h pre-treated OMW with respect to the untreated OMW. This result is in a good agreement with the microtoxicity test that shows toxicity increase after 2-h pre-treatment. The biomethanisation process was found to be stable during 60 days of operation. The biodegradability enhancement of the treated effluent may be attributed to the reduction of the total phenolic compounds concentration of the waste and of the associated toxicity, which facilitates the anaerobic digestion of the OMW. Enhancing biological treatment used as a post treatment for catalytically pre-treated OMW have been already reported in the literature for both wet air catalytic oxidation [1] and WHPCO [16] of raw OMW.

4. Conclusion

The main goal of this study was to investigate the combined process (WHPCO/biomethanisation) performance for OMW treatment. The strong influence of (Al-Fe)PILC/ H_2O_2 system at 50°C on OMW detoxification was clear. Experimental results showed that with WHPCO operated at a reaction temperature of 50°C for more than 2 h, it's possible to improve significantly the biomethanisation of the OMW. The use of this heterogeneous catalyst rapidly transformed the bioresistant phenolic compounds, decreased both initial COD and colour concentrations. As a consequence, the oxidized wastewater was much less toxic and more amenable to further anaerobic digestion if compared to the non-treated wastewater. This resulted in an enhancement in the yield of biogas production. A process combining catalytic oxidation operated at 50°C for OMW detoxification, followed by anaerobic digestion process may be a promising technology for continuous industrial depollution process.

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