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Reduction of olive oil mill waste water phenolic compounds and COD using *Paecilomyces variotii*

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ABSTRACT

The present study aimed to shed light upon the role of microorganisms in biological treatment of phenolic compounds as a one of the widespread pollutants in Egypt. The ability of twenty six different indigenous isolates which isolated from the olive oil mill waste water (OOMW), for growth in media containing 10% and 25% OOMW as sole carbon source was tested. *Paecilomyces variotii* was the most potent isolate among all isolates as it degraded 10.40 % of the phenolic compounds. These isolate was selected to examine its biodegradation activity under different conditions. The maximum degradation of phenolic compounds and chemical oxygen demand (COD) decrease percentage was (68.14 and 59.12, respectively) obtained at 50% dilution of OOMW. The best environmental conditions for phenolic compound biodegradation and COD reduction, in shacked flasks at 150 rpm were, pH 6.0, temperature 37±1˚C and incubation period 12 days, with the supplementation of the degradation media with 150 mg/l sucrose, 2.5 g/l yeast extract and 0.070 mmol/l $CuSO_4$, and 4ml inoculums size. In addition, low dose of gamma radiation (0.25 kGy) enhanced the fungal biodegradation activity, and led to increase the phenolic compounds biodegradation percent 8.7% than the optimum conditions previously mentioned. Finally, the biotreated OOMW was lower toxicity to environment than untreated one.

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1. INTRODUCTION

The olive oil industry has a significant environmental impact due to the production of huge amounts of either a highly polluted olive mill wastewater (OMW) or a solid residue, depending on the olive oil extraction process. The uncontrolled disposal and treatment of OMW is a serious environmental problem because it contains high organic Chemical Oxygen Demand (COD) concentration and high content of microbial growth-inhibiting compound such as phenolic compound and tannins, and so resistance to degradation. Phenols are easily soluble in water and eventually may reach down to streams, rivers, lakes and soil and represent a serious ecological problem due to their widespread and occurrence throughout the environment, and cause toxic effects on aquatic flora and fauna, ultimately affect the ecological balance [1]. The low volatility of phenol and its affinity for water make oral consumption of contaminated water the greatest risk to humans and death among adults has been reported with ingestion of phenol ranging from 1 to 32 g [2]. So, it is necessary to eliminate or reduce the phenolic components in the OMW. The progresses on this problem are made by reducing the chemical oxygen demand (COD), and eliminate or reduce the phenolic compounds as a major OMW pollutant. Several chemical and physical methods have been developed for the phenol degradation but these methods are expensive and inefficient. The by-products from the chemical and physical methods process posse's toxic compounds [3]. Bioremediation is a biological process by which living organisms degrade or transform hazardous organic contaminates to less toxic compounds [4]. Recently, fungi have received

considerable attention for their bioremediation potential which is attributed to the enzyme they produce [5]. Parameters such as pollutant concentrations, temperature, pH and microbial adaptation are the most important parameters that affect phenol biodegradation rate depends on the period in which the culture was adapted to phenol degrading microorganism [6]. The aim of the present study: Isolation of OOMW indigenous microorganisms and selection of the most potent microbial isolate which can degrade the OOMW phenolic compounds, and decrease its COD. Increase the overall efficiency of the selected microorganism by optimization of factors that affecting the biodegradation with the application of low doses gamma radiation.

2. MATERIALS AND METHODS

2.1 Collection of the olive oil mill waste water

Samples of the OOMW were collected from, an olive oil factory, Ismailia, Egypt-Alexandria Desert Road, Egypt. The OOMW was taken from a modern three-phase (one of the olive oil extraction methods). OOMW samples were collected in pre-sterilized glasses from a depth of about 1 meter from the waste water surface.

