

## Olive mill wastewater treatment: an experimental study

E. Bettazzi\*, M. Morelli\*\*, S. Caffaz\*\*, C. Caretti\*, E. Azzari\*\* and C. Lubello\*

\*Dipartimento di Ingegneria Civile, Università di Firenze, Via Santa Marta 3, 50139 Firenze, Italy (E-mail: [cecilia@dicea.unifi.it](mailto:cecilia@dicea.unifi.it))

\*\*Publiacqua, Via Villamagna 39, 50126 Firenze, Italy (E-mail: [s.caffaz@publiacqua.it](mailto:s.caffaz@publiacqua.it))

**Abstract** Olive oil production, one of the main agro-industries in Mediterranean countries, generates significant amounts of olive mill wastewaters (OMWs), which represent a serious environmental problem, because of their high organic load, the acidic pH and the presence of recalcitrant and toxic substances such as phenolic and lipidic compounds (up to several grams per litre). In Italy, traditional disposal on the soil is the most common way to discharge OMWs. This work is aimed at investigating the efficiency and feasibility of AOPs and biological processes for OMW treatment. Trials have been carried out on wastewaters taken from one of the largest three-phase mills of Italy, located in Quarrata (Tuscany), as well as on synthetic solutions. Ozone and Fenton's reagents applied both on OMWs and on phenolic synthetic solutions guaranteed polyphenol removal efficiency up to 95%. Aerobic biological treatment was performed in a batch reactor filled with raw OMWs (pH = 4.5,  $T = 30^{\circ}\text{C}$ ) without biomass inoculum. A biomass rich of fungi, developed after about 30 days, was able to biodegrade phenolic compounds reaching a removal efficiency of 70%. Pretreatment of OMWs by means of oxidation increased their biological treatability.

**Keywords** Fenton's reagent; fungi; olive mill wastewater; ozone; phenolic compounds

### Introduction

Olive oil extraction is one of the most important traditional food industries in the Mediterranean, and it still has a primary importance from an economical point of view. Mediterranean countries account for approximately 95% of the world olive oil production, corresponding to about 11 millions tons of olives produced per year and 1.7 million tons of extracted oil (Aktas *et al.*, 2001). In addition to olive oil, this industry produces by-products, in particular wastewaters and olive husks, which represent a serious environmental problem. Olive oil mill wastewaters (OMWs) are characterised by a high organic load (80–300 g/l of COD) and a low degree of biodegradability due to the acidic pH and, in particular, to the presence of phenolic and lipidic compounds, well known as toxic to bacteria (Beccari *et al.*, 1999; Gernjak *et al.*, 2004).

It is estimated that every ton of milled olives corresponds to about 0.80 ton of OMWs (Aktas *et al.*, 2001). In the Mediterranean countries OMWs total production reaches about 30 million m<sup>3</sup> per year (Dionisi *et al.*, 2005). Italy is one of the major olive oil-producing countries, counting up to 530,000 tons per year, corresponding to 2,200,000 tons of OMWs. Traditional disposal on the soil is still the typical solution adopted in Italy. The Italian law in force (L. 574/96) allows discharge of a maximum of 50 m<sup>3</sup>/ha when OMWs come from a traditional mill and 80 m<sup>3</sup>/ha when OMWs come from a continuous mill.

During recent years, different processes for OMW treatment, alternative to the soil disposal, have been investigated, e.g. chemical–physical technologies, chemical oxidation using ozone and Fenton's reagent and biological digestion performed by yeasts and fungi.

Lime coagulation treatment, without and with bentonite, seems to be an economical and effective OMW pretreatment, able to remove up to 50% of polyphenols and 50% of COD (Beccari *et al.*, 1999; Aktas *et al.*, 2001) and to enhance the OMWs anaerobic digestion efficiency.

