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Improved Combined Chemical and Biological Treatments of Olive Oil Mill Wastewaters

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A novel system was investigated, finalized to reduce the impact of highly polluting wastewaters, and based on combined actions of catalytic oxidations and microbial biotechnologies. Olive oil mill wastewaters (COD 10 000−100 000 mg O_2/L) were oxidized up to 80−90% by stoichiometric amounts of dilute hydrogen peroxide (35%) and in the presence of water soluble iron catalysts, either Fe(II) or Fe(III), at concentrations up to 1% w/w and more, i.e., much larger than those reported for conventional Fenton processes. In the combined action, the mineralization activity of a selected microbial consortium was used to degrade residual volatile and nonvolatile organic compounds into CO_2 and biomass. The results of this search could suggest an improved operational methodology capable to reduce the potential impact of wastewater.

KEYWORDS: Fenton's reagent; iron; hydrogen peroxide; olive oil mill wastewater; microbial degradation

INTRODUCTION

Recent reports continue to point to an increasing and alarming reduction of the quality of waters to be used by humans and also of the level of fertility of soils, especially in the Mediterranean areas, in conjunction with increasing agricultural practices not always concerned to the conservation of the ecosystems. Olivoculture and production of olive oil represent one of the most important and old agricultural activities in the Mediterranean countries; nevertheless, a series of environmental issues, such as the recovery and detoxification of the effluents from olive oil mill plants, appear far to be resolved. These effluents (olive oil mill wastewater or vegetation waters, OOMW) are relatively small in volume but concentrated during the ripening of the olive fruits (usually in late fall/winter) within short time intervals. OOMW is a highly polluting wastewater, with very high values of BOD and COD, low values of pH, and high contents of phenols, polyphenols, and tannins, for which a definite phytotoxicity toward the main agricultural cultivations and, in general, the conventional wastewater treatment plants is ascertained (*1*). The development of environmentally acceptable methods for disposal of OOMW still remains a problem, whereas the degradation of the toxic compounds contained in this wastewater will certainly enhance the quality of the remediated waters and of the sedimentation muds, in view of their safe utilization as fertilizers (*2*, *3*).

Degradation of organic pollutants in wastewater by combined chemical and biological oxidation is becoming a successful alternative to conventional treatment technologies. In this way, the quantitative degradation of the recalcitrant organics into CO_2 and H_2O by strong (and extensive) chemical treatment becomes no more necessary, the new goal being a moderate transformation by chemical means of the recalcitrant organics into more easily attackable end products, which can be subsequently submitted to conventional or advanced (microflora and biomass disperse or adhere) biological treatments (*4*). Many advanced chemical oxidation technologies (*5*) are based on the production of hydroxyl radicals, possessing an extremely high oxidation potential (+2.73 V); to reduce the temperatures (and hence the pressures) required for wet oxidation, it has been proposed for a long time to treat the organically polluted wastewaters with Fenton reactant, i.e., hydrogen peroxide in the presence of iron salts (*6*, *7*). In the Fenton process, the completion of the oxidation is dependent on the hydrogen peroxide/organic pollutant ratio (usually 2 to 1 equiv/equiv), on the catalyst to peroxide ratio (ca. 1 to 10 mol/mol) (*8*), and on pH (between 3 and 4), while the rate of oxidation is determined by the initial iron(II) concentration and the temperature. Usually, the amount of added iron salts is low, less than 20 mM, but there are examples of Fenton processes running at higher concentrations, up to 60 mM (*9*). Comprehensive recent analyses of Fenton reaction for water purification and recovery are reported (*10*−*13*), whose yields and rates can be enhanced by assisting the reaction photochemically (*14*) or electrochemically (*15*).

Given sufficient time, a long list of chemicals can be destroyed completely by the Fenton process, apart, and rather surprisingly, some common carboxylic acids, such as acetic, maleic, and fumaric acid, and acetone, which is often found among the effluent of the Fenton treatment, by being formed

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in situ by oxidation of a variety of precursors. The higher recalcitrance to the Fenton treatment of the above compounds prompted us to extend the study on the detoxification of OOMW to the biological treatment, for which the presence of simple organic compounds in the previously chemically treated reaction mixtures could be beneficial. The main goal of the experimentation was to evaluate, on a laboratory scale, the possible advantages, in terms of efficacy and depolluting effectiveness, of a combined and synergic action of a catalytic oxidation treatment in liquid phase by using an iron/hydrogen peroxide system together with advanced microbial biotechnologies. It should be noted that there is a large amount of recent literature dealing with treatment of OOMW with both Fenton (*16*) and biological methods (*17*).