2.2 Isolation, selection and identification of microbial isolates from the olive oil mill waste water

Ten milliliter of OOMW was serial diluted up to 10-7. One ml of each dilution was plated (in triplicate) on both malt extract agar with 0.05 gm/ml of chloramphenicol at pH 5.5 for 7 days for fungi and 5 days for yeast at 25˚C and Nutrient agar media with 0.05 gm/ml of amrazole at pH 7.0 for one day and 37˚C for bacteria. Selection and separation of colonies was done by repeated subculture [7]. An inoculum from the isolation media was used for inoculation (MEA) medium for fungi and yeast and (NAM) for bacterial isolates, supplemented with 10% of the OOMW. The selected microorganisms was inoculated in Mineral Salt Medium (MSM) for fungi and yeast and Tryptone Glucose Medium (TGM) for bacterial isolates, supplemented with 25% of the OOMW to know the best isolate for degradation of phenolic compounds and decreasing COD to complete this study.

Identification of fungi was carried out by the methods of Moubasher (1993) [8] and confirmed by the help of the Micro Analytical Center, Cairo University, Egypt. Yeast isolates was identified by the methods of Barnett *et al*. (2000) [9]. Identification of bacterial isolates was carried out by the methods of Holt *et al.* (1994) [10].

2.3 Factors affecting the biodegradation of olive oil mill wastewater

The biodegradation was carried out in 250 ml conical flasks, which contained 50 ml of OOMW. To obtain the optimum conditions, different factors were studied.

Different concentrations of OOMW (50%, 75% and 100%) v/v were obtained by adding varying amount of distilled water. Four incubation periods with time intervals 4 days (4, 8, 12 and 16 days). Four incubation temperatures (25±1, 30±1, 37±1, and 45±1˚C). pH was varied between 4 and 10 with an increment of 2. The pH value was controlled by adding 0.1N HCl or NaOH solution. Various carbon sources (glucose, fructose, sucrose and starch) with concentration (100 mg/l). Different concentrations (50, 100, 150 and 200 mg/l) of the best carbon source which recorded from this experiment were investigated. One g/l of different nitrogen sources (sodium nitrate, urea, peptone and yeast extract). Different concentrations of best nitrogen source (0.50, 1.0, 1.5, 2.0, 2.5 and 3 g/l) were investigated. In addition, three agitation speeds (100, 150, and 200 rpm), Mineral salts: (copper sulphate, zinc sulphate and ferric chloride) with concentration of 0.070 mmol/l, and Three inoculum level (0.75, 1.5, 3, 6 and 12 g/l) were investigated with the application of gamma irradiation low doses (0.00, 0.25, 0.50, 0.75 and 1.00 kGy).

2.4 Preparation and adaptation of fungal inoculum

Preparation and adaptation of selected fungal isolate inoculums were determined according to Elsa *et al*. (2004) [11]. and Jacob Alsohaili (2010) [12].

2.5 Analytical methods

Dry weight was measured by drying 10 ml culture broth at 60˚C to constant weight. The pH of the broth media was measured at 25˚C with glass electrode pH meter (type pioneer 10 portable pH meter with a fisher pencil probe). Phenolic compounds concentrations were determined by kits of (Nanocolor® phenol 12.05), Cat. No.91875 and Test 1-75. Chemical oxygen demand (COD) concentration was determined by kits of (Nanocolor® COD 300), Cat. No. 985 033 and Test 0-33.Total suspended solid (TSS) were obtained by centrifugation at 6000g for 20 min, the settled solids were then dried overnight at 105°C. Total dissolved solids (TDS) was determined after evaporation of a 10 ml OOMW sample in a porcelain cup at 105 ºC for 24 hrs. Color of OOMW was evaluated by measurement of absorbance decrease at 395 nm.

Radiation process. Irradiation process was carried out at National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The irradiation facilities used were an experimental Co^{60} Russian gamma chamber. INDIA. The average dose rate of this gamma radiation source was 2.5 kGy/1hr at the time of the experiments.

2.6 Statistical analysis. The data are the means of 3 independent experiments. Experimental data were subjected to analysis of variance (ANOVA) using SAS program [13].

3. RESULTS AND DISCUSSION

Biodegradation is an important means to eliminate toxic wastes from the environment.

