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Phenolic Compounds and COD Removal from Olive Mill Wastewater by Chemical and Biological Procedures

[1]Afify, A.S, [1] Mahmoud, M.A.,[2] Emara, H.A. and [2]Abdelkreem, Khadega I.

[1]Department Biochemistry, Faculty of Agriculture, Cairo University, Egypt.
[2]Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

Abstract: In this study biological and chemical treatments were applied to olive mill wastewater (OMW) to reduce its phenolic compounds and chemical oxygen demand (COD) contents in order to reduce its toxicity. The biological treatment was carried out by using two fungal isolates (*Aspergillus wentii* and *Aspergillus niger*) isolated from the OMW and compared with *Pleurotus ostreatus*. *A. wentii* was more efficient than *A. niger* and *Pleurotus ostreatus* in removing of COD and phenolic compounds from OMW. The optimum OMW dilution was 10%, in which the maximum COD removal (62.2%) and maximum phenolic compounds reduction (80.9%) were obtained by *A. wentii*. This was followed by 59.3% removal of COD and 79.6% reduction of phenolic compounds obtained by *P. ostreatus* at the same OMW dilution (10%). Lower biodegradation percentages of COD (28% at 40% OMW dilution) and phenolic compounds reduction (72.9% at 30% OMW dilution) were obtained by *A. niger*. Different chemical treatments were applied primary on tannic acid solution to select the best of them for OMW application. These treatments were UV alone and combined with H_2O_2, Diefferent Fenton systyems and photocatalysis by titanium dioxide. The maximum degradation of tannic acid obtained in the first treatment was 47% at H_2O_2 concentration 0.11M at pH 9 under UV radiation. The maximum tannic acid degradation obtained by Fenton system was 70%. It was obtained at both Fe^{2+} concentrations (0.0024M and 0.0048M) and 0.11M H_2O_2 in dark and UV radiation at pH 9. While the maximum degradation obtained by photocatalysis by titanium dioxide was 35% at TiO_2 50mg/l and pH 5. The maximum degradation of phenolic compounds (74.4%) was obtained when OMW treated with 0.55M of H_2O_2 in dark after 40 min .

Key word: Olive mill wastewater; Fenton; Photo-Fenton; UV plus hydrogen peroxide and TiO_2

INTRODUCTION

Olive mill wastewater (OMW) composed of many complex substances that are not easily degradable. The acid pH and the high amounts of organic matter and phenols make it very difficult to be purified. Deterioration of natural water bodies due to olive mil wastes is a serious problem as indicated by coloring, appearance of an oily shine, and increased oxygen demand. It affects the soil quality, toxic to plants and soil micro flora when disposed into the soil. Therefore, direct discharge of olive mill wastewater into receiving media is not permissible and certain measures must be taken before disposal of the OMW into the environment (Azbar *et al*., 2004 and Ergu *et al*., 2008).

Several studies carried out to reduce the phenolic compounds and COD content of this waste by using different treatment methods. Biological treatments by different microorganisms may be fungi such as *Aspergillus niger* (Hamdi *et al.,* 1991) and Borja *et al.* (1995); *Phanerochaete chrysosporium* (Ahmadi *et al*., 2006), *Pleurotus ostreatus* (Fountoulakis *et al*., 2002 and Aggelisa *et al.,* 2003); *Lentinula edodes* (Annibale *et al.,* 2004); yeasts such as *Yarrowia lipolytica* and *Candida tropicalis* (Ettayebi *et al*., 2003), bacteria such as *Azotobacter vinelandii* (Piperidou*, et al*., 2000). Chemical treatments such as photocatal ysis using TiO_2 (Oppenländer, 2003; Konstantinou and Albanis, 2004 and Parsons, 2004); by Fenton system (Benitez *et al*., 2001; Gernjak *et al.,* 2004 and Dincer *et al*., 2008). Other treatments were also used such as ultrafiltration (Akdemir and Adem, 2008); electrochemical oxidation (Longhi *et al*., 2001 and Gotisia *et al*., 2005).The present study was aimed to reduce the phenolic compounds and COD contents of olive mill wastewater (OMW) by using biological and chemical treatment methods.