MATERIALS AND METHODS

Sample and Sampling. Fresh OOMW effluent was obtained from a two phase continuous oil mill plant, located at Rosciano (Pescara, Italy). Samples were collected in December at a point of good mixing from the effluent of the horizontal decanter. Fresh samples were stored at 4 °C during the transport to the laboratories and immediately analyzed; aliquots of crude OOMW were diluted with sterile tap water to the required values.

Chemical Oxidation Procedure. Research grade ferrous sulfate heptahydrate, ferric sulfate hydrate, and hydrogen peroxide (35%) were purchased from Aldrich. No preliminary treatment of OOMWs was made prior to the experiments; the samples were not filtered, and the pH was let free to adjust, depending on the concentration of the iron salts added (pH around 3). Typical experiments were carried out in 10 mL vials, systematically repeated also in a 10 L plastic tank, and placed on stirrer plates; in the latter cases, the reaction mixture consisted of 500 mL of OOMW, to which a weighted amount of the appropriate iron salts was added as a solid and dissolved under stirring. All of the oxidation experiments were performed at least in duplicate. Hydrogen peroxide was directly transferred from the original commercial bottle (35%) in the appropriate ratio to the measured CODs of OOMW (usually 1:1 equiv:equiv). Oxidation was usually immediate and highly exothermic, with the temperature rapidly rising up to the boiling point. To prevent excessive heating, hydrogen peroxide was added slowly, by small portions (10% of the total amount), and always waiting until the tumultuous chemical reaction had finished (5−15 min); the reaction exhibited induction periods, particularly if the initial temperature was kept below 18 °C, and may therefore start suddenly, especially if hydrogen peroxide in the reaction mixture is concentrating, due to continued injection of the reagent. After the addition of the oxidant was completed, the reaction mixtures were cooled to room temperature and then analyzed by COD; if residual hydrogen peroxide was still present in the reaction mixtures, its disproportionation into oxygen and water was induced prior to the analyses, by adding small amounts of K_2RuCl_6 (it should be recalled that ruthenium derivatives are among the most effective dismutation agents for hydrogen peroxide) (*18*). Oxidation of acetic and formic acid and of acetone (0.1 M) was carried out in the presence of iron(III) sulfate (50 mM) and of a large excess of H_2O_2 (3.3 M); the reaction (far less exothermic than the previous ones) was followed by gas chromatography−mass spectrometry (GC-MS) and ^{1}H NMR.

Chemical Analyses. Organic analyses were performed on a HP 6890 GC instrument (Hewlett-Packard, U.S.A.) equipped with flame ionization detection, using a 30 m HP-5 capillary column (0.32 mm i.d.; 0.25 film thick) with the injection port thermostated at 250 °C (carrier gas, He) on aliquots withdrawn with a microsyringe from the aqueous reaction mixtures previously diluted 1:10 with acetone. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz (Bruker, Karlsruhe, Germany) equipped with a BBO 5 mm probe; a water suppression (Bruker made−zgcppr) pulse sequence was used; in addition, a reference consisting of a coaxial capillary, inside the NMR tube, filled with a 30 mM D_2O solution of 3-(trimethylsilyl)-2,2′,3,3′-d_4-propionic acid sodium salt, was used. The samples were analyzed after complete removal of dissolved (paramagnetic) iron by precipitation in the presence of alumina and bicarbonate. COD and BOD were measured on IRSA−CNR methods (*19*). Total phenols were determined by colorimetric assay (*20*).