3.1 Physico-chemical characterizations of the freshly untreated OOMW

Table 1 showed a major complexity of OOMW especially phenolic compounds (50.0 mg/l) and chemical oxygen demand (COD) 22800.0 mg/l which were higher levels than normal range (0.05 and 100 mg/l), respectively. It was reported that the total phenolic compounds in OOMW ranged from 10.32-30.99 mg /l [14] and COD has high values reached to 21000 mg/l [15].

Table 1. Physico-chemical characters of untreated olive oil mill wastewater.

Parameters	Measured (mg/l)	Normal range (mg/l)
Total suspended solids (TSS)	94000.0	2000.0
Total dissolved solids (TDS)	22000.0	60.0
Chemical oxygen demand (COD)	22800.0	100.0
Oils and grease	2450.0	10.0
Phenolic compounds	50.0	0.05
pH	5.5	6.0-9.0

3.2 Isolation and identification of microorganisms

Twenty six isolates, ten fungi, nine yeast and seven bacteria, were isolated from OOMWW. From these isolates, three fungi, two yeast and bacteria had ability to overcome toxic chemicals (phenolic compounds) and grow well on medium containing 10% and 25% OOMW. Fungi were identified as *Paecilomyces variotii*, *Ascobolus stercorarius* and *Aspergillus terreus* [8].Seven fungal species able to grow in OOMW, namely *Aspergillus clavatus, A. wentii, A. niger, Mucor strictus hagem*, *Penicillium nigricans*, *P. cilreaviride*, and *P. chermesinum* were isolated from OOMW [16]. Yeasts isolates were identified as *Yarrowia lipolytica* and *Candida tropicalis* [9]. *Candida cylindracea*, *Candida rugosa* and *Yarrowia lipolytica* were isolated from OOMW disposal pond in North region of Portugal [17]. Bacterial Isolates were *Lactobacillus curvatus* and *Bacillus brevis* [10]. *Bacillus brevis* was isolated from OOMW and used it in removing of the phenolic compounds [18]. Also, *Lactobacillus paracasei,* which isolated from OOMW can aerobically degrade phenolic compounds [19].

3.3 Selection of the most potent phenolic compounds degrading microorganisms

Isolated microorganisms had different abilities to degrade OOMW phenolic compounds, the bacterial isolates have the lowest ability (6.40% by *Bacillus brevis*, and 6.0% by *Lactobacillus curvatus*), meanwhile, yeast isolates had medium ability (8.40% by *Candida tropicalis* and 7.60% by *Yarrowia lipolytica*). The results indicate that the fungal isolates had the highest ability which were 8.60 % by *Aspergillus terreus*, 9.20% by *Ascobolus stercorarius* and the maximum reduction of phenolic compounds was 10.40% by *Paecilomyces variotii* which selected to complete the study. It was suggested that fungi produce various metabolites and enzymes so they have effectiveness for removal, reduction and detoxification of industrial effluents [20].

3.4 Factors affecting the biodegradation of phenolic compounds by *Paecilomyces variotii*

3.4.1 Concentration of OOMW

The maximum phenolic compounds reduction was 4.46 mg/l (17.84 %), and the maximum decreased COD 123.9 g/l (10.87%) were obtained at 50% olive oil wastewater Table 2. The dry weight of *Paecilomyces variotii* increased by decreasing the OOMW concentration from 100% (3.30 g/l) to 50% (4.78 g/l). Statistical analysis showed significant difference of results, and the optimum relationship between dry weight and phenolic compounds biodegradation and decreasing COD was achieved, when 50 % OOMW was added to MSM medium instead of distilled water. The increase of degraded phenolic compounds and decreased COD concentration by diluting the olive oil mill waste water may be due to the reduction of phenolic compounds concentration in the samples. In this study, no lower OOMW concentrations were used because the high dilution leads to increase waste volume. When *Coriolus versicolor* and *Funalia trogii* were inculated in OOMW media without any dilution, no growth was observed because of its high COD and phenolic compounds content [21]. Total phenolic compounds removal by *Phanerochaete chrysosporium* decreased with the increased concentration of OOMW from 20% to 40% [22].

Table 2. Effect of olive oil waste water different concentration on OOMW biodegradation by *Paecilomyces variotii*.

