

**Potential of different fungi species in biodegradation
field of phenolic compounds.**

Abstract

Phenolic compounds are dominant pollutants in terrestrial and freshwater environmental, that have toxic effects on living organisms at low concentrations, because it has the ability to persist in the ecosystem. So bio-removal is a good technique that employs the metabolic potential of microorganisms in order to clean up the environmental pollutants and turned into less dangerous or harmless substances. This work aims to the isolating of different species of fungi from wastewater of factories and coast of the red sea to test the ability of these fungi to degrade phenolic compounds. Ten species of fungi and sterile mycelium are used to remove phenol and its derivatives at different concentrations (0.4%, 0.6% and 0.8%). All fungi species have the ability of removing phenol and their derivatives, but *P.chrysogenum*, *Saccharomyces* sp. and sterile mycelium exhibited low ability to break down of hydroxyl-benzene, 2-naphthol and 1,3 dihydroxy benzene, respectively.

Key words: Biodegradation, phenolic compounds, fungi, chlorophyll pigment.

Introduction

Due to the release of phenolic compounds from agro-industrial operations, these compounds have become widespread in the world as environmental pollutants. Many of these aromatic compounds are toxic to the living system and their presence in the aquatic and terrestrial habitats often have serious ecological consequences, Where natural phenolic compounds are considered one of the most important and dangerous pollutants of the current environment ¹. Many industrial effluents and residues contain the structure of phenolic compound such as waste of ships, paper factories,

aluminum factories, wine-distillery, olive oil extraction, green olive debittering, cork preparation, wood debarking, coffee production, coal gasification, coke-oven batteries, refinery and petrochemical plants and other industries that produce things such as synthetic chemicals, herbicides, pesticides, antioxidants, pulp-and-paper, photo developing chemicals, etc.²⁻¹⁰.

These compounds are stable and even at low concentration they may be toxic towards living organisms and cause unfavorable chemical changes in water and soil as- inhibiting the sunlight penetration and decrease the photosynthetic activity of aquatic system¹¹. Phenolic compound is the most toxic and it can persist in the ecosystem for long time due to its long range transportation, bioaccumulation in human and animal tissue and biomagnification in food chain¹². Many serious diseases are caused by pollution phenol for both human and animal who inhalation and dermal contact such as cardiovascular diseases and severe skin damage, while ingestion can cause serious gastrointestinal damage and death. Even short-term application of phenol to the skin can produce blisters and burns in animals¹³. For these reasons several physico-chemical methods are used to remediate phenolic wastes such as ozonisation, adsorption, reverse osmosis, electrolytic oxidation, photocatalysis¹⁴. While all these methods have failing, so some of these methods are very costly like ozonisation, electrochemical, reverse osmosis and photochemical, but the disadvantage of physical adsorption is the elimination of sludge¹⁵, bioremediation by using microbial cells to resolve phenol contamination problem consider one of the cheapest possible solutions¹⁶⁻¹⁹.

The oxidative activities of microorganisms are the principal reason for the biological treatment of industrial wastewaters. Filamentous fungi may be an important supply of phenol degrading species²⁰. Fungi are known for their wide incidence and also the outstanding capability of degrading advanced and inert natural products -such as lignin, chitin and cellulose. Fungi adopt additional simply than bacterium and are capable to grow in extreme conditions, like nutrient deficiency, low pH, restricted water, etc²¹. And not on the least, there comes the ability of fungi to survive within the presence of varied xenobiotics that turn to be toxic to variety of different microorganisms.

The purpose of this study was to investigate the ability of the different species of fungi to degrade some phenolic compounds, usually present in agro-industrial effluents and the effect of input and output degradation on chlorophyll pigments of *chlorella* sp.

Materials & Methods

Samples collection

Ten samples of wastewater were collected from different sites of red sea beach and factories in Upper Egypt during summer 2017. Wastewater samples were collected in sterile bottles (100 ml) and in plastic bags, respectively, transferred directly to the laboratory and preserved at 4°C until used.

Fungal isolation and identification

Fungi were isolated from the wastewater collected from red sea beach and factories in Upper Egypt using Chapek-Dox salts medium. The most common fungi were recultivated using Czapek's Dox medium until pure colonies were obtained. These fungi were identified using the methods described by ²².

Microorganisms

Eighteen isolates belonged to 6 genus and 10 species (*Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *penicillium aurantiogriseum*, *P. chrysogenum*, *Phoma* sp., *Saccharomyces* sp. and sterile mycelium) were isolated from wastewater and used for degradation of phenol and its derivatives.