Corresponding Author: Khadega I.A. Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.Phone: 202 33887729.
E-Mail: Gabbrykhadega@yahoo.com

MATERIALS AND METHODS

Materials:

Olive mill wastewater (OMW) was obtained from Horticultura department, Agricultural Research Center, Giza, Egypt. *Pleurotus ostreatus* (NRRL–2366) and *Phanerocheate chrysosporium* (NRRL-6361) were provided from Northern Regional Research laboratory, Illinois, Peoria, USA and used as standard strains in the present study. The microorganisms were subcultures every 30 days. Rose-Bengal chloramphenicol agar medium (The Oxoid, 1979) used for isolation of fungi from OMW. Potato Dextrose Agar medium (PDA) (Martin; 1950) used for maintenance of the isolated fungi. Some of fungal isolates isolated from OMW on the same medium.

Methods:

Isolation and Identification of Fungal Isolates from OMW:

According to the method described by Gilman (1982) the specialists in plant pathology department (A.R.C), Giza, Egypt identified the fungal isolates that were under study. Ten milliliter of OMW was serial diluted up to 10^{-7}. One ml of each dilution was plated (in triplicate) on both Rose-Bengal chloramphenicol and Martin's media. The plates were incubated at 28°c for 24 hr.

Determination of Chemical Constituents of OMW:

Total lipids were determined according to the method described by Halmiton and Shiela (1992). Total nitrogen was determined by Kjeldahl method (Cottenie *et al*., 1982).Total sugars were determined by the method of (Dubois *et al*., 1956). Ash content was determined according to (AOAC, 1990).Tannins were determined according to the procedure described by Hagir *et al.* (1997). Phenolic compounds were extracted according to the method of (Elena De Marco *et al*., 2006). 10.0 ml of OMW was mixed with 15 ml of hexane; the mixture was vigorously shaken and centrifuged for 5 min at 3000 rpm. The phases were separated and the washing was repeated two times successively. Extraction of phenolic compounds was then carried out with 10 ml of ethyl acetate after acidification by HCl to pH 2. The phases were separated and the extraction was repeated four times successively. The ethyl acetate was evaporated under vacuum and the dry residue was dissolved in 3 ml of methanol and this solution was used for determination of phenolic compounds**.** Total phenols were determined spectrophotometrecally as described by Swain *et al.* (1959). Minerals (K, Ca, Na, Zn, Mn, Cu, Fe, Mg, and P) were determined according to the method described by Cottenie *et al.* (1982) using Atomic Absorption Spectrophotometer (Perkin-Elmery model: 372). Biochemical oxygen demand (BOD_5) was determined as the method described by Lenore *et al.* (1992).Chemical oxygen demand (COD) was determined according to the method described by Arnold *et al.* (1992).

Biological Treatments:

According to Warcup (1957) the ability of isolated fungi to degrade the phenolic compounds and reducing the COD contents in OMW was examined as the following: Seven fungal species that were previously identified namely *Aspergillus clavatus, Mucor strictus hagem, Penicillium nigricans, Penicillium cilreaviride, Aspergillus wentii, Penicillium chermesinum* and *Aspergillus niger* were tested. Each fungus was inoculated into 250 ml conical flasks containing 50 ml of sterilized OMW (20%). The flasks were shaken (150 rpm) for 1 week at 25°C. Phenolic compounds and COD content were determined as descried above.

Chemical Treatments:

Several chemical oxidation treatments were carried out primarily on tannic acid solution and one of these treatments was chosen for OMW treatment: It was treated as the method described by Fotiadis *et al*. (2007) and Benitez *et al*. (2001).