Iron(III) Quantitation. Quantitation of the solubilized iron(III) was carried out as follows: an appropriate aliquot of the reaction solution (containing 1−30 mg of Fe) was neutralized with 0.1 M NaOH and filtered to separate $Fe(OH)_3$; the precipitate was redissolved with 0.1 M HCl, and the clear resulting solution was treated with 1 mL of 10% w/w aqueous hydroxylamine hydrochloride solution; after the pH was adjusted around 3.5 with CH_3COONa or CH_3COOH (both 20% w/w), 10 mL of a 0.1% w/w aqueous solution of 1,10-phenanthroline was added, diluted with water to 100 mL, and kept in the dark for 15 min. The red/orange resulting solution was analyzed with a spectrophotometer set at 510 nm, and the quantitation was performed by comparing the absorbance with a calibration curve.

Hydrogen Peroxide Evaluation. After precipitation of the iron by neutralization with 1 M NaOH, the resulting mixture was centrifuged and the obtained clear solution was reacidified with 1 M sulfuric acid down to pH 2; an excess of 1 M KI solution and 2−3 drops of 1% starch solution were added, and the whole mixture was kept in the dark; after 15 min, the formed iodine was titrated by a standard solution (0.1 M) of $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$. In the presence of a hydrogen peroxide/iron ratio ≥100, i.e., at the beginning of the reaction, the neutralization step is not necessary.

Biological Oxidation Procedure. The work was carried out using aerobic microbial degradation: the effluents after the chemical treatment were loaded into reactor, and the experiments were conducted as described above. OOMW samples (150 mL) enriched with fresh OOMW reinoculum (10% v/v) were transferred to 500 mL flasks and agitated in a rotary shaker at 300 rpms for 15 days. All tests were done at 28 °C.

Media and Microbial Groups. Total viable cell number of aerobic heterotrophic bacteria was evaluated in Standard Plate Count Agar at 37 °C after 72 h of incubation, for yeast and fungi in YPD, and for Malt Agar (Oxoid) at 28 °C, after 48 h, respectively (*21*). The quantitative determination of the viable microflora was done on several samples by a traditional culture technique, on the basis of colony forming units (CFU) and expressed as log CFU/mL. Ten-fold serial dilutions in 0.1% (w/vol) peptone water were carried out in triplicate in Petri dishes.

When required, the strains isolated were identified on the basis of physiological and biochemical characteristics according to the *Shorter Bergey's Manual of Determinative Bacteriology* (*22*). Besides the standard staining procedures (Gram staining), the biochemical characterization was performed using API test kits API 20 NE, API STAPH, and API 50 CHB by following the instructions of the supplier (bioMérieux Italia, Rome, Italy). Microbial growth and strains identification were confirmed by optical microscope (Axioplan, Zeiss) and scanning electron microscopy observation (*23*).

Biochemical and Enzymatic Assays. The ATP assay was performed according to Ahmed et al. (*24*), including a specific enzymatic kit (NRM/Lumit-Qm, code 9332-l; Lumac B. V., Landgraaf, The Netherlands) to release ATP from microbial cells in media and in matrixes. Samples of 1.0 mL were diluted (1:10 or more, if necessary) in a buffer solution (Tris-HCl, 0.025 M, pH 7.75). An aliquot (100 μL) of the solution was placed in a transparent vessel, to which 100 μL of NRM reagent was added for ATP extraction and delicately stirred. A 100 μL aliquot of Lumit-QM (luciferin−luciferase) reagent was added after 30 s, and after agitation for 10 s, the vessel was placed in the luminometer counting chamber. Luminescence, expressed in digital relative luminescence units (RLU), was then converted to ATP content according to the calibration curve obtained from pure standards. The luminometer used was a Biocounter 1500 P (Lumac B. V.) equipped with a photomultiplier tube set at 7.200 RLU with 200 pg of ATP in 100 μL of Lumit buffer and Lumit-QM reagent (*25*, *26*).

Impedometric Measurements. An aliquot (100 μL) of the above solutions (10^{-1} dilutions of primitive samples) was inoculated in 1.9 mL of sterile Standard Plate Count broth medium (bioMérieux Italia), by using sterile disposable modules consisting of 16 independent wells at the bottom of which were the measurement electrodes. Measurements of changes in impedance, expressed as detection time (DT) in hours

Table 1. Physical, Chemical, and Biological Parameters of OOMW Used in the Present Study

parameters	unit	values
pH		4.9–5.5
COD[a]	g/L	60–180
BOD_5[b]	g/L	20–55
total phenolic compounds	g tannic acid/L	4.0–5.0
total bacterial counts	log CFU/mL	1.7
total yeast counts	log CFU/mL	5.1
total fungi counts	log CFU/mL	2.0
ATP content	ng/mL	3.5
detection time	h	11.2
GI	%	26.0

[a] Chemical oxygen demand. [b] Biochemical oxygen demand.