Concentration of OOMW**	Initial phenolic compounds (mg/l)*	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%) ***	Initial COD (g/l)*	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
Undiluted (100%)	50.0±0.363^{a}	5.94±0.23^{a}	11.88	2280±10.713^{a}	1612.00±40.501^{a}	7.07	3.30± 0.152^{c}
75%	37.5±0.450^{b}	5.17±0.12^{b}	13.78	1710±8.566^{b}	1410.00±22.679^{b}	8.25	4.06±0.120^{b}
50%	25.0±0.535^{c}	4.46±0.24^{c}	17.84	1140± 6.362^{c}	1238.73±19.767^{c}	10.87	4.78±0.113^{a}

Incubation period: 8 days. Incubation temperature: 25˚C. Inoculum size: 1 ml
Paecilomyces variotii spore suspension with optical density (A_{600}= 0.75).
* Means in the same column with different letters have significant differences between each other.
**: Dilution rate: v/v (waste/ water).
*** Degraded phenolic compounds or decreased COD %= (Degraded phenolic compounds or decreased COD /Control)*100.
Degraded phenolic compounds or decreased COD = Control- Remained phenolic compounds or Remained COD.
* * * *Data represents mean ±SE for 3 replicate flasks at P<0.05 (n=3).
* * * ** a, b, c: significantly different from the corresponding control.

3.4.2 Incubation periods

The degraded phenolic compounds and decreased COD concentration increased through time progress until reaching the maximum value of degraded phenolic compounds (19.73%) and decreased COD concentration (11.44%), respectively with the highest dry weight (5.70 g/l) at the 12th day of incubation, longer incubation periods resulted in a significant decrease for each one of them Table 3. *Aspergillus wentii, P. ostreatus* and *Aspergillus niger* obtained the maximum COD 74.5%, 43% and 13.8% after two weeks, three weeks and first week, and the maximum phenolic compounds reduction 81.3%, 88% and 64.1% after two weeks, two weeks and first week, respectively [16].

Table 3. Effect of different incubation periods on the olive oil mill waste water phenolic compounds biodegradation by *Paecilomyces variotii*.

Incubation period (days)	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
4	3.47±0.13^{bc}	13.87	963±11.85^{b}	8.45	3.80±0.06^{c}
6	3.89±0.10^{bc}	15.56	1146.7±52.78^{a}	10.06	4.37±0 .90^{b}
8	4.46±0.24^{a}	17.84	1238.7±19.77^{a}	10.87	4.78±0.11^{c}
12	4.93±0.24^{ab}	19.73	1303.6±33.96^{c}	11.44	5.70± 0.12^{a}
16	4.24±0.15^{ab}	17.45	1206.33±24.74^{a}	10.58	4.2±0.17^{b}

Legend as Table 2. Initial phenolic compounds: 25 mg/l.
Initial COD: 11400 mg/l (1140 g/l). Dilution rate: 50% (waste/ water).

3.4.3 Incubation temperature

The optimum incubation temperature for *Paecilomyces variotii* was 37±1˚C with degraded phenolic compounds (5.82 mg/l), decreased COD (1769.33 mg/l), respectively and maximum growth (6.82 g/l) Table 4. Dry weight increased with increasing in temperature; accordingly *Paecilomyces variotii* could be specified as a thermo tolerant. De Hoog *et al*. (2000) reported that *Paecilomyces variotii* are thermotolerant and can grow well at temperatures as high as 50˚C and possibly 60˚C. It was found that the maximum phenol degradation by *paecilomyces variotii* exhibited at 37 ˚C [23]. *Pseudomonas aeruginosa* growth and phenol degradation rate increased with the increase in temperature up to 37±1˚C, but above 37±1˚C it decreased [24].

3.4.4 pH values

The extra cellular pH plays an important role in biodegradation processes and metabolic process. The degraded phenolic compounds and decreased COD concentration increased gradually and reached the maximum value at *p*H 6 (8.55 mg/l (34.19%) and 2180 mg/l (19.13%), respectively. Higher pH values (> pH6) resulted in a gradual decrease in the degraded phenolic compounds and decreased COD concentration as well as the dry weight Table 5. It was found that the optimum phenol degradation was exhibited by *paecilomyces variotii* at optimum pH 6 [23].