Different oxidative methods have been applied in order to reduce OMWs organic load and toxicity. The high oxidating agents, like hydroxyl radicals, produced by ozone, Fenton's reagent, UV and advanced oxidation processes are very effective in degrading phenolic compounds. As regards the Fenton's process, literature data (Rivas *et al.*, 2001; Gernjak *et al.*, 2004) give an average OMW polyphenol removal up to 99%, and a COD removal efficiency of about 70%. Concerning the OMWs oxidation with ozone, it is possible to reach a COD reduction efficiency of 80% and a total polyphenols reduction above 90% (Andreozzi *et al.*, 1998; Kestioglu *et al.*, 2005).

Referring to biological treatment, literature data show that yeasts and different kinds of fungi (white-rot and brown-rot fungi) are able to reduce the organic load and the polyphenol content of the OMWs. The removal efficiency ranges from 40–70% for polyphenols and from 60–70% for COD (Borja *et al.*, 1995; Fountoulakis *et al.*, 2002; D'Annibale *et al.*, 2004). Literature data generally refer to laboratory pure fungi cultures applied to OMWs pretreated by means of sterilisation, dilution and thermal processes (D'Annibale *et al.*, 1998; Aggelis *et al.*, 2003).

This study is aimed at investigating and comparing different treatments in order to find a process suitable to treat olive mill effluents in an economical and feasible way. The processes we studied are: centrifugal dewatering, chemical lime coagulation, ozonation, Fenton's process and biological degradation by fungi. Trials have been carried out both on OMWs and on synthetic solutions of phenolic compounds: gallic acid, which is the most commonly used compound for the calibration of the polyphenol colorimetric analytical method; and p-coumaric acid, which is one of the most recalcitrant compounds present in the OMWs.

## Methods

The raw OMWs used for our experimental research have been supplied by one of the largest three-phase mills of Italy, located in Quarrata (Tuscany). The samples have been taken from two 5 m<sup>3</sup> storage tanks. Raw OMWs samples have been centrifuged at 4,000 rpm in order to separate the liquid phase from the solid phase. All the trials have been carried out on the liquid phase. Table 1 shows the raw and the centrifuged OMWs main characteristics.

The solid phase has been used for the gross calorific value (GCV) and the lower calorific value (LCV) assessment. Mean values of such characteristics are: LCV = 7,300 kJ/kgTS, GCV = 31,000 kJ/kgTS, humidity = 80%.

Further centrifuged OMW characteristics are: BOD<sub>5</sub> = 25,500–27,015 mg/l, BOD<sub>20</sub> = 40,000–43,000 mg/l, TKN = 286–438 mg/l and P<sub>tot</sub> = 275–463 mg/l. For the synthetic solutions preparation, demineralised water has been added with 0.3–3 g/l of gallic acid or 0.3 g/l of p-coumaric acid. These concentrations have been chosen

**Table 1** Raw and centrifuged OMWs characteristics

	Raw OMWs	Centrifuged OMWs
pH	4.4–4.8	4.6–5.12
COD [mg/l]	262,750–301,600	48,850–72,720
Total Polyphenols [g/l]	9.6–10.6	2.36–2.93
TSS [g/l]	113.5–128.4	2.19–3.02
VSS [%]	91.63–94.5	95.5

according to the OMW Total Polyphenols (TP) content and the maximum solubility value of the p-coumaric acid.

TP content were determined spectrophotometrically according to the Folin–Ciocalteu method. COD, TSS, VSS, TKN and BOD have been assessed according to the *Standard Methods for the Examination of Water and Wastewater* (2005). Residual hydrogen peroxide concentration has been evaluated by a respirometric method, based on the oxygen produced by activated sludge catalase (Rozzi *et al.*, 2003).

Regarding OMW lime coagulation, treatment has been carried out in a 1 litre batch reactor comparing different lime concentrations by means of jar tests. The tested lime concentrations have been varied from 6.5–20 g/l, corresponding to sample pH values ranging from 8–12.2. Jar tests have been carried out considering 10 minutes at 100 rpm, 10 minutes at 35 rpm and 2 hours of sedimentation.

