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RESEARCH ARTICLE

# Sequential treatment of olive oil mill wastewater with adsorption and biological and photo-Fenton oxidation

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**Abstract** Olive oil mill wastewater (OMWW), a recalcitrant pollutant, has features including high phenolic content and dark color; thereby, several chemical or physical treatments or biological processes were not able to remediate it. In this study, the treatment efficiencies of three treatments, including adsorption, biological application, and photo-Fenton oxidation were sequentially evaluated for OMWW. Adsorption, biological treatment, and photo-Fenton caused decreasing phenolic contents of 48.69 %, 59.40 %, and 95 %, respectively. However, after three sequential treatments were performed, higher reduction percentages in phenolic (total 99 %) and organic contents (90 %) were observed. Although the studied fungus has not induced significant color reduction, photo-Fenton oxidation was considered to be an attractive solution, especially for color reduction. Besides, toxicity of OMWW treatment was significantly reduced.

**Keywords** Sequential treatment · Olive oil mill wastewater · Adsorption · Biological treatment · Photo-Fenton oxidation · Toxicity

## Introduction

Olive oil mill wastewater (OMWW) is a highly toxic effluent obtained from the extraction process by the olive oil industry and creates a major problem in Turkey as in other Mediterranean countries (Carlos Ruiz et al. 2002). This effluent is characterized by an unpleasant smell, dark color, and high organic matter content such as sugars, tannins, polyphenols, polyalcohols, pectins, and lipids (Dias et al. 2004). As reported by several authors, more than 30 phenolic compounds have been detected in OMWW (De Marco et al. 2007). Besides, phenolic and aromatic compounds have been considered as important contributors to toxicity of OMWW (Martínez et al. 2005; Yeşilada et al. 1998, 1999). OMWWs are potential sources of pollution and eutrophication for natural water bodies. The presence of pollutant organic matter in OMWW puts microbial activities at risk in the environment, when it is applied integrally to the soil or discharged in large amount directly into rivers and/or lakes (Sabbah et al. 2004). The organic load of OMWW is so high with biological oxygen demand (BOD) up to100 g $L^{-1}$ and chemical oxygen demand (COD) up to 200 g$L^{-1}$. These values may be found as 300 times higher than in a typical municipal sewage (Saez et al. 1992).

Proposed physicochemical processes include evaporation ponds and lagoons (Kotronarou and Mendez 2003). According to Canizares et al., several advanced oxidation technologies including conductive-diamond electrooxidation, ozonation, and Fenton oxidation were studied (Canizares et al. 2007) and have not been efficient in

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decreasing the high COD and toxicity of OMWW. Several authors have already combined chemical and biological processes for this wastewater treatment (Justino et al. 2009; Ergul et al. 2011). A well-designed sequential treatment consisting of various chemical, physical, and biological processes represent a better solution (Bettazzi et al. 2006). Adsorption, as a simple and relatively economical method, is a widely used technique in the removal of pollutants. Various inexpensive minerals (i.e., clays, zeolite, etc.) have been used for removal of color and phenol adsorption from OMWW (Santi et al. 2008; Eroğlu et al. 2008).

In general, biological treatment of OMWW has been studied using lignin-degrading organisms known as white-rot fungi that have an ability to remove phenolic substances which have lignin-like structure and recalcitrant compounds through their biomasses or extracellular enzymes including laccase, manganese-dependent peroxidase, and lignin peroxidase produced by these fungi. Several white-rot fungi, *Phanerochaete chrysosporium* (Justino et al. 2009; Dhouib et al. 2006), *Trametes versicolor*, *Funalia trogii* (Ergül et al. 2009; Yesilada et al. 1999; Aytar et al. 2011; Apohan and Yesilada 2011), *Pleurotus* spp. (Justino et al. 2009), *Lentinula edodes* (D'Annibale et al. 1998), *Pycnoporus coccineus* (Jaouani et al. 2005), and *Coriolopsis polyzona* (Jaouani et al. 2005), and species of *Cerrena*, *Byssochlamys*, *Lasiodiplodia*, and *Bionectria* (Mann et al. 2010) have been used for potential applications in the biological treatment of OMWW.