Table 4. Effect of different incubation temperatures on the olive oil mill waste water phenolic compounds biodegradation by *paecilomyces variotii.*

Temperature (˚C)	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
25	4.93±0.24^{b}	19.73	1303.60±33.96^{c}	11.44	5.70±0.12^{c}
30	5.09±0.10^{ab}	20.32	1493.01±17.94^{b}	13.09	6.20±0.12^{b}
37	5.82±0.26^{a}	23.27	1769.33±31.80^{a}	15.52	6.82±0.27^{a}
45	2.88±0.18^{c}	11.52	1034.80±19.77^{d}	9.08	3.23±0.12^{d}

Legend as Table 3. Incubation period 12 days.

Table 5. Effect of initial pH value on the olive oil mill waste water phenolic compounds biodegradation by *paecilomyces variotii*.

pH	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
4.0	4.89±0.229^{c}	19.57	1587.60±31.24^{c}	13.93	5.52± 0.105^{c}
5.5	5.82±0.26^{c}	23.27	1769±31.80^{bc}	15.52	6.82± 0.27^{b}
6.0	8.55±0.20^{a}	34.19	2180±153.10^{a}	19.13	8.89±0.28^{a}
8.0	6.93±0.23^{b}	27.71	1883±29.05^{b}	16.52	5.19± 0.35^{a}
10	5.50±0.46^{c}	22.00	1370±42.18^{d}	12.02	4.59±0.31^{d}

Legend as Table 4. Incubation temperature: 37˚C.

3.4.5 Carbon sources

All added carbon sources (100 mg/l) had enhancement effect on phenolic compounds degradation. The most preferable carbon source which supported maximum degraded phenolic compounds (12.22 mg/l (48.88%) and decreased COD concentration 2801 mg/l (24.57%), respectively was sucrose Table 6. When different sucrose concentrations (50, 100, 150 and 200 mg/l) was added, degraded phenolic compounds concentration was linearly increased with increasing sucrose concentration till reached its maximum value at 150 mg/l then decreased with increasing the concentration of sucrose than the optimum value Table 6.

When both phenolic compounds and added carbon source were present together, the tolerance of the cells to substrate inhibition increases. The additional conventional carbon source may aid in reducing toxicity of toxic chemical, provide reducing power or act as inducing agents for biodegradation enzyme [1].

Table 6. Effect of different carbon source on the olive oil mill waste water phenolic compounds biodegradation by *paecilomyces variotii*.

Carbon source	Phenolic compounds				
	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
Control	8.54±0.199^{c}	34.16	2180±153.10^{e}	19.13	8.89±0.28^{c}
Glucose	9.65±0.23^{b}	38.61	2319.67±144.82^{d}	20.35	10.97±0.26^{b}
Mannitol	10.06±0.21^{b}	40.26	2661.00±217.48^{c}	23.35	11.79±0.59^{ab}
Sucrose	12.22±0.18^{a}	48.88	2801.00±340.54^{a}	24.57	12.95±0.49^{a}
Starch	11.66±0.20^{a}	46.66	2780.00±138.96^{b}	24.39	12.03±0.33^{ab}
Sucrose (mg/l)					
50	10.11±0.0.23^{b}	40.44	2511.33±101.85^{a}	22.03	11.78±0.28^{a}
100	12.22± 0.18^{a}	48.88	2801.00±340.54^{a}	24.57	12.95±0.49^{a}
150	12.62±0.39^{a}	50.48	3007.00±235.74^{a}	26.37	13.09±0.55^{a}
200	10.95± 0.35^{b}	43.80	2707.67±99.53^{a}	23.75	11.52±0.57^{a}

Legend as Table 5 pH 6 Control =MSM medium without adding carbon source

3.4.6 Nitrogen sources

The nutrients, particularly nitrogen are required to improve the aromatic compounds biodegradation. Yeast extract (1 g/l) was the superior nitrogen source and the most suitable organic nitrogen source for the biodegradation (degraded phenolic compounds; 14.55 mg/l, decreased COD; 4844 mg/l and the dry weight; 16.18 g/l, respectively). Peptone showed significant effect, and the inorganic nitrogen sources showed lower values on degraded phenolic compounds, and decreased COD in comparison with the control Table 7.

