JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 5151−5154

Olive Oil Mill Wastewater Treatment Using a Chemical and Biological Approach

ANTONIO FIORENTINO,[†] ALESSANDRA GENTILI,[†] MARINA ISIDORI,*,[†]
MARGHERITA LAVORGNA,[†] ALFREDO PARRELLA,[†] AND FABIO TEMUSSI[‡]

Dipartimento di Scienze della Vita, Seconda Università di Napoli, Via Vivaldi 43,
I-81100 Caserta, Italy, and Dipartimento di Chimica Organica e Biochimica, Università Federico II,
Via Cinthia 4, I-80126 Napoli, Italy

Olive oil mill wastewaters (OMW) are recalcitrant to biodegradation for their toxicity due to high values of chemical oxygen demand (COD), biological oxygen demand (BOD), and phenolic compounds. In the present study OMW, collected in southern Italy, were subjected first to a chemical oxidative procedure with $FeCl_3$ and then to a biological treatment. The latter was performed in a pilot plant where mixed commercial selected bacteria, suitable for polyphenols and lipid degradation, were inoculated. The effect of treatments was assessed through COD removal, reduction of total phenols, and decrease of toxicity using primary consumers of the aquatic food chain (the rotifer *Brachionus calyciflorus* and the crustacean *Daphnia magna*). The results showed that the chemical oxidation was efficacious in reducing all parameters analyzed. A further decrease was found by combining chemical and biological treatments.

KEYWORDS: Polymer supported oxidation; biological treatment; OMW; toxicity testing

INTRODUCTION

Italy and other countries of the Mediterranean basin are the main olive oil producers. During olive oil production large quantities of strongly polluted waters, known as olive oil mill wastewaters (OMW), are generated (40−55 L/100 kg of olives processed by traditional batch press or 80−120 L/100 kg of olives processed by continuous solid−liquid centrifuge system). OMW are resistant to biological degradation because of their volatile solids, inorganic matter, and high concentration of phenolic compounds (*1*). Due to these characteristics, which increase the organic load of chemical oxygen demand (COD, 80−200 g/L) and biological oxygen demand (BOD, 50−100 g/L) to values ∼200−400 times higher than those of a typical municipal sewage (*2*), the annual disposal of several million cubic meters of OMW is a major environmental problem for agriculture in the Mediterranean area. Indeed, the discharge of large quantities of this pollutant in the sewage system is not possible without any treatment, and Italian regulations allow its spreading on agricultural soil up to 50 m^3/ha for OMW obtained by press and 80 m^3/ha for OMW obtained by centrifuge (*3*). The untreated release of OMW on land produces potential danger for the surrounding environment, and several studies have shown that simple OMW phenolic compounds of low molecular weight are responsible for toxicity on seed germination (*4*), aquatic organisms (*5*−*7*), and bacteria (*8*). For these reasons, different biological and chemical/physical methods have been proposed to reduce the organic matter, polyphenols, and tannins present in OMW in order to avoid the toxic effects on the environment. Thus, oxidation systems have been often used as pretreatment to decrease OMW toxicity and allow biological degradation. Beltran et al. (*9*) utilized ozone alone and combined with hydrogen peroxide or UV radiation for the chemical OMW oxidation as useful pretreatments before an aerobic biological oxidation step. These processes allowed high COD reductions, nearly complete disappearance of aromatic content and color, and moderate total carbon reductions. Other studies demonstrated the utility of photo-Fenton pretreatment for recalcitrant wastewaters (*10*, *11*). Most of the chemical processes have proved their worthiness in the elimination of toxic compounds. On the other hand, these kinds of treatments have been shown to be expensive when compared with biological ones. Furthermore, the biological treatments are, at present, the most compatible with the environment. Thus, Pinto et al. (*12*) reported the removal of low molecular weight phenols from olive oil mill wastewater using microalgae, and, in a recent work, OMW were subjected to biological degradations with aerobes and facultative aerobic bacteria without strict anaerobes (*13*). However, it is important for biologically recalcitrant compounds to develop efficient chemical pretreatment processes that reduce the toxicity, increase the biodegradability of the substances, and lead the pretreated wastewaters to a biological treatment. Different studies have combined chemical oxidation and biological systems to treat biorecalcitrant pollutants with potential advantages for water treatment (*14*, *15*).

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* Corresponding author (telephone +39 0823274565; fax +39 0823 274571 e-mail marina.isidori@unina2.it).
[†] Seconda Università di Napoli.
[‡] Università Federico II.