(h), were recorded using a Bactometer M 128 (Bactometer Processing Unit, bioMérieux, Italia), which performs simultaneous incubation and reading every 6 min for each analysis on eight modules.

Phytotoxicity Test. The germination assay of selected seeds (*Lepidum sativum* L.) in water solution (50 and 75%) from sample extracts was measured according to the Italian Society of Soil Science and Ranalli et al. (*27*). The germination index (GI) at different dilutions (eq 1) and their averages (eq 2) were carried out according to the following relations:

$$\text{GI}\ (50-75\%)\ \% = \frac{\text{Gc} \times \text{Lc}}{\text{Gt} \times \text{Lt}} \times 100 \qquad (1)$$

where Gc = mean seeds germinated on sample, Gt = mean seeds germinated on control, Lc = mean length roots on sample, and Lt = mean length roots on control.

$$\text{GI}\% = \frac{\text{GI50\%} + \text{GI75\%}}{2} \qquad (2)$$

RESULTS

Chemical Treatments. Fresh OOMW samples were characterized according to the procedures described in Materials and Methods (**Table 1**). Initial kinetic studies on Fenton's reaction were conducted under conventional conditions, i.e., with iron(II) content in the 1−20 mM range (<0.1%, w/w). OOMW samples with COD varying between 10 000 and 38 000 mg/L (1.25−4.7 equiv/L) were treated with hydrogen peroxide, at oxidant to oxidizable ratios between 1 and 2 (equiv/equiv). In all examined cases, the reactions practically stopped within the first 24 h, leaving significant amounts of residual oxidizable and unreacted oxidant (**Figure 1a**); COD removals were always smaller than 30%, even after 1 week of stirring; the small COD removal observed in the absence of added iron can be attributed to the presence of small but significant amounts of iron salts in the original OOMW samples or, more simply, to the uncatalyzed oxidation.

Progressive addition of fresh iron(II) led to step by step removal of COD, up to 90% (**Figure 1b**), in agreement with the accepted role of Fe(II) as the effective catalyst for the oxidation; however, the role of Fe(III) cannot be underestimated, as revealed by the experiments described later. The good results obtained with the above procedure prompted us to set another series of experiments, aimed to examine the effects of higher concentrations of iron salts on COD removal, in the presence of stoichiometric amounts of H_2O_2 (equiv/equiv with respect to the amount of oxidizable material present in the samples, as measured by COD). The influence of the increasing iron concentrations on COD removal was not monotonic (**Figure 2**). In the presence of the same quantity of hydrogen peroxide (9.8 equiv/L, i.e., 4.9 mol/L), iron concentrations smaller than