As regards ozone treatment, lab-scale trials have been carried out in a 400 ml batch reactor (glass mariotte vessel). The O<sub>3</sub> mass flow rate used was 7.5 mg/min. Contact times used varied from 1–5 hours for the OMW samples and from 1–2 hours for the synthetic solutions. Remaining ozone in the off gas was determined iodometrically by KI solution (2% w/w). OMW samples have been oxidised both without pretreatment and after a lime coagulation process. The addition of 10–20 g/l of lime, reduced the TSS content and increased the pH value at 8–12. The pH value was varied by adding H<sub>2</sub>SO<sub>4</sub> (0.5 M) or NaOH (1 M), in order to highlight the different oxidation pathways and to compare their efficiency for the ozonation of gallic and p-coumaric solutions. The initial pH values ranged from 2–9.

Regarding Fenton's process, trials have been carried out in a 1 litre batch reactor. OMW samples have been previously centrifuged and the pH value has been adjusted at around 3 using H<sub>2</sub>SO<sub>4</sub> (98% w/w). The reagents used are hepta-hydrated FeSO<sub>4</sub> (8% w/w) and H<sub>2</sub>O<sub>2</sub> (35% w/w). Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> dosages have been chosen by testing different Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> ratios: 1/12, 1/24 and 1/60. Considering these ratios, the reagent concentrations that we used are summarized in Table 2.

In order to highlight the catalytic effect of the Fe<sup>2+</sup>/Fe<sup>3+</sup> ions, the Fenton's process has been compared to the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) oxidation. For this latter treatment we used 12 g/l of H<sub>2</sub>O<sub>2</sub>. All the experiments lasted 24 hours. Samples were analysed before the treatment and after a reaction time of 2 hours, 4 hours and 24 hours. Considering our experimental results and the literature data, Fenton's process on phenolic synthetic solutions has been performed using a 1/12 Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> ratio and a 1/3 H<sub>2</sub>O<sub>2</sub>/acid ratio.

In all the trials, oxidative efficiency has been assessed measuring the COD and TP removal after filtering the treated samples. In order to avoid residual hydrogen peroxide interference, COD of the OMW samples treated by Fenton's process has been measured after adding NaOH (4 M) and achieving a pH value above 11. This pH value guarantees hydrogen peroxide decomposition and favours ferric ion precipitation.

The completely mixed batch reactor used for aerobic treatment was composed of a 4 litre glass vessel (Applikon) and a mechanical stirrer with variable speeds. The speed was controlled within the range of 200–500 rpm in order to ensure no limiting oxygen conditions. Oxygen, temperature (fixed at 30 °C) and pH (fixed at 4.5) control and monitoring of the lab-scale reactor were performed by a biocontroller *ADI 1010 Applikon*. On-line pH control was ensured by NaOH (4 M) and H<sub>2</sub>SO<sub>4</sub> (1 M) addition with peristaltic pumps.

**Table 2** Chemical concentrations chosen for OMW Fenton's treatment

Reagent	Value					
Fe <sup>2+</sup> [g/l]	0.5	0.67	0.75	1.0	0.5	0.5
H <sub>2</sub> O <sub>2</sub> [g/l]	6	8	9	12	12	30

The batch reactor was filled with OMW after centrifugal dewatering and oil extraction. Nutrient solution ( $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NaH}_2\text{PO}_4$ ) was added periodically in the batch reactor.

Fungi presence was detected by means of microscope observations.

Respirometric tests were performed in a 1.5 batch reactor in order to measure biomass activity, specific growth rate ( $\mu_{\text{max}}$ ), affinity constant ( $K_s$ ), yield factor ( $Y_H$ ), endogenous decay coefficient ( $b_H$ ) considering different substrates: centrifuged OMWs (3, 5, 50, 100 ml), gallic acid and p-coumaric acid (1, 5, 50, 100, 150 ml).