Chemical oxidation of tannic acid solution was conducted for 2.5 hours at 25°C by using several oxidizing agents:

a- UV radiation at 254 nm.

b- Combination of UV radiation with two different concentrations of hydrogen peroxide (0.05*M* and 0.11*M*) at three pH values 5, 7 and 9.

c- Single Fenton's reagent: A solution consists of hydrogen peroxide and ferrous sulfate. Two different concentrations of ferrous sulfate 4.6 X 10^{-3} and 2.3 X 10^{-3} *M* were used with 0.05 *M* and 0.11 *M* H_2O_2 (four treatments) at three pH values 5, 7 and 9.

d- Combination of Fenton reagents with UV radiation at three pH values 5, 7 and 9.

e- Titanium dioxide treatments: different amounts of TiO_2 (25, 50, 75, 100 and 125 mg) were added to one liter of tannic acid solution and exposed to UV radiation.

In all of the previous experiments the tannic acid concentration was determined in a sample every 30 min along the treatment period (150 min).

Chemical Treatment of OMW:

The OMW is oxidized by three concentrations of H_2O_2 (0.11, 0.55 and 2.0 *M*) at pH 9 in the presence and absence of UV radiation. A sample was taken every 30 min. along the treatment period (150 min) for determination of total phenols.

RESULTS AND DISCUSSION

Chemical Composition of OMW:

Olive mill wastewater was analyzed for its content. Tables (1) and (2) show the chemical composition of OMW samples as g/100 ml OMW. While the mineral analysis showed that the major element in this waste was potassium (475 mg/100ml), followed by calcium and sodium (250 and 175 mg/100ml, respectively). These results were in agreement with the results obtained by Paredes *et al.* (1999) who analysed the OMW in deferent localities from deferent verities and recorded a range for each measurement parameter. He found that total phenols ranged were from 1.32-3.99 mg %, while these results were recorded 3.46 mg % for total phenoles.

Biological Treatment of OMW:

As shown in Table (3), the results indicate the maximum reduction of COD was 68.4% by *A. wentii* while the minimum COD reduction (7%) was obtained with *P. chrysosporium*. Where as the maximum degradation of the phenolic compounds (70%) was obtained by *A. niger*. It was similar to that obtained by *P. ostreatus* and *P. chrysosporium their values* 70.6%, 75.0% and 70.2% respectively. On the other hand, *P. nigricans* recorded the minimum reduction of phenols (54%). These results were in agreement with the results obtained by Hamdi *et al.* (1991). They reported that *Aspergillus niger* removes 61% of COD and 58% of total phenolic compounds of OMW. Also Chwei *et al.* (2003) found that *A. niger* removes 35% of COD of OMW in batch culture. Sayadi and Radhouane (1993) reported that *p. chrysosporium* removes 73% of COD content of OWW.

Table 1: Chemical composition of olive mill wastewater

Component	Amount (g / 100 ml)
Moisture	87.18
Total solids	12.82
Ash	1.00
Total nitrogen (without centrifugation)	3.96
Total nitrogen (after centrifugation)	1.43
Total carbohydrates.	5.00
Total lipids	5.00
Phenolic compounds	3.46
Tannins	0.00
Lignin	2.60
COD	10.00
BOD	3.30
Organic carbon	8.18
Organic matter	14.10

Table 2: Minerals analysis (mg/100 ml) of OMW

Element	Concentration (mg / 100 ml)
Potassium	475
Calcium	250
Sodium	175
Zinc	10.0
Magnesium	50.0
Iron	25.0
Manganes	2.0
Phosphorus	0.17
Copper	0.0

Table 3: Effect of different fungal isolates on the reduction COD and phenolic compounds of OMW as compared by *P. ostreatus* and *P. chrysosporium*.