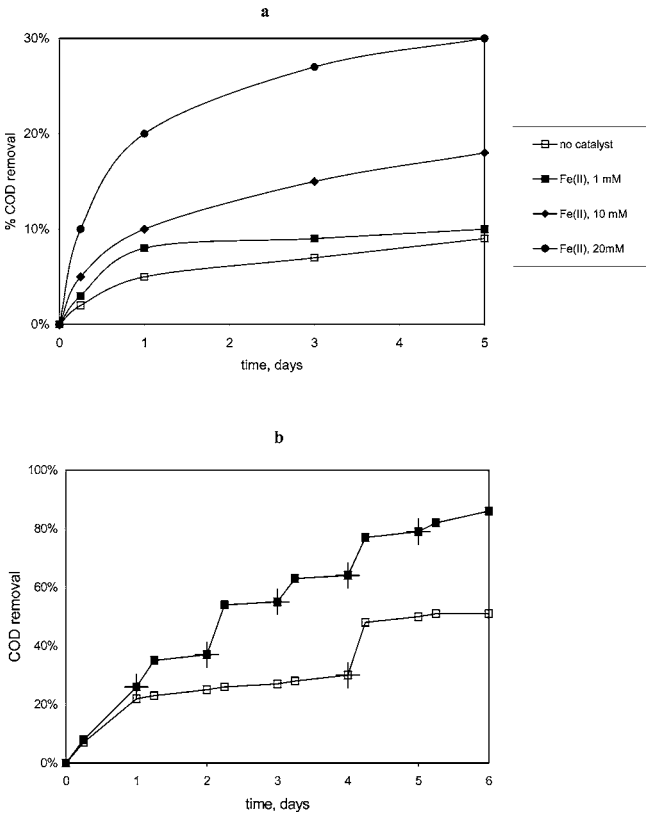

Figure 1. Time courses of Fenton treatment of OOMW. Reaction conditions: 10 mL of OOMW, 4.7 equiv/L; H_2O_2, 4.7 equiv/L (1:1 equivalent ratio) in the presence of $FeSO_4$. (**a**) Catalyst concentrations: solid circles, 20 mM; solid diamond, 10 mM; solid squares, 1 mM; and void squares, no catalyst added. (**b**) Catalyst concentration, 10 mM; crossed points indicate further addition of 10 mM portions of Fe(II).

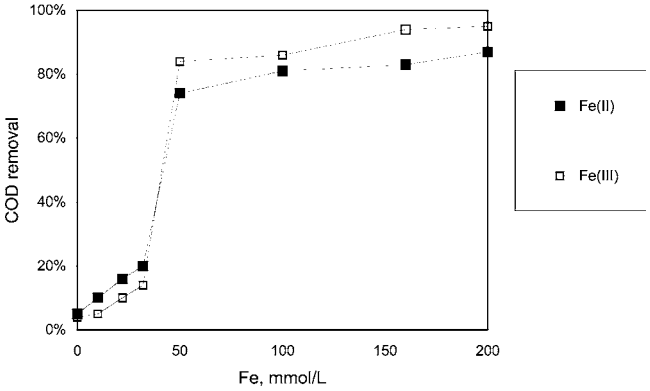

Figure 2. Fenton treatment of OOMW. Reaction conditions: 500 mL of OOMW, 9.8 equiv/L; H_2O_2, 9.8 equiv/L (1:1 equivalent ratio) added by portions; solid squares, Fe(II) added as $FeSO_4$; void squares, Fe(III) added as $Fe_2(SO_4)_3$; data after 1 h of reaction.

30 mM ($Fe:H_2O_2$ < 1:170, mol/mol) gave rise to limited COD removals, whereas in the presence of iron salts concentrations ≥ 50 mM ($Fe:H_2O_2$ ≥ 1:100, mol/mol), a dramatic enhancement of oxidation was observed. In these conditions, the oxidation took place almost instantaneously, with a massive release of CO_2 and violent heating of the reaction mixture, up to the boiling point of the solutions. A very effective COD removal (80%, in the presence of 50 mM iron) was thus achieved in very short times, even if yields improved only slightly with larger iron concentrations, up to 200 mM ($Fe:H_2O_2$ = 1:25 mol:mol) (**Figure 2**). It is important to note that the observed conspicuous heating of the reaction mixtures did not affect the efficacy of

Table 2. COD Removal and Chemical and Microbiological Parameters on OOMW after Chemical Treatment[a]

added H_2O_2 (%)[b]	COD residual (equiv/L) (COD removal, %)	TOC (mM)	CFU/mL (log)	total ATP (ng/mL)	DT (h)	total phenol (%)	GI (%)
0	11.40 (0)	nd	1.70	3.5	11.2	1.24	26.0
15	4.90 (59)	nd	absent	<0.001	>18	0.98	7.5
30	4.35 (62)	1260	absent	<0.001	>18	0.90	9.5
60	2.20 (81)	430	absent	<0.001	>18	0.40	10.0
100	1.70 (85)	135	absent	<0.001	>18	0.32	13.5

[a] Reaction conditions: OOMW, 500 mL (COD 11.4 equiv/L); Fe(III) added as $Fe_2(SO_4)_3$, 100 mM; H_2O_2, 35%, added by portions; parameters monitored after 1 h of reaction; nd, not determined. [b] Equiv of H_2O_2 per equiv of initial COD.