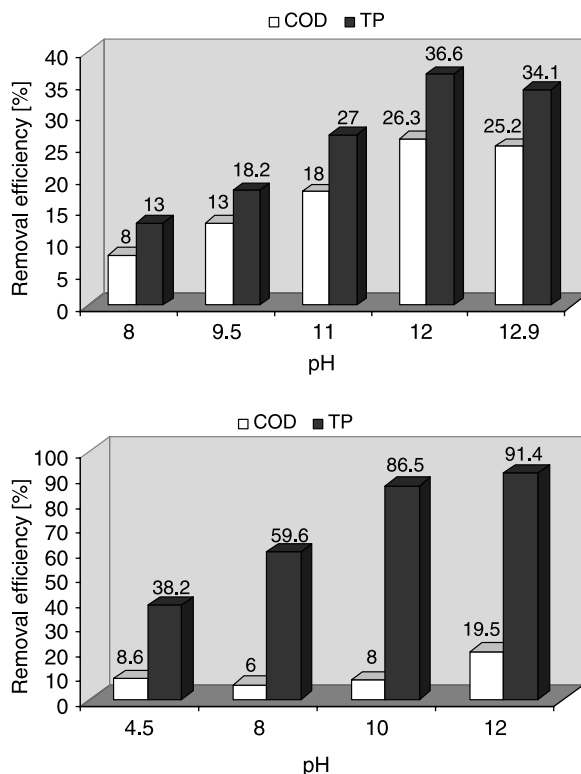
Respirometric tests have been carried out in order to assess the short term BOD ( $\text{BOD}_{\text{st}}$ ) of centrifuged OMWs, oxidized (Fenton's or ozone) OMWs using activated sludge taken from a municipal WWTP and using biomass from the aerobic batch reactor.

### Results and discussion

As regards lime coagulation our experimental trials, in accordance to literature data, showed a maximum COD and TP removal efficiency corresponding to the optimal pH value of 12. For this condition we reached the following removal efficiencies: 26% for COD, 37% for TP and 88% for TSS.

Ozone oxidation has been carried out on centrifuged OMWs (pH = 4.5) and lime pre-treated OMW samples (pH = 8–12.9). Figure 1 shows the average COD and TP removal efficiency obtained for lime coagulation and ozone treatment at the different experimental conditions.

Ozone removal percentages refer to a contact time of 4 hours. Higher contact time values did not significantly increase the process efficiency. According to our experimental results we can assess that ozone efficiency is greatly influenced by the pH value.



**Figure 1** COD and TP removal at different pH values obtained with lime coagulation and ozone treatment

TP removal efficiency significantly increases for pH values above 8, reaching the maximum of 92% at pH equal to 12. This result confirms other researchers evidence, even if the optimal pH found in this study is greater than the optimal value of 11 suggested by the literature data. COD reduction shows a similar trend, but the maximum removal efficiency never exceeded 20%. Concerning the synthetic solutions, the TP maximum removal reached a value of about 95% for pH equal to 5, and it did not change for higher pH values. This means that, according to the literature data, the direct ozonation pathway positively effects pure phenolic solution oxidation. On the other hand, COD removal is greatly influenced by the pH values, increasing from 7% at a pH value of 2, to about 30% at a pH value of 7, reaching the maximum removal efficiency of 60% for pH value equal to 9. These results correspond to a contact time of 2 hours.

Concerning the Fenton's process applied on OMWs,  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  dosages have been chosen by testing different  $\text{Fe}^{2+}/\text{H}_2\text{O}_2$  ratios (1/12–1/60). From our experimental results, the best  $\text{Fe}^{2+}/\text{H}_2\text{O}_2$  ratio was 1/12. For this ratio the best results have been reached with 1 g/l  $\text{Fe}^{2+}$  and 12 g/l  $\text{H}_2\text{O}_2$ . Figure 2 shows the average COD and TP removal corresponding to OMWs and synthetic solutions treatment at the aforementioned experimental conditions.

OMW maximum removal efficiency was about 60% for TP and 23% for COD. These results, according to the literature data, highlight the greater efficiency of ozone for TP degradation and the slightly higher efficacy of Fenton's process for COD removal.