Fungal isolate	% of COD reduction	% of phenols reduction
Aspergillus clavatus	13.5	64.1
Mucor strictushagem	12.2	59.1
Penicillium nigricans	16.3	54
Penicillium cilreaviride	22.4	60.6
Aspergillus wentii	68.4	67.4
Penicillium chermesinum	30	54.3
Aspergillus niger	24.6	70.6
p. ostreatus	32	75
p.chrysosporium	7.1	70.2

Effect of Incubation Time on Biodegradation of Phenolic Compounds, COD Reduction of OMW by Selected Fungi Isolates:

From Figure (1) both *Aspergillus niger* and *A. wentii,* showed the maximum COD and phenolic compounds reduction, they were chosen for studding the optimum incubation period in which it gave the maximum COD and phenolic compounds reduction of OMW. The effect of these two fungi showed that *P. ostreatus* was higher efficiency than *P. chrysosporium*.

The maximum COD reduction (74.5%) was obtained by *A. wentii* after two weeks of incubation; this was followed by (43%) that obtained by *P. ostreatus* after three weeks . *A. niger* showed the lowest COD reduction (13.8%) at the first week. The maximum phenolic degradation (88%) was obtained by *P. ostreatus* after two weeks. This was followed by (81.3%) that was obtained by *A. wentii* after two weeks, while *A. niger* showed the lowest degradation percentage (64.1%) that obtained after one week (Fig. 2).

The obtained results showed that *A. wentii* was more efficient than *A. niger* in removing of COD and phenolic compounds from OMW. It showed the maximum COD removal (74.5%) after two weeks of incubation, *A. wentii* also showed better results in removing of phenolic compounds than *A. niger*. It gave 81.3% reduction after two weeks.Our resultus were in accordance with those obtained by (Hamdi *et al*. 1991) who explained that the highest biomass and the grittiest COD and phenolic compound removal were obtained by *Aspergillus sp*. In general and A. niger in particular. This matched with our results that conformed that *A. wintii* recorded the optimum COD and phenolic reduction.

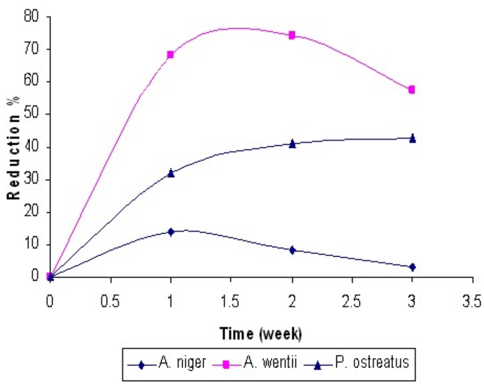

Fig. 1: Reduction of COD content of OMW by *A. niger, A. wentii* and *P. ostreatus* as affected by incubation time.

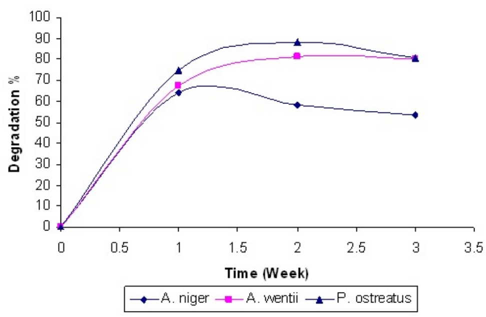

Fig. 2: Biodegradation of phenolic compounds content of OMW by *A. niger, A. wentii* and *P. ostreatus* as affected by incubation time.

Effect of OMW Dilution on Biodegradation of Phenolic Compounds and COD Removal by the Selected Fungal Isolates:

This experiment was carried out to show the optimum dilution of OMW which used on biodegradation of phenolic compounds and COD by selected fungal serial dilutions 10, 20, 30, 40, 50, and 60%. The maximum COD removal (62.2%) was obtained by *A. wentii* grown in 10% OMW. This was followed by 59.3% removal of COD obtained by *P. ostreatus* at the same dilution .The maximum COD removal obtained by *A. niger* was 28% at 40% concentration of OMW (Fig. 3), while the maximum phenolic compounds degradation (80.9%) was obtained by *A. wentii* grown in 10%. This was followed by 79.6% that obtained by *P. Ostreatus* in the same dilution (10%). The least biodegradation percentage (72.9) was obtained by *A. niger* in 30% dilution. (Fig. 4).