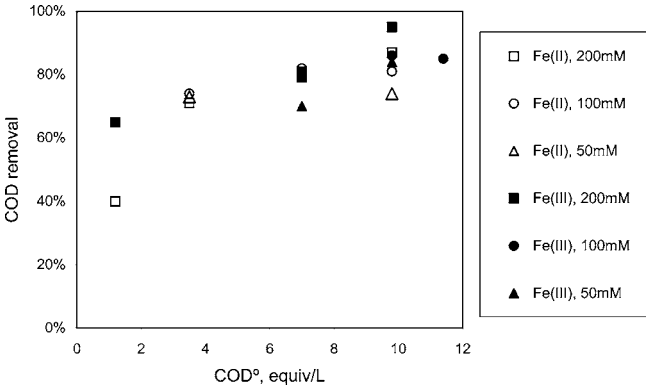

Figure 3. Fenton treatment of OOMW. Reaction conditions: 500 mL of OOMW; H_2O_2 added at small portions in 1:1 equivalent ratio. Fe(II) added as $FeSO_4$: void squares, 200 mM; void circles, 100 mM; and void triangles, 50 mM. Fe(III) added as $Fe_2(SO_4)_3$: solid squares, 200 mM; solid circles, 100 mM; and solid triangles, 50 mM. Data after 1 h of reaction.

the treatment, contrary to the common findings of a declining efficiency of peroxide utilization at temperatures over 40−50 °C, due to the accelerated dismutation of the oxidant. It was not possible to check the actual concentration of hydrogen peroxide during the reactions, due to the fact that the oxidant was always added at small portions; nevertheless, we always checked the residual amounts of hydrogen peroxide at the end of each addition, by finding that it was virtually absent. Moreover, it should be noted that Fe(II) and Fe(III) gave almost superimposable results; on the other hand, analysis of the oxidation states of iron in the reaction mixtures always indicated Fe(III) as the dominant species. Finally, addition of hydrogen peroxide in less than the stoichiometric amount always led to a COD removal higher than the oxidant added, thus suggesting involvement of aerial oxygen in the oxidation process (**Table 2**). On the contrary, addition of hydrogen peroxide in excess to the 1:1 equivalent ratio with respect to the oxidizable never led to 100% removal of COD, the excess of oxidant undergoing quick dismutation to oxygen and water. The efficiency of COD removal in the described process was highly dependent on the initial COD contents (**Figure 3**), as commonly found in Fenton treatments (*28*). Oxidation was indeed more effective with the most concentrated OOMW samples, strongly suggesting a delicate kinetic competition between oxidizing action and dismutation of hydrogen peroxide; arguably, oxidation rates of less polluted samples, i.e., with COD smaller than 2−4 equiv/L, were not competitive enough with dismutation, thus leaving a substantial amount of oxidizable untreated.

The total organic carbon (TOC) of the treated samples smoothly decreased with COD removal, indicating that the oxidation state of the residual organics increased during the reaction. Very tiny amounts of residual organic carbon were detected (**Table 2**), and this means that only a partial transformation to water and carbon dioxide occurred. A correct measure, for a quantitative purpose, of the TOC value in raw wastewater was not reliable because of the very high content of organics and the heterogeneous nature of the untreated samples. To ascertain the nature of the oxidized organic species, the reaction mixtures were carefully, but unsuccessfully, analyzed by GC-MS and ^{1}H NMR, thus indicating, as expected, the presence of highly oxidized and polar or ionic species, such as polycarboxylic acid. More information on the fate of simple organics was obtained by investigating the behavior of some model compounds, i.e., formic, fumaric, and acetic acids and acetone under the reaction conditions described in Materials and Methods. The experiments demonstrated the peculiar sensitivity of each substrate to the oxidation system, whereas formic and fumaric acid were almost immediately destroyed, with no organics identified both at NMR and at GC; acetone was oxidized very slowly (90% conversion after 6 h), giving rise to substantial amounts of acetic acid (18−20%); the latter was indeed the most recalcitrant of the examined substrates, with only a 60% conversion to CO_2, after 6 h.