These removal efficiencies are much greater than the ones reached by the hydrogen peroxide oxidation. As a matter of fact, the addition of 12 g/l hydrogen peroxide led to a removal efficiency of about 30% for the TP and of about 10% for COD.

Referring to the phenolic solutions, gallic and p-coumaric acids showed a very different behaviour when oxidised by means of Fenton's process. For the gallic acid solutions, TP and COD removal efficiency reached 80% and 40% respectively. On the other hand p-coumaric acid solution was less degradable than both gallic acid and OMW samples. The maximum removal efficiency was about 36% for TP and 20% for COD.

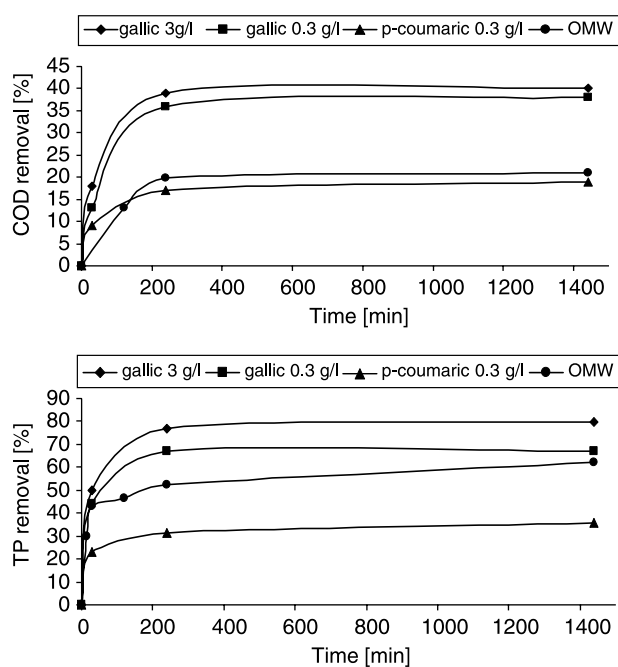


Figure 2 Fenton's COD and TP removal efficiency

As regards the aerated batch reactor trials, main results are shown in Figure 3.

In the first period, when the process was conducted without the addition of other organic substrates, a biomass rich of fungi was observed. In a second period, we added pure polyphenol compounds in order to enhance the fungi activity. After 70 days, the aerobic reactor was conducted in a fed-batch mode in four different phases: (1) centrifuged OMW load; (2) aeration (2–3 days); (3) clarification; and (4) supernatant extraction. The duration of the clarification phase decreased from 1 day to 3 hours when structured sludge flocs formed.

The removal efficiency of organic compounds are shown in Table 3.

Respirometric results on the fungi biomass are shown in Figure 4.

Figure 4(a), which represents the respirometric curve obtained with high  $S_0/X_0$  ratio shows the complexity of the OMW sample due to the presence of several organic substrates. Figure 4(b), which was obtained with pure gallic acid injection, highlights the fungi capacity to completely degrade the acid. Similar results have been found for the p-coumaric acid. These respirograms enable the  $Y_H$  determination at low  $S_0/X_0$  ratio and the  $\mu_{max}$  determination at high  $S_0/X_0$  ratio. The decay coefficient was estimated using an endogenous respirometric curve (exponential model). These data are summarised in Table 4.

Figure 5 shows the respirometric tests obtained with injections of: (a) centrifuged OMWs; (b) Fenton's pretreated OMWs; and (c) ozone pretreated OMWs. Centrifuged samples have been tested using both activated sludge and fungi biomass, whereas the other samples have been tested using only activated sludge.

Table 5 shows the aerobic biodegradability of centrifuged and pretreated OMWs.