Biodegradation media

Biodegradation was conducted on 4 combined media with 3 concentrations of phenol and their derivatives (0.4% , 0.6% and 0.8%), containing the following ingredients as single carbon and energy sources: hydroxy benzene, 2-naphthol, 4-nitrophenol and 1,3 dihydroxy benzene. The total concentration of phenolic compounds in each 100 ml were 0.4%,0.6% and 0.8%. Media contained also Chapek-Dox salts (in %), as follows: NaNO₃ – 0.2, KH₂PO₄ – 0.1, KCl – 0.05, MgSO₄.7H₂O – 0.05, FeSO₄.7H₂O –0.001. The starting pH of culture media was 5.5 ²³.

Determination of phenol degradation potential

The isolates showing growth on Chapek-Dox salts were used for further studies on bioremediation of phenol. Ten ml Chapek-Dox salts broths were inoculated with a 8-

d-old culture of the isolates, and the flasks were incubated under shake culture condition on a rotary shaker for 8 days at 28° C. After an appropriate incubation period, the cells were removed by centrifugation and the cell-free supernatants were used for estimation of residual phenol . The residual phenol was estimated by ²⁴.

Folin –Ciocalteu reagent

The method employed the Folin –Ciocalteu phenol reagent (BDHL td) which was attended by the method of ²⁵. The general method involved the successive addition of 1.5 ml sodium carbonate (200 g ⁻¹) and 0.5 ml Folin-Ciocalteu phenol reagent to 10 ml sample. After 60 min at 20° C, the absorbance was measured at 725 nm against distilled water and correct for the absorbance of a distilled water reagent blank.

$$\% \text{ Phenol Removal Efficiency (PRE)} = \frac{Ci - Cf}{Ci} \times 100$$

Wherever, Ci is the initial concentration of phenol (mg/L) and Cf is the final concentration of phenol. All experiments of phenol biodegradation were done three times; the results were expresses as average ± standard deviation (SD) ²⁶.

Biological assay:

***Chorella* sp. test**

Each extract of *Aspergillus niger* and *Penicillium chrysogenum* (0.05 mg) was applied to 0.8 cm diameter filter paper disc (Whatman No.3), each disc was placed in test tube contained 10 ml of *Chorella* sp. The tubes were kept at a temperature of 25 ±1° C. A control tube with only *Chorella* sp. was also made, which incubated for 8 days.

Determination of pigments (chlorophyll a, chlorophyll b and carotenoids):

Pigment fractions were determined spectrophotometrically according to ²⁷. A known volume (10 ml) of *Chorella* sp. suspension was centrifuged at 3000 rpm and the growth medium was decanted. Pigments were extracted in hot methanol (70°C) for 10 minutes. Cell debris was removed by centrifugation and the clear supernatant which contains the pigments was aspirated and diluted to a definite volume. The extinction coefficient was measured using spectrophotometer (Spectronic 601) against a blank of methanol at the wavelengths of 452, 644 and 663 nm. Taking into consideration the dilution

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made, the content of pigment fractions ($\mu\text{g/ml}$ algal suspension) were calculated using the following equations²⁸:

$$\text{Chlorophyll } a = 10.3 E_{663} - 0.918 E_{644}$$

$$\text{Chlorophyll } b = 19.7 E_{644} - 3.87 E_{663}$$

$$\text{Carotenoids} = 4.2 E_{452.5} - [0.0264 \text{ Chl. } a + 0.4260 \text{ Chl. } b]$$

Statistical analysis

The experimental data were subjected to multivariate analysis of variance using anova. Means were compared using Duncan's test at the 5% level using the SPSS program (SPSS Inc., -Chicago, IL, USA).

Results

Ten species and sterile mycelium belonging to 6 genera were collected in the present study (table, 1). *Aspergillus* was represented by 4 species, and *Penicillium* was displayed by 2 species, while the other genera were introduced by one species. *Saccharomyces* was the common ~~genus in this study~~ genus in this studies, which comprising 46.68% of total fungi and recovered from 20% of the total samples. *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus*) followed by *Saccharomyces* sp., which contributed by 16.67% of total fungi and isolated from 40% of total samples. *Penicillium* ranked the third place in the count, which comprising 13.33% of total fungi. The remaining species (*Alternaria alternata*, *Cladosporium cladosporioides*, *Phoma* sp. and sterile mycelium) were contributed collectively 23.33% of total fungi and isolated only from one sample.