In the wastewaters submitted to the chemical treatment, an increase of the phytotoxic effect was observed, with reduction of the GI parameter from the initial value of 26% to inclusive values between 7 and 14%, depending on the different intensities of the treatment; total phenols decreased markedly, down to 1−0.3%, even if they never disappeared. Moreover, complete absence of viable microorganisms (0 CFU/mL) was observed, even after mild treatments, i.e., with small amounts of added H_2O_2 (15% of the required); accordingly, ATP values were below 0.001 ng/mL with a DT over 18 h (**Table 2**).

Biological Treatment. The elevated content in total phenols in the OOMW samples, together with the acidic values of pH, contributed to the development of phytotoxicity, as indicated by the low GI values (materials with a GI under 40% are generally considered phytotoxic, whereas materials with GI > 70% are to be regarded free from phytotoxicity effect) (*27*).

The results obtained with a biological process based upon aerobic microbial degradation are reported in **Table 3**. The biological treatment of the fresh OOMW samples (**Table 3**) induced a variation (increase) of both the total bacterial position (log CFU/mL), from 1.7 to 5.48, and the content of ATP, from 3.5 to 9.01 ng/mL, with a significant decrease of the DT parameter, from 11.2 to 4.2 h. Moreover, an increase of GI was measured, from 26 to 45.5%, with a parallel decrease of the total phenols (from 1.24 to 0.76%) and a significant demolition of the COD (from 11.4 to 5.8 equiv/L).

OOMWs were also treated by a combined chemical−biological process, involving initial chemical treatments, as described previously, followed by an aerobic microbial degradation, in the presence of aeration and crude OOMW as reinoculum. Main chemical and microbiological parameters were monitored and are reported in **Table 3**. COD values were further abated by the biological treatment, with an apparent upper limit of 90% removal, which was achieved already by a "soft" chemical pretreatment (requiring 60% of stoichiometric hydrogen peroxide). Bacterial population of the resulting mixtures markedly increased (up to 3 orders of magnitude or more), accompanied, as expected, by an increase of ATP values. A microbiological investigation on the microflora selected during the biological treatment pointed to the presence of a bacterial population, both Gram-positive and Gram-negative, dominated by species belonging to *Bacillus* and *Pseudomonas* genera,

Table 3. COD Removal and Chemical and Microbiological Parameters on OOMW after Combined Chemical and Biological Treatments

chemical treatment[a]		biological treatment[b]	parameters after both treatments				
added H_2O_2 (%)[c]	COD residual (equiv/L) (COD removal, %)	COD residual (equiv/L) (COD removal, %)	CFU/mL (log)	total ATP (ng/mL)	DT (h)	total phenol (%)	GI (%)
	untreated sample	untreated sample					
0	11.40 (0)	11.40 (0)	1.7	3.5	11.2	1.24	26.0
	untreated sample						
0	11.40 (0)	5.80 (49)	5.48	9.01	4.2	0.76	45.5
15	4.90 (59)	4.30 (62)	4.62	8.60	4.9	0.44	71.5
30	4.35 (62)	3.25 (71)	5.39	8.72	4.0	0.32	78.5
60	2.20 (81)	1.20 (90)	5.24	8.10	4.6	0.27	81.0
100	1.70 (85)	1.15 (90)	4.39	8.44	7.3	0.21	76.5

[a] Reaction conditions as in **Table 2**. [b] Conditions, aeration (50 mL/min), inoculum of crude OOMW (30 mL); parameters monitored after 15 days of treatment. [c] Equiv of H_2O_2 per equiv of initial COD.

respectively. The Gram-positive rods (spore forming) were attributed to *Bacillus subtilis*; two Gram-positive cocci were attributed to *Micrococcus roseus* and *Micrococcus luteus*; among Gram-negative dominated *Pseudomonas fluorescens* and *Pseudomonas cepacia*. The bioelectrical test responses, based on impedometric changes due to microbial activities, exhibited low values after the combined treatment (4.9−4.0 h), in agreement to the well-known reverse correlation between DT response and microbial activity (*25*). The phytotoxicity results, expressed by responses of micronucleus *Vicia faba* apical roots, were around 80%, clearly related to the decrease of the total phenols content (down to 0.2%).