Fenton's reagent is a well-known advanced oxidation process involving $Fe^{2+}$- and $Fe^{3+}$-mediated catalytic decomposition of $H_2O_2$ under acidic pH (2–5) and combines with ultraviolet radiation to produce the highly oxidative $OH^-$ radical at room temperature. The reaction produces free radicals such as $HO^-$ and $HO_2^-$, which almost nonselectively attack organic as well as inorganic pollutants at diffusion-controlled reaction rates (Justino et al. 2009).

The elimination of phenolic compounds contained in OMWW by ozonation or ozone/UV have increased effluent biodegradability (Amat et al. 2003; Lafi et al. 2009). Similarly, one of the advanced oxidation process, Fenton's reagent, is often used as a pre- or post-treatment phase in combination with any microbial process for degrading residual volatile and nonvolatile organic compounds into $CO_2$ and biomass (Bressan et al. 2004). According to the studies of Khoufi et al., an innovative process for the treatment of olive mill wastewater has been recently upscaled from lab-scale to pilot plant (Khoufi et al. 2006, 2009). This process combines the electro-Fenton reaction followed by anaerobic digestion and ultrafiltration as a post-treatment to completely detoxify the anaerobic effluent and remove its high molecular mass polyphenols.

In this study, sequential treatment of OMWW, composed of adsorption with various inexpensive mineral adsorbents, aerobic treatment with *T. versicolor*, and photo-Fenton oxidation, was carried out. Finally, we obtained the comparative results with literature findings. According to our results, 99 % of phenol removal and 90 % decrease in organic contents were totally obtained, not including the requirement of adaptation phase and addition of any nutrients, which these findings, using three remediation methods including adsorption, biological operation, and photo-Fenton reaction, demonstrated; the same findings were not demonstrated in the previous studies relating to this wastewater in the literature. Besides, toxicity values were significantly reduced after all treatments.

## Materials and methods

### Characteristics of OMWW

The experiments were performed with OMWW that was provided from a three-phase plant (Edremit, a part of Marmara region of Turkey) exhibiting the following characteristics: pH, 4.8; COD, 59.149 $gL^{-1}$; total phenols 3.653 g $L^{-1}$. OMWW was centrifuged (9,000×*g*, 20 min), filter-sterilized, and stored at −20 °C until the next experiment process. Characteristics of OMWW were shown in Table 1.

### First treatment of OMWW with different adsorbents

Montmorillonite, clinoptilolite, pumice, perlite, and fly ash have been used for removal of color and phenol adsorption from OMWW which was obtained from Balıkesir, Turkey. Typical analyses of adsorbents were given in Table 2. Batch adsorption studies were performed using crude OMWW in 250-mL Erlenmeyer flasks with 100 mL of working volume for agitated culture conditions at 250 rpm. The particle sizes of all

**Table 1** Composition of OMWW during the experimental period

| Parameter | Mean value |
|---|---|
| Total COD (mg $L^{-1}$) | 59,149 |
| Dissolved COD (in liquid phase) (mg $L^{-1}$) | 33,070 |
| COD (in precipitate) (mg $L^{-1}$) | 26,079 |
| $BOD_5$ (mg $L^{-1}$) | 29,930 |
| Total phenol (mg $L^{-1}$) | 3,659 |
| Total solid content (mg $L^{-1}$) | 36,580 |
| Suspended solid content (mg $L^{-1}$) | 14,080 |
| $NH_4^+$ | 3.45 |
| $NO_3^-$ | 108 |
| Oil-gres | 115 |
| Conductivity (mS/cm) | 11.3 |
| pH | 4.85 |

adsorbents were about 300 µm. The effect of initial pH (1.0–12.0) on adsorption of OMWW was examined for 150 min. The pH of the solution was adjusted using concentrated $H_2SO_4$ and NaOH. The effect of different montmorillonite amounts (1–150 $gL^{-1}$) on adsorption was examined at pH 10.0. All the experiments were carried out in triplicate. At the end of the study, color, phenol removal, and COD were determined by a spectrophotometer (Shimadzu UV–Vis Spectrophotometer 2550).