The obtained results confirmed that *A. wentii* was the best fungus that able to reduce both COD and phenolic compounds from olive mill wastewater after one week of incubation (Hamdi *et al.,* 1991) whereas (Ahmadi *et al.,* 2006) reported that the changed levels of the OMW concentration, i.a.20-40% increasing the pollutant removal.

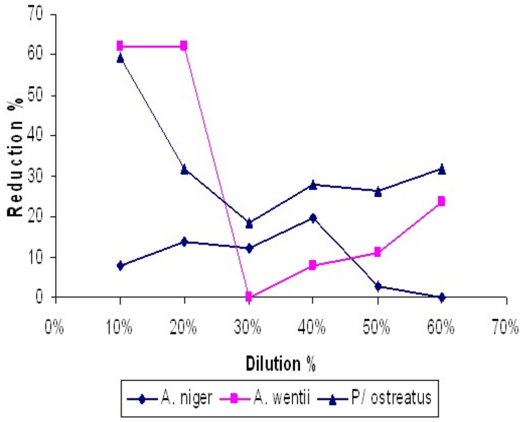

Fig. 3: Effect of OMW dilution on COD reduction by *A. niger, A. wentii* and *P. ostreatus*

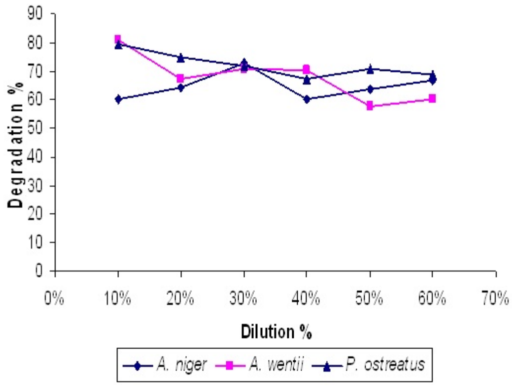

Fig. 4: Effect of OMW dilution on phenolic compounds degradation by *A. niger, A. wentii* and *P. ostreatus.*

Chemical Treatments:

Oxidation of Tannic Acid by UV Radiation in the Presence and Absence of Different Concentrations of H_2O_2 at three pH Values:

Data in table (4) showed that the presence of H_2O_2 increases the degradation of tannic acid in all experiments. Also, the two concentrations of H_2O_2 showed similar results. In addition to that, the degradation percentage increased as the pH was increased from 5 to 9. The maximum degradation percentages at 0.05*M* H_2O_2 concentration were 28.2%, 37% and 44% at pH values 5, 7 and 9 respectively, while, they were 32.6%, 37.4% and 47% at 0.11*M* H_2O_2 concentration and pH 5, 7 and 9 respectively.In the same line, (Chiou *et al.*, 2008 and Borghei and Hossenini 2008) who worked on furfural and phenol mentioned, that H_2O_2 readily produced hydroxyl radicals under UV irradiation, thereby increasing photodegradation, whereas irradiation process, where no oxidants are present, has little effect on decomposition of furfural.

Oxidation of Tannic Acid by Fenton System as Affected by UV Radiation and pH Value:

Effect of Fenton system in the presence and absence of UV radiation at different pH values, two ferrous ions concentrations and 0.05 *M* H_2O_2 as shown in Table (5). The best degradation of tannic acid (57.7%) was obtained at pH 5 and Fe^{2+} (0.0024 *M*) in absence of UV radiation. This is followed by 52 % that obtained in the presence of UV and pH 9 at Fe^{2+}(0.0048 *M*). On the other hand, the degradation percentages of tannic acid were increased as the concentration of H_2O_2 has been increased from 0.05*M* to 0.11*M* in all treatments. In addition to that, the maximum degradation was increased from 20%, 46.5% and 70% as the pH increased from 5, 7 to 9, respectively.