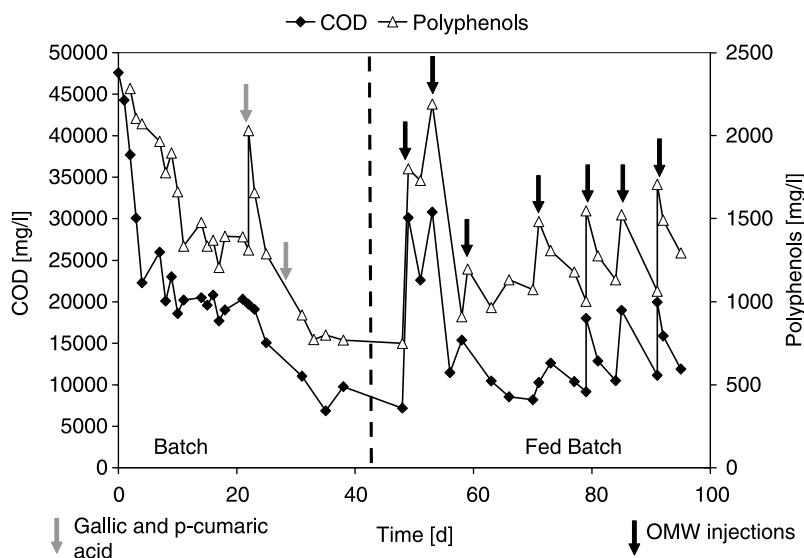
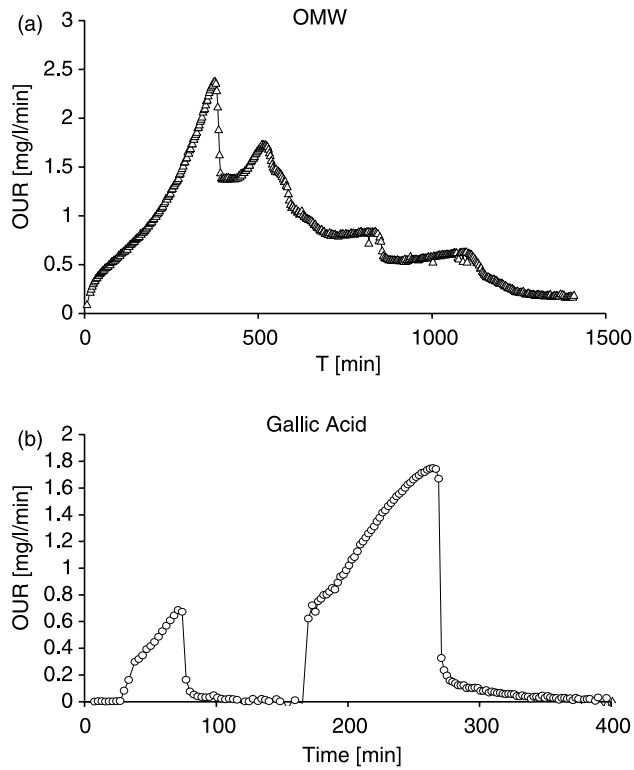


Figure 3 COD and polyphenols concentration trends in the aerobic batch reactor

Table 3 COD and TP removal in the aerobic reactor

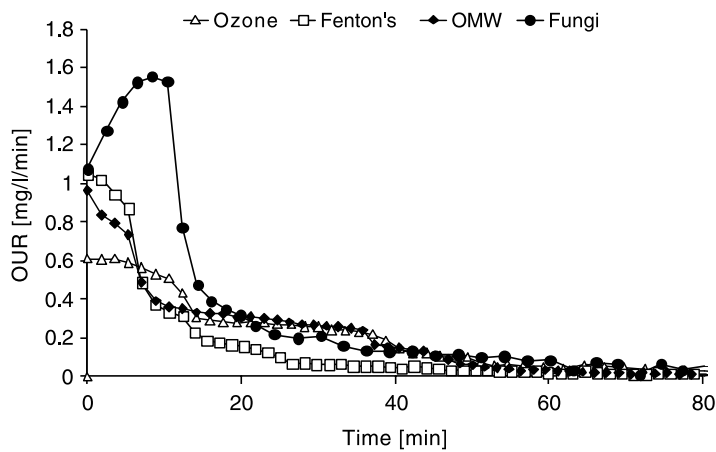
	Centrifuged OMW (mg/L)	Supernatant (mg/L)		Maximum removal (%)
		Mean value	Minimum value	
COD <sub>filtered0.45 μm</sub>	50,940	10,268	6,881	86
Polyphenols	2,550	1,066	750	70