10.1021/jf049799e CCC: $27.50

In this paper we first describe the application of two oxidation methods by polymer-supported reagents and then the combination of the most effective one with an advanced biological process based on the use of selected bacteria. The use of the immobilized oxidants on solid phase (*16*) is an advantageous technique due to the simple handling of the reagents, the opportunity of recycling, and the possibility to control the reaction and the product yields, reducing the formation of oxidation byproducts.

The aim of this work was to verify if the combination of chemical and biological processes could decrease the high potential hazard of OMW.

To verify the efficiency of all the processes COD and total phenols analyses were performed for the different approaches. Furthermore, the toxic potential and its reduction were evaluated through tests on typical consumers of the freshwater food chain: the rotifer *Brachionus calyciflorus* and the crustacean *Daphnia magna*, determining the $L(E)C_{50}$ (OMW percentage able to have an effect on 50% of the exposed organisms, lethality for rotifers, and immobilization for crustaceans).

MATERIALS AND METHODS

OMW Collection. Olive oil mill wastewaters were collected from a mill located in Campania, southern Italy, during the oil-harvesting season (from November to February) in plastic containers and kept in the dark at 4 °C. The main physicochemical characteristics of the utilized OMW were as follows: pH 4.2 ± 0.4; COD $= 150{,}000 \pm 12{,}000$ mg/L; BOD $= 80{,}000 \pm 10{,}000$ mg/L; total phenolic compounds $= 1200 \pm 170$ mg/L; total nitrogen $= 930 \pm 70$ mg/L; toxicity units (TU) $= 100/L(E)C_{50}) = 4000 \pm 130$ for rotifers and 4200 ± 160 for crustaceans. The reverse osmosis (RO) fraction was obtained after OMW fractioning as previously described (*4*). In this fraction the total phenolic compounds were 1000 ± 160 mg/L, low molecular weight phenols < 300 Da.

Periodate-Supported Polymer Preparation. To prepare the polymer-supported periodate, 20 g of Amberlite anion exchange (IRA-416), containing a quaternary ammonium group, were washed with several solvents in the order 500 mL of distilled water, 500 mL of dichloromethane, 500 mL of acetone, and 250 mL of toluene and successively dried in vacuo.

Then 500 mL of an aqueous solution of sodium periodate (6×10^{-3} M) was added to the resin, and the mixture was kept in magnetic stirring for 3 h at room temperature. The reagent was then filtered, washing first with 250 mL of water and then with 250 mL of acetone.

OMW Treatment with Polymer-Supported Periodate. Three hundred grams of polymer-supported periodate reagent was added to 1 L of OMW. The mixture was kept in magnetic stirring for 5 h, at 70 °C and successively filtered on a Gooch funnel.

Polymer-Supported Fe(III) Preparation. To prepare the polymer-supported Fe(III), 20 g of Amberlite cation exchange (IR-120), containing a sulfonic group, was washed with several solvents in the order 500 mL of distilled water, 500 mL of dichloromethane, 500 mL of acetone, and 250 mL of toluene and successively dried in vacuo.

Subsequently 500 mL of an aqueous solution of ferric chloride (0.4 M) was added to the resin, and the mixture was kept in magnetic stirring for 3 h at room temperature. The reagent was then filtered, washing first with 250 mL of water and then with 250 mL of acetone. Titration of polymer-supported Fe(III) was performed with a standardized $Na_2S_2O_3$ solution (0.050 N). One gram of reagent contains 0.7 mequiv.

OMW Treatment with Polymer-Supported Fe(III). One hundred grams of polymer-supported Fe(III) reagent was added to 1 L of OMW. The mixture was kept in magnetic stirring for 5 h, at 70 °C, and successively filtered on a Gooch funnel.

This procedure was utilized for the oxidation of the reverse osmosis fraction (RO), the low molecular weight fraction of OMW obtained as previously described (*4*).

HPLC Analysis. The RO treatment with polymer-supported Fe(III) reagent was followed, injecting an aliquot in an HPLC-UV system utilizing an RP-18 column. The column was equilibrated with a mixture of A (H_2O containing 1% acetic acid)/B (methanol containing 1% acetic acid) 9:1 (v/v) and using the following program: isocratic run for 25 min, followed by an increase of B to 60% in 30 min and a decrease to 10% in 5 min. The detector wavelength was set at 260 nm.