These results are higher than that obtained from the previous experiment in which the degradation of tannic acid was tested by UV and H_2O_2 only without ferrous ions; whereas the maximum degradation was 47% at pH 9 and H_2O_2 0.11M. Therefore, it was noticed here that addition of ferrous ions increased the degradation of tannic acid in its solution in the presence of H_2O_2.

Our results are similar to (Primo *et al*, 2008). Who mentioned that, The photo-Fenton process ($UV/Fe^{2+}/H_2O_2$) was found to be the most efficient treatment, combining the use of iron as catalyst and hydrogen peroxide in presence of UV radiation, for reduction of COD (78%) compared to the other processes investigated ($UV/Fe^{2+}/H_2O_2 > Fe^{2+}/H_2O_2 > UV/H_2O_2 > UV$). In the contrary to (Dincer *et al.*, 2008) who reported that the optimum reduction of COD was at pH 3.

Table 4: Summery of the maximum percentages of tannic acid degradation as affected by H_2O_2 concentrations at different pH values.

H_2O_2 concentration (*M*)	pH value		
	5	7	9
0.05	28.2	37	44
0.11	32.6	37.4	47

Table 5: The maximum percentages of tannic acid degradation as affected by Fenton systems at different pH values.

Treatments		In the dark		Under UV radiation	
		Fe^{2+} (0.0024 *M*)	Fe^{2+} (0.0048 *M*)	Fe^{2+} (0.0024 *M*)	Fe^{2+} (0.0048 *M*)
H_2O_2 0.05M	pH 5	57.7	46.6	35.0	40.1
	pH 7	37.0	37.0	37.5	35.0
	pH 9	47.0	52.0	45.0	46.0
H_2O_2 0.11M	pH 5	17.0	20.0	9.0	15.0
	pH 7	45.0	43.0	43.0	46.5
	pH 9	70.0	70.0	70.0	70.0

Photo Catalysis of Tannic Acid by Titanium Oxide:

At pH 5 the degradation was increased along the time in all concentrations of titanium oxide. The maximum degradation of tannic acid reached to the end of experimental period (150min.). It was 33%, 35%, 33%, 33% and 32% at the titanium oxide concentrations 25, 50, 75, 100 and 125 mg/L respectively. Therefore, the maximum degradation (35%) was obtained at 50mg/l titanium oxide after the same period (150) under UV radiation. This is much lower than that obtained in the previous experiments (Fig. 5).

Photocatalysis of tannic acid by titanium oxide at pH 7 showed that the degradation was also increased during the time in all concentrations of titanium oxide. The maximum degradation of tannic acid reached at the end of experiment period (150min.). It was 17%, 22%, 23%, 23% and 23% at the titanium oxide concentrations 25, 50, 75, 100 and 125 mg/L respectively. The maximum degradation (23%) was obtained at titanium oxide concentration 75mg/l after 150min. This result is lower than that obtained at pH 5 (35%), Figure (6).

Photocatalysis of tannic acid by titanium oxide at pH 9 showed no degradation of phenolic compounds by this treatment method. It was noticed that the degradation of tannic acid was decreased as the pH was increased from 5 to 9. The maximum degradation percentages that obtained at pH 5, 7 and 9 were 35%, 23% and 0%, respectively.

In a comparison between the three methods which that used in the degradation of tannic acid: the treatment by H_2O_2 under UV radiation showed maximum degradation percentage 47% at H_2O_2 concentration of 0.11*M* and pH 5. While the maximum degradation percentage that obtained when Fenton system was used was 70% at the two Fe^{2+} concentrations in dark and UV. This was much higher than that obtained when phtocatalysis by titanium oxide was used. Titanium oxide phtocatalysis method showed only 34% degradation of tannic acid as the maximum degradation percentage.