**Figure 4** Respirometric tests on the fungi biomass with: (a) OMWs and (b) gallic acid

**Table 4** Kinetic and stoichiometric parameters ( $T = 30^{\circ}\text{C}$ ) of polyphenols degraded by fungi biomass

Polyphenols	$\mu_{\max}$ [ $\text{d}^{-1}$ ]	$b_H$ [ $\text{d}^{-1}$ ]	$Y_H$ [COD/COD]
Gallic acid	$5.3 \pm 0.5$	$0.81 \pm 0.08$	$0.58 \pm 0.05$
p-coumaric acid	$5.9 \pm 0.7$	$0.81 \pm 0.08$	$0.48 \pm 0.05$



**Figure 5** Respirometric tests on centrifuged and pretreated OMWs

**Table 5** Aerobic biodegradability of centrifuged and pretreated OMWs

Sample 0.45 $\mu\text{m}$ filtered	Biomass	COD (mg/L)	BOD <sub>st</sub> (mg/L)	Y <sub>H</sub> (COD/COD)	BCOD <sub>st</sub> /COD (%)
Centrifuged	Activated sludge	51,250	8,879	0.65	49.5
Fenton's	Activated sludge	35,000	6,413	0.65	59.1
Lime + Ozone	Activated sludge	39,600	10,862	0.65	78.3
Centrifuged	Fungi	50,940	17,928	0.53	74.8

The experimental results highlight the increase of pretreated OMWs aerobic biodegradability compared to the value obtained with untreated centrifuged OMWs. The BOD<sub>st</sub> of centrifuged OMWs evaluated on fungi biomass (about 18,000 mg/l) was twice as much as the BOD<sub>st</sub> obtained with non-acclimated activated sludge (about 9,000 mg/l). Regarding the activated sludge biomass, Y<sub>H</sub> value has been calculated by measuring the filtered COD degraded during the respirometric test. Referring to the fungi biomass, Y<sub>H</sub> value has been set equal to the mean value of the results of pure compounds as shown in Table 4.

## Conclusions

According to the experimental results obtained, the following conclusions can be drawn:

- (1) OMWs contain highly recalcitrant compounds and a combination of physical, chemical and biological processes is necessary in order to remove them.
- (2) OMWs ozone treatment is greatly influenced by the initial pH value. At the optimal pH value of 12, the maximum removal efficiency of the combined treatment lime coagulation/ozonation was 95% for TP and 26% for COD.
- (3) TP removal efficiency of the ozone process for synthetic solutions treatment is less affected by the pH, reaching the maximum value of 95% at pH value equal to 5. On the other hand, COD removal is influenced by the initial pH value, reaching the maximum removal efficiency of 60% at pH value equal to 9. Gallic acid and p-coumaric acids were similarly affected by the ozone treatment.
- (4) OMWs Fenton's treatment guarantees a TP removal of 60% and a COD removal of 23%.
- (5) Removal efficiency of Fenton's process for synthetic solutions depends on the organic acid considered. As regards the gallic acid, the maximum percentage removal obtained was 80% for TP and 40% for COD. On the other hand, referring to the p-coumaric acid, the maximum percentage removal obtained was 36% for TP and 20% for COD.
- (6) In an aerobic batch reactor, a biomass rich of fungi guarantees up to 86% of COD removal and up to 70% of TP removal. The fungi biomass was able to completely degrade the gallic and the p-coumaric acids.
- (7) Respirometric tests show the higher biodegradability of all the pretreated OMW samples compared to the one of the raw OMWs.

After having assessed the chemical and biological efficacy of the chosen treatments in reducing OMWs total polyphenol content, the next step in the research will be a cost analysis in order to compare the different chosen processes from an economic point of view.

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