### Biological treatment of OMWW with *T. versicolor*

The microorganisms used for biological treatment of OMWW, nonadapted *T. versicolor* ATCC (200801) was provided from ATCC. Biological treatment was carried out with 25–50 % concentrations and nondiluted OMWW samples pretreated by montmorillonite after pH value was reduced to 4.8, which is appropriate for biological process. After preculture incubation at 30 °C on Malt broth (Merck) for 7 days, obtained fungal pellets were weighed at equal amounts (25 $gL^{-1}$) and were used as inoculum. Pellets were transferred into 100 mL OMWW samples in a 250-mL flask for agitated culture conditions (150 rpm). *T. versicolor* was incubated at 30 °C for 24 h and spectrophotometric measurements of phenol removals were measured. After determining dilution of OMWW, the effect of biomass amount (5–100 $gL^{-1}$) was examined for biological treatment. To evaluate the effect of incubation time (1–10 days), dephenolization with 10 $gL^{-1}$ of biomass was studied. Spectrophotometric measurements of color, phenol removals, and COD were measured in the tenth day of the incubation period. All experiments were carried out in triplicate and noninoculated controls were incubated in parallel under the same conditions.

### Photo-Fenton oxidation of OMWW as post-treatment

Photo-Fenton treatment was carried out after biological treatment was performed with *T. versicolor* of 50 % OMWW samples. In this study, 200 mL of homogenous and representative OMWW sample was transferred into a 500-mL glass beaker for continuous magnetic stirring (100 rpm) at room temperature which was followed by biological treatment. Then, 6 mL of $FeSO_4$ $5{\cdot}H_2O$ (0.5 M) was progressively added into a beaker. After the OMWW samples have been mixed for 10 min, the mixture pH was adjusted to 4.0 (with $H_2SO_4$ or NaOH). Four milliliters of $H_2O_2$ (30 %v/v) was added every 10 min within a period of 1 h. During this period, a UV lamp was switched on after the first $H_2O_2$ has been added and switched off after 2 h (Justino et al. 2009). After 4 days of incubation period, spectrophotometric measurements of phenol removals were measured for every day. At the end of the study, color, phenol removal, and COD were determined by a spectrophotometer.

### Analytical methods

Total phenol was determined by Folin–Ciocalteau reagent with gallic acid as the standard (Slinkard and Singleton 1977). Concentration of COD was determined according to APHA (1992). The net surface charge of the montmorillonite at different pH values was determined from the zeta potential measurements by a Malvern Zetasizer instrument.

### Toxicity evaluation

Acute toxicity was investigated by determining the luminescent inhibition of the *Vibrio fischeri* NRRL number B-11177. In order to test toxicity, a vial of this commercial bacterium was purchased in freeze-dried form from Strategic Diagnostics, Inc.

**Table 2** Typical analysis of adsorbents

| Physical properties | Pumice | Perlite | Clinoptilolite | Montmorillonite | Fly ash |
|---|---|---|---|---|---|
| Density ($g/cm^3$) | 1.88 | 2.34 | 1.425 | 2.537 | 1.99 |
| Specific surface ($cm^2/g$) | 4.814 | NA | 1.783 | NA | 3.340 |
| Void fraction (*e*) | 3.48 | NA | NA | 1.09 | NA |
| Porozite (*n* %) | 58 | 98 | 56 | 52.23 | NA |
| Chemical properties (%) | | | | | |
| $SiO_2$ | 69 | 73.54 | 67.6 | 51.14 | 45.18 |
| $Al_2O_3$ | 14.65 | 15.23 | 11.3 | 19.76 | 20.94 |
| $Fe_2O_3$ | 2.51 | 1.02 | 0.77 | 0.83 | 7.99 |
| CaO | 1.11 | 0.41 | 3.26 | 1.62 | 1.22 |
| MgO | 0.55 | 0.43 | 1.18 | 3.22 | 2.79 |
| $K_2O$ | 3.52 | 4.73 | 2.17 | 0.04 | 2.24 |
| $Na_2O$ | 2.48 | 3.56 | NA | 0.11 | 0.57 |
| $SO_3$ | 0.4 | 0.12 | NA | NA | 5.63 |
| Combustion losses | 4.96 | 0.78 | 13.59 | 22.8 | NA |

(SDI, Newark, DE, USA) and activated by reconstitution solution (Azur). The light emission of this bacterium in contact with both untreated and treated samples and 5 and 15 min of exposure times was measured using the Microtox® 500 analyzer according to the Basic Test Protocol. The luminescence was recorded at 490 nm. The data were processed using the MicrotoxOmni Software. The concentration of the sample produced by a 50 % decrease in light after exposure for 15 min was defined as the effective concentration (EC$_{50}$).