The organic nitrogen sources (0.5% and 1% peptone or yeast extract) were considerably better than inorganic sources as ammonium sulphate for the growth and biodegradation of hydrocarbons by *Acinetobacter faecalis*, *Staphylococcus sp*., *Pseudomonas putida* and *Neisseria elongate* [25].Increasing the concentration of yeast extract led to a significant increase in both degraded phenolic compounds (15.45 mg/l) and decreased COD (5644 mg/l) concentration, up to 2.5 g/l and decreased thereafter Table 7. the presence of 0.01% yeast extract at pH 7 in medium contained 6 bacterial strains showed the highest phenolic compounds removal efficiency (99 %) but when urea was substituted for yeast extract the efficiency reduced to 75 %. The replacement of yeast extract with tryptone and urea affected the degradation efficiency of phenolic compounds, this might be due to the structure of yeast extract as it is readily available as amino acids in the mineral salts medium. Using yeast extract as nitrogen source was more efficient in degrading phenolic compounds [26].

Table 7. Effect of different nitrogen sources on the olive oil mill waste water phenolic compounds biodegradation by *Paecilomyces variotii.*

Nitrogen source	Phenolic compounds				
	Degraded phenolic compounds (mg/l)*	**Degraded phenolic compounds (%)*** **	**Decreased COD (mg/l)***	**Decreased COD (%)*** **	**Dry weight (g/l)***
Control	12.62±0.39^{bc}	50.48	3007±235.74^{c}	26.37	13.09±0.55^{c}
Sodium nitrate	11.00±0.32^{d}	44.00	2333±34.76^{d}	20.46	11.05±0.21^{d}
Urea	11.99±0.20^{c}	47.96	2628±49.54^{d}	23.06	12.89±0.20^{c}
Peptone	13.26±0.21^{b}	53.04	3845±38.12^{b}	33.72	14.73±0.16^{b}
Yeast extract	14.55± 0.12^{a}	58.00	4844±37.85^{a}	42.49	16.18±0.21^{a}
Yeast extract (g/l)					
0.50	13.88±0.240^{b}	55.22	3994.0±168.321^{c}	35.04	14.10±0.387^{d}
1.00	14.55± 0.115^{ab}	58.00	4844.0±37.846^{b}	42.49	16.18± 0.21^{bc}
1.50	14.69± 0.439^{ab}	58.75	4928.0±163.623^{b}	43.22	16.68±0.345^{ab}
2.00	14.76± 0.394^{ab}	59.04	5357.0±211.179^{ab}	46.99	17.03±0.323^{ab}
2.50	15.45± 0.239^{a}	61.79	5644.0±351.304^{a}	49.51	17.59±0.295^{a}

Legend as Table 6. Sucrose: 150 mg/l Control=MSM medium without external nitrogen source

3.4.7 Agitation and static conditions

The results in Table 8 indicated that the shaking condition accelerated the biodegradation process of OOMW in comparison with the static condition. The maximum degraded phenolic compounds (16.49 mg/l) and decreased COD (6011 mg/l) with dry weight (18.22 g/l) was at 150 rpm, with no significance difference between 100 rpm and 200 rpm, but under static conditions they decreased. The agitation rate of 150 rpm were the optimal for achieving the higher degradation of phenolic compounds by Actinobacillus species, it could be attributed to increased amounts of dissolved O_2 as a result of agitation. [27].

3.4.8 Mineral salts

The addition of $CuSO_4$ caused activation in phenolic compounds degradation (16.88 mg/l), decreased COD (6643.67 mg/l) and dry weight (20.47 g/l). Furthermore, $ZnCl_2$ showed a noticeable delaying in degradation process where degraded phenolic compounds were 13.39 mg/l Table 9. reported that the enzymes which were responsible for phenolic compounds degradation activated by copper ions Cu^{2+} [28].