OMW Biological Treatment. The biological treatment was carried out in two activated sludge pilot plants in bench scale (Bio Kontrol mark2, Isco, Italy). These plants consisted of two Plexiglas tanks (oxidation and sedimentation ponds) filled with 12 L of the synthetic wastewater as indicated by the procedure 82/243 CEE/82 [160 mg of peptone, 110 mg of beef extract, 30 mg of $CO(NH_2)_2$, 7 mg of NaCl, 4 mg of $CaCl_2 \cdot 2\,H_2O$, 2 mg of $MgSO_4 \cdot 7H_2O$, and 28 mg of K_2HPO_4, per liter of dechlorinated drinking water], to which 2 g/L of bacterial inoculum was added. Octel Gamlen Industries, Vernon, France, supplied lyophilized bacteria. The inoculum was constituted by the formulation GTX plus IP20, the first containing microorganisms selected for degradation of oil and lipids, the second for degradation of phenols and polyphenols. GTX plus IP20 was found to be suitable for biological degradation of OMW in a previous work (*13*). The pilot plants were fixed with air flow of 2 L/min to guarantee no less than 60% oxygen saturation in the oxidation tank. Because of the high polluting load, the pilot plants were run with the total recycling of sludges from the sedimentation pond (60 mL/min). Both sludge-free and oxidized OMW were poured in the respective pilot plant at concentrations that did not interfere with bacteria activity (COD $= 3000-5000$ mg/L; pH $7.2-7.4$; total phenols < 30 mg/L; bacterial load $= 10^7$ UFC/mL). These conditions were used as starting time, and the treatment was protracted for up to 4 days. Samples of sludge-free and oxidized OMW were drawn, after sedimentation, to analyze COD, total phenols, and toxic potential every 24 h.

Chemical Parameters. The COD was determined by oxidation with potassium dichromate solution at 148 °C for 2 h and titration with ferrous ammonium sulfate of remaining unreduced dichromate as reported by 5220B of Standard Methods (*17*), whereas total phenols were determined by 4-aminoantypirine in the presence of potassium ferric cyanide by photometry as reported by 5530D of the same methods (*18*).

Toxicity Assessments. Acute toxicity bioassays were performed on two consumers of the freshwater chain, the rotifer *Brachionus calyciflorus* and the crustacean cladoceran *Daphnia magna* supplied, respectively, in cysts and in ephippia by MicroBioTest, Nazareth, Belgium. Organisms were exposed to the filtered wastewater exiting the pilot plants every 24 h for 4 days.

Exposures of daphnids were initiated with organisms <24 h old and conducted for 24 h at 20 °C following the 6341 International Standard Organization procedure (*19*) for acute toxicity tests. The effective endpoint was immobilization (EC_{50}).

Rotifers (age $= 0-2$ h) were exposed for 24 h at 25 °C in the dark following the E1440 American Society for Testing and Materials procedure (*20*). The effective endpoint was mortality (LC_{50}).

Data were analyzed using the Toxcalc (Toxicity Data Analysis and Database Software, 1996) to find the LC_{50} for rotifers and the EC_{50} for daphnids. Toxicity values were then converted into toxic units (TU) $= 100/L(E)C_{50}$.

RESULTS AND DISCUSSION

To investigate the possible reduction in toxicity of the olive oil mill wastewaters, two oxidation methods using polymer-supported reagents were performed in preliminary investigations. These processes were followed by the evaluation of toxicity of untreated and treated OMW for the rotifers.

Literature data reported that $NaIO_4$ is a good selective oxidant (*21*), when immobilized on alumina, oxidizing alcohols and sulfates, whereas when immobilized on silica, transforming hydroquinones to quinones (*22*). In the first oxidative method, OMW was treated with polymer-supported periodate. To immobilize the oxidant reagent, we prepared the periodate-supported polymer by adding an aqueous solution of sodium periodate to an anion-exchange resin. The polymer-supported

Table 1. Phenol Removal Calculated by HPLC

compound	removal (%)
protocatechuic acid	20
hydroxytyrosol	72
catechol	53
tyrosol	10
4-hydroxybenzoic acid	10
vanillic acid	2

periodate reagent was added to OMW, and the mixture was kept in magnetic stirring for 5 h at 70 °C and successively filtered. A slight clarification of the oxidized OMW was observed after the filtration. The treated wastewaters, assayed on *B. calyciflorus*, showed a reduction in toxicity compared with untreated OMW by 45.8%. Chemical oxygen demand is also an important parameter, which was followed to determine how substances' degrees of oxidation change, such as the concentration of phenols, in order to know the amount of remaining pollutants. In this treatment COD and phenols were reduced by 33 and 41.8%, respectively.