## Results and discussion

Establishing adsorption/desorption equilibrium needs special care while organic matter and phenols start to desorb after a certain contact time. Characteristics of OMWW were given in Table 1. A typical analysis of the adsorbents in this study obtained from the Balikesir of Turkey is given in Table 2. Table 3 indicates effects of OMWW pH variation throughout adsorption with different adsorbents on phenol removal. Phenol removal in alkaline pH rather than acidic conditions was exhibited to be both more efficient and stable in this study. Because of the fact that phenol is a weak acid (p$K_a$=10), OMWW will be adsorbed to a higher extent at neutral to basic pH values due to the binding forces prevailing between the phenol oxide and the positively charged surface of modified bentonite (Al-Asheh et al. 2003). Comparison of the phenol removal efficiency obtained at varying dosages of adsorbents such as montmorillonite, clinoptiolite, an fly ash at optimal pH levels of 10, 7, and 9, respectively, is represented in Table 4. In the case of using adsorbents studied, the adsorbed amount of OMWW increased when the pH level was higher. However, changing the pH to acidic reduces the uptake of OMWW. The same trend was obtained in the literature (Al-Asheh et al. 2003; Lizhong et al. 1996). It can be noted that increasing montmorillonite amount from 0.1 up to 15 gL$^{-1}$ caused phenol removal efficiency to rise correspondingly, reaching maximum values of 48.69 % at 15 gL$^{-1}$. Based on the results obtained in all the adsorption experiments, montmorillonite with a dosage of 15 gL$^{-1}$ at pH 10 was chosen as the first treatment for the OMWW and used in further experiments. According to zeta potential measurements, the surface charge of the montmorillonite varied from −20.05 to −10.9 mV when the pH was altered from 1.0 to 12.0. At pH 10.0, the selected optimum value of adsorption, zeta potential was −11.9 mV (Fig. 1). These results demonstrate the adsorption of the studied OMWW onto the montmorillonite as a function of pH.

Biological processes for the treatment of wastewaters, which can remove organic matter and inorganic nutrients, are considered ecofriendly, reliable, and, cost-effective. As an alternative method, dilution is very often used prior to biological treatment for reducing toxicity to the microorganisms. In this study, biological treatment of OMWW was conducted in batch system. After adsorption treatment, by reducing pH to 4.8, as evaluated at our previous study (Aytar et al. 2011), a growing cell of *T. versicolor* ATCC200801 was added into nondiluted and diluted (25 % and 50 %) OMWW's without any addition of nutrients which caused important phenolics and COD removal during incubation. The rates of 30.96 %, 24.28 %, and 18.41 % of phenol removal were observed at the dilution rates of 25 % and 50 %, and nondiluted OMWW, respectively. To observe efficiency of concentration as high as possible, 50 % dilution of OMWW was chosen. In the case of the effect of biomass amount on phenol removal of OMWW with growing cell having *T. versicolor*, 10 g of biomass amount was chosen for further experiment related to tolerance to the initial polyphenol concentration of *T. versicolor* growing cell. According to our results, 59.40 % of dephenolization and 36 % of COD removal with 10 g of *T. versicolor* pellets at the end of 10 days of the incubation period were determined at 15 % OMWW in the absence of external organic supplements without spending adaptation period (Table 5). OMWW pH variation occurred throughout biological treatment and was changed from 4.8 to 7.3.

**Table 3** % phenol removal of OMWW with various adsorbents at different pH

| pH values | Pumice | Perlite | Clinoptiolite | Montmorillonite | Fly ash |
|---|---|---|---|---|---|
| pH 1 | 3.97 | 19.54 | 2.40 | 29.03 | 3.36 |
| pH 2 | 7.08 | 7.48 | 2.62 | 19.86 | 13.33 |
| pH 3 | 1.66 | 5.75 | 4.12 | 9.97 | 2.06 |
| Own pH | 1.38 | 0.20 | 14.59 | 19.71 | 6.47 |
| pH 7 | 2.60 | 9.16 | 22.28 | 24.71 | 11.85 |
| pH 9 | 16.76 | 30.99 | 6.80 | 27.53 | 33.97 |
| pH 10 | 25.51 | 38.32 | 19.35 | 40.20 | 26.20 |
| pH 12 | 5.71 | 6.39 | 0.38 | 41.66 | 11.52 |