Table 8. Effect of different shaking rate on the olive oil mill waste water phenolic compounds biodegradation by *paecilomyces variotii*.

Shaking rate (rpm)	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
0 (Static)	15.45± 0.24^{b}	61.79	5644.33±351.3^{c}	49.51	17.67±0.30^{b}
100	15.75±0.14^{b}	63.00	5797.33± 55.68^{b}	50.85	17.79±0.14^{ab}
150	16.49± 0.25^{a}	65.97	6011.00±123.52^{a}	52.73	18.22±0.16^{a}
200	15.97±0.22^{ab}	63.88	5865.67 ±82.67^{b}	52.04	7.82±0.14^{ab}

Legend as Table 7

Table 9. Effect of different mineral salts on the olive oil mill waste water phenolic compounds biodegradation by *paecilomyces variotii*.

Mineral salts (mmol/l)	Degraded Phenolic compounds (mg/l)*	Degraded Phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
Control	16.49±0.25^{a}	65.97	6011±123.52^{b}	52.73	18.22± .16^{a}
$FeSO_4$	15.69±0.20^{b}	62.76	5977.67±66.34^{b}	52.44	16.78±0.15^{c}
$ZnCl_2$	13.39±0.22^{c}	53.56	2791±137.39^{c}	24.48	14.63±0.32^{d}
$CuSO_4$	16.88± 0.24^{a}	67.52	6643.67±182.14^{a}	58.28	20.47±0.28^{a}

Legend as Table 8. Shaking rate: 150 rpm and adding 0.070 mmol/l from each mineral salt
Control = Combination of all factors with no addition of external mineral salts on MSM media

Table 10. Effect of different inoculum sizes on the olive oil mill waste water phenolic compounds biodegradation by *paecilomyces variotii*.

Inoculum size (ml)	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
0.5	16.20±0.25^{ab}	64.80	6220.33±117.25^{a}	54.56	16.59±0.17^{b}
1.0	16.88± 0.24^{a}	67.52	6643.67±182.14^{a}	58.28	20.47±0.28^{a}
2.0	16.90±0.24^{a}	67.60	6659.6 ±182.14^{a}	58.42	20.97±0.28^{a}
4.0	17.04± 0.25^{a}	68.15	6740.33±212.10^{a}	59.12	21.05±0.32^{a}
6.0	15.84 ±0.31^{b}	63.36	6280.67±147.56^{a}	55.09	16.27±0.33^{b}

Legend as Table 9. Mineral salt (copper sulphate)= 0.070 mmol/l.

Table 11. Effect of different gamma radiation doses on the olive oil mill waste water phenolic compounds biodegradation by *paecilomyces variotii*.

Gamma radiation (kGy)	Degraded phenolic compounds (mg/l)*	Degraded phenolic compounds (%)***	Decreased COD (mg/l)*	Decreased COD (%)***	Dry weight (g/l)*
0.00	17.04± 0.25^{b}	68.15	6740±212.10^{ab}	59.12	21.72±0.17^{a}
0.25	19.21± 0.39^{a}	76.84	6864 ±107.97^{a}	60.21	21.05±0.32^{a}
0.50	15.76± 0.12^{c}	63.08	6238±124.61^{b}	54.72	18.23±0.317^{b}
0.75	13.07± 0.25^{d}	52.28	5095±119.47^{c}	44.69	16.67±0.28^{c}
1.00	11.64± 0.20^{e}	46.52	4476±283.88^{d}	39.26	15.32±0.17^{d}

Legend as Table 10. Inoculum size: 4 ml *Paecilomyces variotii* spore suspension with OD (A_{600}= 0.75).

3.4.9 Inoculum size

The maximum degraded phenolic compounds (17.04 mg/l) and maximum decreased COD concentration (6740.33 mg/l) with maximum dry weight (21.05 g/l) were obtained at inoculum size 4 ml, then OOMW biodegradation decreased with higher inoculum size (6 ml) Table 10. The optimal inoculum size of white rot fungi *Coriolus versicolor* and *Funalia trogii* were 5 ml for COD removal [21].