Table (2) explained the Effect of different species of fungi on degradation of phenol derivatives -at 0.4% concentrations, so the results showed that the ability of different fungi species on biodegradation of phenol derivatives were differed according to the type of phenol derivatives, so hydroxy-benzene and 1,3dihydroxy benzene exhibited the highest biodegradation by fungi species, but 2-Naphthol and 4-Nitrophenol showed the moderate bio ~~removal~~ byremoval by fungi species. The highest degradation of 1,3dihydroxy benzene ~~were~~ was done by *Cladosporium cladosporioides*

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158 (90.40 %). While the lowest bio removal occurred by sterile mycelium for
159 1,3dihydroxy benzene (1.01%).

160 In general, table 3 showed that the ability of fungi species to degrade the phenol
161 derivatives at 0.6% concentrations, so all species of fungi exhibited the degradation of
162 phenol compounds by different proportions. Fungi species degrade phenol derivatives
163 in range between 1.1 to 87.08 %). *A. flavus* appeared highest ability for analysis of
164 1,3dihydroxy benzene compound (87.08), while *Saccharomyces* sp. showed lowest
165 degradation of 2-Naphthol compound (1.1%).

166 Table 4 . *A* appeared the potency of different species of fungi on degradation of phenol
167 compounds, where the ability of degradation of phenol and their derivatives increased
168 with increased the concentration of phenol based on the results detected in many
169 species of fungi. All species exhibited the potency to degrade phenol and their
170 derivatives, but the potency differed from species to species, and from derivatives to
171 derivatives. So *Cladosporium cladosporioides* showed the highest degradation of
172 hydroxyl benzene (95.00%), while sterile mycelium appeared the lowest degradation
173 of 1,3dihydroxy benzene (1.59 %).

174 In this study, we choose two species of degraded fungi of phenol and their
175 derivatives (*A.niger* and *P.chrysogenum*) at different concentration of phenol to find
176 out the ability of input and output of phenolic compounds degradation on
177 photosynthetic pigments of chlorella sp. (table, 5). Furthermore the value of
178 chlorophyll pigments was increased under output effects of phenolic compounds
179 degradation than input bioremoval. The results in table- explained that the highest
180 value of chlorophyll was showed at concentration 0.8% of 2-naphthol which degrade
181 by *P.chrysogenum* (12.83 mg/g fresh wt.), followed by concentration 0.8% of
182 hydroxyl-benzene which removal by *A.niger* (12.05 mg/g fresh wt.). While low value
183 of photosynthetic pigments of chlorella sp. was observed at concentration 0.4% for
184 control sample (3.75 mg/g fresh wt.).

185 Discussion

186 The ability of microorganisms to eliminate injurious chemicals from contaminated
187 environments powerfully depends on the presence of different compounds. Most
188 industrial wastes embrace totally different organic mixtures creating vital the

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189 investigation on the microbial destruction of composite substrates. The bioremoval or
190 degradation of one or all elements are often delayed and/or discontinued depending
191 on the composition of the studied mixture. Wastewaters from oil refineries, mining
192 business and variety of industrial chemical syntheses contain several aromatics as
193 phenol, cresols, nitrophenols, etc ²⁹. The metabolism of aromatic compounds, notably
194 phenol and their derivatives explained in prokaryotic microorganisms ³⁰⁻³¹. Ten
195 species and sterile mycelium belonging to 6 genera were collected in the present
196 study. *Aspergillus* was represented by 4 species, and *Penicillium* was displayed by 2
197 species, while the other genera were introduced by one species. *Aspergillus*,
198 *Penicillium* and *Neurospora* attack aromatics and a variety of soil and wood-rotting
199 fungi dissimilate the aromatic polymer lignin, as well as other plant phenolics ³².
200 Another fungus, the *Penicillium* strain Bi 7/2 has been shown the ability of growth
201 on phenolic compounds as sole source of carbon and energy, including
202 protocatechuic and gallic acids ³³.

203 *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus*) was followed
204 *Saccharomyces* sp., which contributed by 16.67% of total fungi and isolated from
205 40% of total samples. Three species of fungi (*H. bergeri*, *F. oxysporum* and *A. flavus*
206 var. *coulmnanis*) were the most common fungal species from the 25 samples of -soils
207 collected from the three Governorates (El Gharbia, Kafre El Sheikh and El-Menofia)
208 ¹³.