**Table 4** Effects of adsorbent amount on OMWW adsorption with selected adsorbents

| Adsorbent amount (g) | Montmorillonite (pH 10) | Fly ash (pH 9) | Clinoptiolite (pH 7) |
|---|---|---|---|
| 0.1 | 7.82 | 16.03 | 0.87 |
| 0.5 | 14.48 | 25.45 | 0.84 |
| 1.0 | 24.08 | 24.14 | 5.80 |
| 2.0 | 33.77 | 21.40 | 6.59 |
| 4.0 | 29.94 | 27.92 | 1.83 |
| 6.0 | 38.81 | 32.29 | 11.51 |
| 8.0 | 37.74 | 36.91 | 13.36 |
| 10.0 | 43.32 | 39.04 | 3.02 |
| 15.0 | 48.69 | 38.11 | 11.85 |

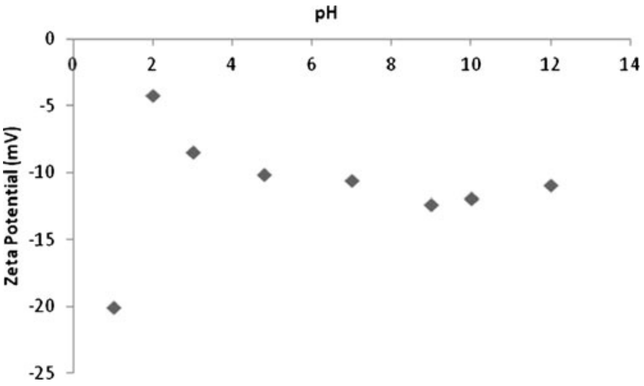


**Fig. 1** Zeta potential measurement of montmorillonite

A rate of 50 % diluted OMWW samples treated with *T. versicolor* was submitted to photo-Fenton oxidation. Such advanced oxidation processes are very hopeful techniques for the treatment of wastewaters having recalcitrant environmental pollutants (Scott and Ollis 1995; Gogate and Pandit 2004). Photo-Fenton process caused a very remarkable color reduction in OMWW which was not achieved by biological treatment. Furthermore, COD was also altered from 31.1 $gL^{-1}$ to 3.1 $gL^{-1}$ and changed the percentage of reduction in total phenolic content (85.8 % after 1 day and 95 % after 2 days) through photo-Fenton oxidation (Fig. 2). Similar results were also observed in the literature (Justino et al. 2009).

The combination of four treatment steps composed of settling, centrifugation, filtration, and activated carbon adsorption provided 94 % phenol removal and 83 % organic matter removal (Azzam et al. 2004). A study of the effect of lime treatment on various OMWW after a classic coagulation/flocculation/sedimentation/filtration process using a range of lime doses from 10 to 40 $gL^{-1}$ revealed that this application showed 62–73 % phenol removal (Aktas et al. 2001). Adsorption on granular activated carbon after coagulation/flocculation/sedimentation gave about 30 % COD reduction and a requirement of 50 $kg\,carbon\,m^{-3}$ effluent (Kestioglu et al. 2005).

According to the study of Justino et al., the pretreatment of samples by photo-Fenton oxidation has decreased the ability of fungi to reduce OMWW sample toxicity probably due to the presence of metabolites derived from the oxidation of the organic compounds present in OMWW, despite those that are bioaccumulated in fungal mycelia (Justino et. al. 2009).

While ozonation and aerobic treatment were combined, Benitez et al. reported a total COD reduction of 82.5 %, a percentage higher than either of the two technologies could achieve alone, which indicated that ozonation increased the biodegradability of the OMWW (Benitez et al. 1999). In another literature data, a similar trend was also observed. Varying inlet ozone concentrations between 10 and 45 $mgL^{-1}$ and having ozonation times of up to 2.5 h, COD removals of up to 70–80 % were observed as well as 40–50 % phenol reduction. After ozonation, active sludge treatment led to a further 60–80 % COD reduction (Rivas et al. 2000). Fenton's reagent can also increase the biodegradability of an OMWW. Beltran-Heredia et al. carried out Fenton's oxidation coupled with an aerobic post-treatment and was able to reach up to 70 % of total COD reduction and over 90 % phenol reduction (Beltran-Heredia et al. 2001). Similar results were observed in the study of Bressan et al. (2004).