3.4.10 Gamma irradiation

The irradiation dose 0.25 kGy gave the maximum degraded phenolic compounds (76.84%) and maximum decreased COD (60.21%) with dry weight (21.05 g/l), then they decreased with increasing the irradiation dose above 0.25 kGy Table 11. The viable count of *Aspergillus terreus* MAM-F23 and MAM-F35 gradually decreased as the gamma radiation dose increased, doses 5.0 and 4.0 kGy reduced the viable count of *Aspergillus terreus* MAM-F23 and MAM-F35 completely [29].

3.5 The physico-chemical characteristics of OOMW before and after treatments

The physico-chemical characteristics of olive oil mill waste water before and after treatments are reported in Table 12. The total content of phenolic compounds is reduced from 50.0 mg/l to 5.8 mg/l by *Paecilomyces variotii* Table 12. Using *Geotrichum sp*., *Aspergillus sp*. and *Candida tropicalis* enables the removal of polyphenolic compounds in percentages of 46.6%, 44.3%, and 51.7%, respectively [30].

The results in Table 13 indicated that COD was high (22800.0 mg/l) but after the treatment by *Paecilomyces variotii* it reduced (6864mg/l). *Candida boidinii*, *Geotrichum candidum*, a *Penicillium sp*. and *Aspergillus niger* HA37 which were isolated from OOMW were used in reducing 45–78% COD [31].
The total suspended solids (TSS) were (94000.0 mg/l) before treatment and it decreased (860.0 mg/l) after treatment, also oil and grease decreased from 2450.0 mg/l to 68.0 mg/l Table 12. The inoculated raw sludge with fungal beads (*Penicillium expansum*, BS30) showed suspended-solids degradation of 64% [32].

Table 12 cleared that the total dissolved solids (TDS) decreased from 22000.0 mg/l to 4000.0 mg/l after the treatment. Waste Testing and Quality Assurance reported that the routinely diluted to any samples reduce the amount of dissolved solids to less than 0.1 – 0.2% TDS [33].

It is evident from the results in Table 12 that the values of pH were increased from 5.5 to 7.5 after microbial treatment. It was found that the aerobic biodegradation of OOMW by *Candida holstii* increased the pH from 4.76 to 6.75 [34].

Paecilomyces variotii had effect in removing the color of OOMW. After optimization of all factors, *Paecilomyces variotii* remove 75.435% color and changed the OOMW from dark red-brown color to brown-yellow color Table 12. *Phanerochaete flavido-alba* removed 70% color and 52% aromatic compounds of OOMW after 14 days in batch fermenter [34]. *Coriolus versicolor* removed approximately 63% COD, 90% phenol and 65% color within 6 days [21].

Table 12. Physico-chemical composition of untreated and treated OOMW

Parameters	Before treatment	After treatment	Normal range
TSS	94000.0 mg/l	860.0 mg/l	60.0 mg/l
TDS	22000.0 mg/l	4000.0 mg/l	2000.0 mg/l
COD	22800.0 mg/l	6864 mg/l	100.0 mg/l
Oils and grease	2450.0 mg/l	68.0 mg/l	10.0 mg/l
Phenolic compounds	50.0 mg/l	5.80 mg/l	0.05 mg/l
pH	5.5	7.5	6-9
Color (O.D. 390 nm)*	5.17	1.27	-

Color samples diluted 10 times.

4. CONCLUSION

The biodegradation treatment is less expensive than other technologies that are used for cleanup of hazardous waste and safe to environment comparing with chemical and physical methods. The current study suggested that 88.4% of phenolic compounds and 70% COD were removed by *Paecilomyces variotii* from olive oil mill wastewater samples containing phenolic compounds. Although, these microorganisms suffer from substrate growth inhibition at higher concentration of phenol, the efficiency of the phenol degradation under such a condition could be further enhanced by the process of media optimization**.** The treated effluent may be recycled and reused in different purposes such as irrigation.

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