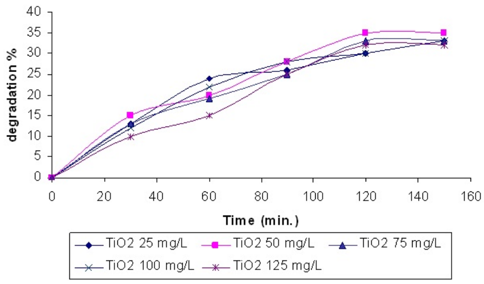

Fig. 5: Effect of titanium oxide concentration on the degradation of tannic acid at pH 5.

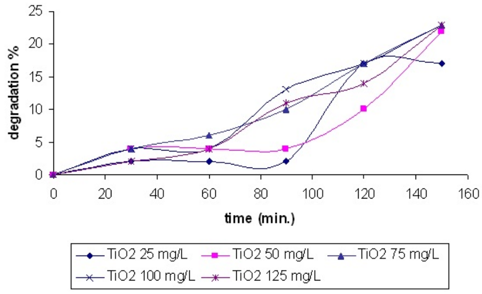

Fig. 6: Effect of titanium oxide concentration on the degradation of tannic acid at pH 7.

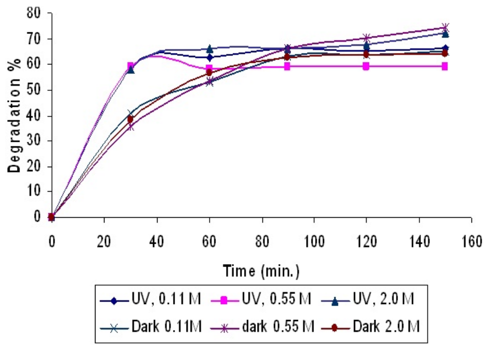

Fig. 7: Effect of H2O2 concentrations on the degradation of phenolic compounds in OMW as affected by UV and dark.

Therefore, it is recommanded to treat the olive oil wastewater by hydrogen peroxide only (without ferrous ions) in presence and absence of UV radiation despite the higher degradation in presence of Fe^{2+} ions. This is due to prevent usage of ferrous ions which contaminate the environment as it is heavy metal ions. Our findings agree with those optioned by (Boroski *et al*., 2008) who reported that The subsequent photocatalysis: $UV/TiO_2/H_2O_2$ (mercury lamps), pH 3.0, 4 h irradiation, 0.25 g /L TiO_2 and 10 mmol /L H_2O_2 shows high levels of inorganic and organic compounds eliminations. The obtained COD values: 1753 mg L^{-1} for the sample from the factory, 160 mg /L after EC and 50 mg L^{-1} after EC/photocatalyzed effluents pointed out that the combined treatment stresses this water purification.

Treatment of Olive Oil Wastewater by Hydrogen Peroxide in Presence and Absence of UV Radiation:

The degradation of phenolic compounds increased at the first 30min. In both UV and dark treatments at all H_2O_2 concentrations. It was then slowly increased after that. The maximum degradation percentages that obtained from the UV irradiated OMW at H_2O_2 concentrations 0.11*M*, 0.55*M* and 2.0 *M* were 66, 59.2 and 72.3%, respectively. While,they were 65%, 74.4% and 64.4% in dark at the same H_2O_2 concentrations respectively. Therefore, the maximum phenolic compounds degradation percentage that obtained from this experiment was 74.4%, that obtained at H_2O_2 0.55*M* in the dark after 150 min (Figure 7).

Finilly it can be concluded that *Aspergillus wentii* can be used for biological treatment of OMW as it degrades 74.5% of COD and 81.3% removal of phenolic compounds after two weeks of incubation. On other hand the best chemical treatment was hydrogen peroxide (0.55M) as it removes 74.4% of total phenolic compounds after 150min. without requirement of UV radiation. The single photochemical process produces a 35% removal of organic matter present measured as COD after 6 h of reaction, while the hydroxyl radicals enhance significantly this reduction with values ranges from 41to76%. At the same time, a removal of aromatic compounds in the oxidation by single UV radiation and combined UV+H_2O_2 was ranged from 20 to 52%.

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