DISCUSSION

The present paper deals with an enhanced Fenton process for the oxidative degradation of organic pollutants in wastewaters, followed by a biological treatment. The chemical process is characterized in that the oxidation is carried out in the presence of large amounts of iron salts, either ferric or ferrous, higher than 0.05 mol per liter, and therefore far beyond the amounts usually reported for conventional Fenton processes. Under these conditions, and contrary to the common belief, oxidation of strongly polluted wastewater is definitely competitive with the dismutation of hydrogen peroxide, so that almost all of the added hydrogen peroxide is consumed to perform the abatement of COD, with very few organics left in the reaction mixtures. Moreover, because oxidation is very fast, no photochemical or electrochemical assistance is necessary. The marked exothermicity of the process can be controlled by gradually adding either the iron salt or the oxidant (hydrogen peroxide) to wastewater.

The observed break in the efficiency of the Fenton treatment at iron concentrations between 30 and 50 mM (around 0.1% w/w of Fe) is the most unexpected finding of this investigation, strongly suggesting that different mechanisms are taking place, when smaller or larger concentrations of iron are used. It is well-known that along with the generally accepted mechanism of Fenton reaction leading to the formation of hydroxyl radicals, in the recent years, several alternative hypotheses have been proposed, pointing to the participation of high valent oxo−iron complexes (oxygenated Fenton chemistry, *29*−*33*), as indeed earlier proposed by Bray and Gordon (*34*). Parallel reactions can be envisaged also for Fe(III), leading to high valent and highly reactive oxo−iron species (*35*).

However, the extreme efficiency of the present reaction does not allow us to distinguish whether a radical mechanism is operating, since common hydroxyl scavengers, such as 2-propanol, disappear almost immediately upon oxidation. Moreover, it should be stressed that the present Fenton system works effectively with a very unusual iron/hydrogen peroxide ratio (around 1/100, at a 1:1 oxidant to COD ratio, equiv:equiv).

The chemical treatment based on the described Fenton reaction indicates the possibility to abate rather effectively the polluting load of OOMW, up to 80−90% in terms of COD. However, the treatment results in the total disappearance of the viable microflora ("sterilization effect"), likely as a result of the direct action of hydrogen peroxide or of the formation of toxic intermediates. Substoichiometric amounts of the oxidizing reagent (modulated Fenton treatment) leads to a partial removal of COD and alters the chemical composition of the OOMWs in a more favorable way to the subsequent biological action. Even if longer times (15 days) are necessary, the biological treatment not only allows the attainment of further, even if less significant, demolition of the COD (up to 90%) but finally offers the immediate possibility to overcome the intrinsic low germinability of the wastewaters. Under our operational conditions, the final values of the GI parameter are always >70%, a limit that attests the absence of chemicals capable to inhibit the germination of the seeds test; these GI data must be compared not only with those of the samples treated only chemically (around 10%) but also with those of the samples treated only biologically, i.e., without a chemical pretreatment, still unsatisfactory, even if higher (around 40%). It is therefore reasonable that the chemical pretreatment effectively removes important organic compounds that inhibit the biological oxidation.

FINAL REMARKS

The main component of the costs of the proposed treatment is that of hydrogen peroxide (presently ca. 0.2 Euro per kg of 35% solution, i.e., 0.009 Euro/oxidation equivalent); therefore, a "soft" chemical pretreatment (60% of the stoichiometric demand) of an average OOMW with COD 80 000 mg/L (10 equiv/L) requires 54 Euro/mc, in terms of hydrogen peroxide consumed.

The other weak point of the treatment consists of the huge amounts of iron salts necessary, 50−100 mM, i.e., 2−5 g Fe/L, well beyond the standard accepted for iron in wastewater (2−4 mg/L) (*36*). However, it should be noted that a large part of the added iron separates from the solution at the end of the treatment as insoluble iron(III) hydroxide, which could be recovered in high yields (even quantitatively by slightly raising the oberved final pH, ca. 3, to 4). This point must not be underestimated, since the addition of the iron salts to the initial OOMW samples always yields clear solutions, likely because

of the complexing ability of the very concentrated organics present therein; in the course of the reaction, however, almost all of the organics is destroyed and the small residual amounts of the complexing agents are not able to maintain in solution significant quantities of iron.

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