The second oxidative method used was the treatment with polymer-supported Fe(III). It is well-known that Fe(III) may be utilized for oxidations of organic compounds in aqueous phase (*23*). To immobilize the oxidant reagent, we prepared the Fe(III)-supported polymer by adding an aqueous solution of ferric chloride to a cation-exchange resin. Also in this case the polymer-supported Fe(III) reagent was added to OMW, and the mixture was kept in magnetic stirring for 5 h at 70 °C and successively filtered. A more evident clarification of the treated OMW was observed after the filtration. The treated wastewaters, tested on *B. calyciflorus*, showed a toxicity reduction by 82.5%. Furthermore, the COD and the total phenols analyses showed a decrease of COD by 53% yield and a rate of phenol removal by 76.4%. It must be emphasized that the resin can be reused after appropriate washings with water.

To understand which phenols were removed in the treatment with polymer-supported Fe(III), we performed this oxidation method also on a fraction obtained from OMW by reverse osmosis containing low molecular weight compounds. The oxidized RO fraction was injected in an HPLC system, and we observed a great decrease of the phenols, particularly of catechol and hydroxytyrosol (**Table 1**). These results are very meaningful because catechol and hydroxytyrosol are among the most abundant, recalcitrant, and toxic phenols present in OMW (*4*, *7*).

The results of toxicity reduction indicate that OMW oxidation with $FeCl_3$ is the most efficient among the proposed oxidative treatments. The oxidative action inactivates the most toxic phenols toward rotifers so that from 4.07 TU/mg of total phenols in untreated OMW, this value decreases to 1.68 TU/mg of total phenols in oxidized OMW.

The high abatement efficiency, achieved by the latter process, determined that only $FeCl_3$-oxidized OMW have been subjected to the subsequent biological degradation to verify the effectiveness of the combined chemical and biological treatment.

As already reported under Materials and Methods, the selected microbial mixture (GTX plus IP20) used in pilot plants was found to be suitable for the biological degradation of OMW. In fact, the results reported in a previous paper (*13*) showed that the COD of crude OMW was reduced by 85% with a final concentration comparable to that of urban wastes, generally between 250 and 1000 mg/L. In the same work, phenols were reduced by 67.4%. Accordingly, toxicity, prevalently due to phenol content, was reduced by 43.3% for rotifers, among the major organisms present in a sewage treatment plant, and by 86.2% for crustaceans, even if the initial toxic units were comparable (TU rotifers = 111; TU crustaceans = 114). From this starting point, $FeCl_3$-pretreated OMW were put into the pilot plant and processed in the conditions above-reported. The COD reduction, total phenols removal, and the toxicity have been followed during 4 days, and the results are reported in **Table 2**. The combined chemical and biochemical oxidation was effective for obtaining high COD abatement, demonstrating the modification of persistent organic compounds into biodegradable fractions. In fact, the biological treatment of the oxidized OMW reduced the COD by a further 62.5% when compared with the only chemical process. Phenols, which are particularly resistant to the biological degradation, were reduced by an additional 60.2%, showing that the chemical oxidation is a useful method to treat recalcitrant pollutants before the aerobic biological oxidation step. Because of the high toxic potential of phenols on aquatic organisms, their reduction also determines the decrease of OMW toxicity on rotifers and crustaceans by a further 30% in TUs.

Table 2. Percent Removal of the Chemically Oxidized OMW in the Biological Pilot Plant after the Indicated Time

	biological treatment after chemical oxidation				
		mean reduction in %			
parameter	starting value	1 dd	2dd	3dd	4dd
COD (mg/L)	4000 ± 210	37.5	51.3	60.9	62.5
total phenols (mg/L)	16.2 ± 7	14.8	25.0	46.1	60.2
TU *B. calyciflorus*	27.3 ± 4.6	5.6	19.4	22.2	30.6
TU *D. magna*	28.2 ± 6.8	9.8	21.4	26.8	33.0

In conclusion, among the utilized chemical approaches, OMW oxidation with $FeCl_3$ supported on Amberlite IR-120 determined a considerable decrease of toxicity (by ∼82%) in 5 h. Chemical oxidation with $FeCl_3$ is particularly efficacious on the phenolic fraction. This chemical pretreatment process, reducing the toxicity and increasing the biodegradability of the recalcitrant compounds, permits an efficient biological treatment of OMW. The biological treatments are, at present, the most compatible with the environment and the least expensive, even if not always applicable. The combination of chemical and biological processes may result in the best compromise between time and cost for the treatment of OMW.

Beneficial effects of such two-step treatments, obtained from laboratory studies, suggest potential advantages for a future use in agricultural waste management.

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Received for review February 5, 2004. Revised manuscript received June 15, 2004. Accepted June 20, 2004. This work was supported by MIUR (L. 488/92) within the framework of Project P2 "Ambiente terrestre: Chimica per l'Ambiente" (Cluster 11-A) of Consorzio Interuniversitario "La Chimica per l'ambiente".

JF049799E