In this study, as a summary, the efficiencies of three treatments, including adsorption, fungus treatment, and photo-Fenton oxidation, sequentially applied to OMWW were analyzed especially for total phenolics and COD content. After effective adsorption of OMWW with montmorillonite, biological process was also treated. Although the treatment with the fungus of diluted OMWW samples allowed phenolics and

**Table 5** Biological treatment experiments after adsorption

| Phenol removal at different OMWW dilutions with biological treatment after adsorption (5 g of biomass amount, 150 rpm of agitation rate, 25±1 °C, 100 mL of total volume) | | | | | |
|---|---|---|---|---|---|
| | OMWW dilutions | | | | |
| | 25 % | 50 % | Nondiluted OMWW | | |
| % phenol removal | 30.96 | 24.28 | 18.41 | | |
| Phenol removal at different biomass amounts with biological treatment after adsorption (50 % OMWW, 150 rpm of agitation rate, 25±1 °C, 100 mL of total volume) | | | | | |
| | Biomass amount (g) | | | | |
| | 0.5 | 2 | 5 | 8 | 10 |
| % phenol removal | 22.70 | 23.25 | 26.08 | 28.50 | 31.03 |
| Phenol removal at different contact time with biological treatment after adsorption (50 % OMWW, 150 rpm of agitation rate,10 g of biomass amount, 25±1 °C, 100 mL of total volume) | | | | | |
| | Days | | | | |
| | 1st | 3rd | 5th | 8th | 10th |
| % phenol removal | 30.76 | 35.69 | 52.08 | 58.56 | 59.40 |

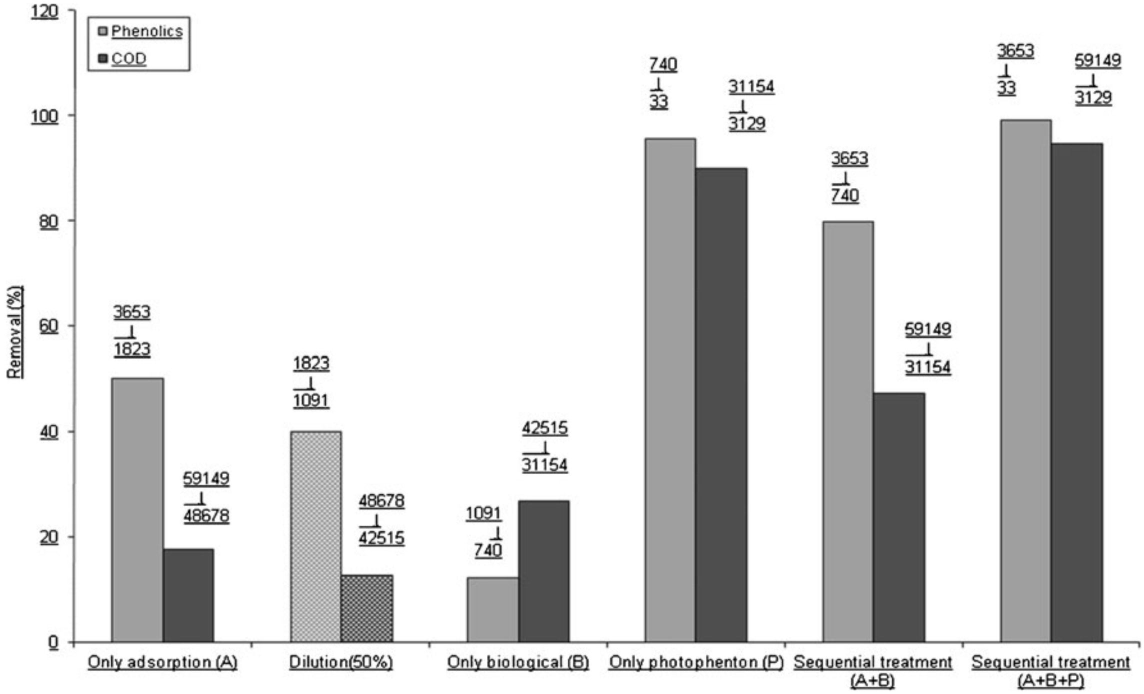
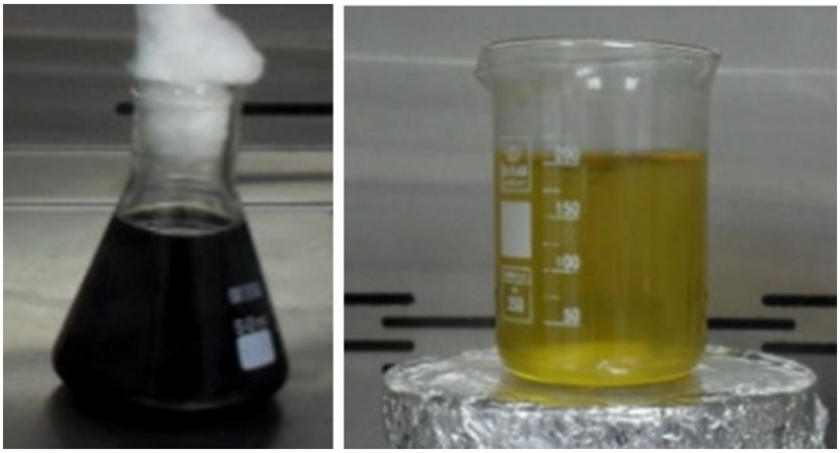