209 ———From results in tables (2, 3 and 4), we have a tendency to showed that every
210 one fungal species used have the ability to degrad the phenol and their derivatives.
211 The microorganisms have the flexibility of removing phenol depended on the action
212 of sort of enzymes. In bioremoval of phenol under aerobic conditions, the
213 degradation is started by oxygenation in which the aromatic ring is —initially
214 monohydroxylated by a mono oxygenase phenol hydroxylase at a position ortho to
215 the pre-existing radical to compose catechol. Catechol is that the main intermediate
216 ensuing from metabolism of phenol by completely different microbial strains. Betting
217 on the sort of strain, the catechol then undergoes a ring break down which will occur
218 either at the ortho position so initiating the ortho pathway that results in the
219 formation of succinyl Co-A and ethanoyl radical Co-A or at the meta position so
220 initiating the meta pathway that results in the formation of pyruvate and

acetaldehyde¹. The results obtained from this investigation explained that, in generally the ability of different species of fungi for degradation of hydroxyl-benzene, 2-naphthol and 4-nitrophenol increased with increased the concentration of phenol and their derivatives. A lot of fungi species have ability to degrade phenol. Consequently, two species of fungi (*Mucor* sp. and *Rhizopus* sp.) have the ability with highest degradation of phenol at initial concentration 100 mg/l. There is a relationship between the ability of fungi to analyze phenol with its concentration²⁶. Many species of fungi produce extracellular enzymes for the metabolism complex carbohydrates into simple carbohydrates used by fungi as a source of sugar, for this reason, it has become possible to degrade pollutants such as phenol³⁴.

At the first three days, the ability of *Mucor* sp. and *Rhizopus* sp. to remove phenol had appeared slightly difference, because the longtime of acclimation period, where the organisms need a time to adapted for the use of phenol as a sole carbon source. The other reason for this way may be referred to sporulation stage which have a period of time to enter mycelium stage. After the first three days, after the first three days, the increased of phenol degradation by fungi was directly proportional with increase period of incubation. Then, bioremediation efficiency was slightly different. The reveal increased degradation efficiency can be explained by the availability of a carbon source which improves the fungi performances and growth and thereafter, the reduction of carbon source in the solution which is reflected as decrease or an inhibition in the bioremoval process (mortality of the cells)²⁶.

While the ability of fungi to degrade 1,3 dihydroxy benzene decreased when increased the concentration of it. the results of initial concentration effect of phenol within the range of 10-150 mg L⁻¹. The uptake of phenol increased with the initial concentration up to 120 mg L⁻¹. Then uptake decreased as the initial phenol concentration was increased. The higher uptake at lower concentrations may be due to the presence of more available sites on the adsorbent than the number of phenol ions which are available in solution. The maximum uptake was determined at 120 mg L⁻¹ as 30 mg g⁻¹³⁵.

Conclusion

—The results indicated that a wide range of fungi species have the ability to degrade phenolic compounds. It can be say the bioremoval a wonderful technique for bioremediation of wastewater.

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355 **Table 1.** Total counts (TC, calculated per 30 colonies), percentage of fungal counts (%C,
356 calculated per total fungi) and frequency of fungal species (%F, calculated per 10 samples)
357 of various fungal genera and species recovered from 10 samples of wastewater.

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Genera and species	TC	C%	NCI	F%
<i>Alternaria alternata</i>	3	10.00	1	10
<i>Aspergillus</i>	5	16.67	4	40
<i>A. flavus</i> Link	1	3.33	1	10
<i>A. fumigatus</i> Fresenius	1	3.33	1	10
<i>A. niger</i> Van Teighem	2	6.67	2	20
<i>A. terreus</i> var. <i>africanus</i> Fennell and Raper	1	3.33	1	10
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	2	6.67	1	10
<i>Penicillium</i>	4	13.33	2	20
<i>P. aurantiogriseum</i> Dierckx	3	10.00	2	20
<i>P. chrysogenum</i> Thom	1	3.33	1	10
<i>Phoma</i> sp.	1	3.33	1	10

<i>Saccharomyces</i> sp.	14	46.68	2	20
Sterile mycelium	1	3.33	1	10
Total account	30	100.00		

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361**Table 2.** Potency of different species of fungi on degradation of phenol derivatives at 0.4%
362concentrations, incubated at 28° C for 8 days.