**Fig. 2** Evaluation of the quality of the OMWW after treatments: adsorption, biological, photo-Fenton, sequential treatment I (adsorption + biological), and sequential treatment II (adsorption + biological + photo-Fenton)

COD reduction, this fungus species did not induce important color reduction. Dark color of OMWW made disposal difficult. However, after biological treatment, photo-Fenton oxidation seemed to be an attractive solution, especially for color reduction. The obtained results and color change were shown in Figs. 2 and 3, respectively. It is a sequential method that can compete with the literature findings above. Figure 3 illustrated that contribution to sequential treatment of each treatment.

Acute toxicities of diluted OMWW samples, before and after all treatment compared to the parent compound, were assessed with *V. fischeri*. Toxicity of OMWW, after all treatment, was observed as $EC_{50}$ of 9.00 %. Due to the fact that a nondiluted sample was sufficiently toxic to test bacterium, *V. fischeri*, it was not possible to evaluate the toxicity of nontreated and treated OMWW samples. During toxicity measurement, it was observed as an $EC_{50}$ of 18.00 % for 1/100 of dilution-treated OMWW. However, toxicity of OMWW was found as an $EC_{50}$ of 9.00 % for 1/1,000 of dilution-nontreated OMWW. These results indicated that toxicity of OMWW was reduced through all treatment processes such as adsorption, biological application, and photo-Fenton oxidation. Similar values of OMWW toxicity were reported by Justino et al. for *D. manga* species (Jaouani et al. 2005). As it was observed by Martirani et al., OMWW treatment with *P. ostreatus* promoted effluent detoxification to *Bacillus cereus* with concomitant phenol content decrease (Martirani et al. 1996). However, according to some authors, such biological processes alone were not effective enough for

**Fig. 3** Color change at OMWW after sequential treatment



detoxification of OMWW especially nonbiodegradable organic compounds because these organic compounds apart from the phenols, namely, medium or high molecular polymers, resulted from low molecular weight polymerization and phenolic autooxidation with recalcitrant and toxic nature and were probably responsible for the remaining OMWW toxicity (Lathasree et al. 2004). However, in this study, the detoxification process could be evaluated as successful to satisfy three treatment procedures.

## Conclusions

OMWW effluent is one of the most persistent pollutants, mainly in the Mediterranean countries. High phenolic content, dark color, and recalcitrant organic compounds were not overcome by single chemical treatment or biological process. Therefore, as it was suggested by several authors, sequential treatment including adsorption, fungal treatment, and photo-Fenton process was carried out in this study and outstanding results were observed. In this study, after especially photo-Fenton process, both phenolic and organic contents were reduced and well decolorized. Besides, toxicity of OMWW before and after the sequential treatment process was successfully reduced.

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