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Species	Degradation (%) of phenol derivatives at derivatives at 0.4% concentrations			
	hydroxy- benzene	2- Naphthol	4- Nitrophenol	1,3dihydroxy benzene
<i>Alternaria alternata</i>	85.37 [*]	46.02 [*]	40.23 [*]	84.37 [*]
<i>A. flavus</i> Link	85.57 [*]	33.88 [*]	38.31 [*]	87.08 [*]
<i>A. fumigatus</i> Fresenius	83.11 [*]	37.88 [*]	54.46 [*]	88.02 [*]
<i>A. niger</i> Van Teighem	82.10 [*]	19.33 [*]	37.97 [*]	84.08 [*]
<i>A. terreus</i> var. <i>africanus</i>	87.68 [*]	31.07 [*]	42.49 [*]	87.29 [*]
<i>Cladosporium cladosporioides</i>	87.97 [*]	30.58 [*]	48.59 [*]	90.40 [*]
<i>P. aurantiogriseum</i> Dierckx	84.26 [*]	39.26 [*]	38.53 [*]	87.81 [*]
<i>P. chrysogenum</i> Thom	2.2 [*]	31.96 [*]	50.51 [*]	87.23 [*]
<i>Phoma</i> sp.	88.05 [*]	41.37 [*]	43.50 [*]	88.20 [*]

<i>Saccharomyces</i> sp.	83.94 [*]	30.44 [*]	16.05 [*]	86.65 [*]
Sterile mycelium	87.38 [*]	27.14 [*]	54.80 [*]	1.01 [*]

*. The mean difference is significant at the 0.05 level.

Table 3. Potency of different species of fungi on degradation of phenol derivatives at 0.6% concentrations, incubated at 28° C for 8 days.

Species	Degradation (%) of phenol derivatives at 0.6% concentrations			
	hydroxy-benzene	2-Naphthol	4-Nitrophenol	1,3dihydroxy benzene
<i>Alternaria alternata</i>	81.87 [*]	30.61 [*]	37.72 [*]	81.98 [*]
<i>A. flavus</i> Link	83.21 [*]	21.55 [*]	73.70 [*]	87.08 [*]
<i>A. fumigatus</i> Fresenius	82.20 [*]	29.59 [*]	77.36 [*]	84.55 [*]
<i>A. niger</i> Van Teighem	82.21 [*]	44.43 [*]	15.32 [*]	80.52 [*]
<i>A. terreus</i> var. <i>africanus</i>	81.87 [*]	29.34 [*]	37.62 [*]	67.80 [*]
<i>Cladosporium cladosporioides</i>	83.51 [*]	50.09 [*]	72.03 [*]	86.47 [*]
<i>P. aurantiogriseum</i> Dierckx	81.69 [*]	34.41 [*]	81.02 [*]	86.29 [*]
<i>P. chrysogenum</i> Thom	26.40 [*]	20.79 [*]	59.38 [*]	85.51 [*]
<i>Phoma</i> sp.	83.19 [*]	30.50 [*]	76.80 [*]	72.59 [*]

<i>Saccharomyces</i> sp.	67.88 [*]	1.1 [*]	50.06 [*]	84.21 [*]
Sterile mycelium	83.86 [*]	38.10 [*]	33.96 [*]	82.55 [*]

*. The mean difference is significant at the 0.05 level.

Table 4. Potency of different species of fungi on degradation of phenol derivatives at 0.8% concentrations, incubated at 28° C for 8 days.

Species	Degradation (%) of phenol derivatives at 0.8% concentrations			
	hydroxy-benzene	2-Naphthol	4-Nitrophenol	1,3dihydroxy benzene
<i>Alternaria alternata</i>	89.27 [*]	35.33 [*]	67.52 [*]	75.82 [*]
<i>A. flavus</i> Link	94.60 [*]	29.90 [*]	51.50 [*]	83.82 [*]
<i>A. fumigatus</i> Fresenius	90.27 [*]	25.43 [*]	57.52 [*]	89.11 [*]
<i>A. niger</i> Van Teighem	66.71 [*]	24.75 [*]	8.05 [*]	65.41 [*]
<i>A. terreus</i> var. <i>africanus</i>	79.59 [*]	30.10 [*]	5.58 [*]	59.10 [*]
<i>Cladosporium cladosporioides</i>	95.00 [*]	37.79 [*]	56.64 [*]	89.94 [*]
<i>P. aurantiogriseum</i> Dierckx	93.17 [*]	32.79 [*]	54.87 [*]	91.26 [*]
<i>P. chrysogenum</i> Thom	26.74 [*]	34.14 [*]	45.84 [*]	86.53 [*]

<i>Phoma</i> sp.	81.71 [*]	22.55 [*]	60.00 [*]	24.54 [*]
<i>Saccharomyces</i> sp.	72.88 [*]	32.85 [*]	46.37 [*]	88.42 [*]
Sterile mycelium	93.67 [*]	31.33 [*]	46.90 [*]	1.59 [*]

*. The mean difference is significant at the 0.05 level.

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Table 5. Concentrations of photosynthetic pigments of *chlorella* sp. (mg/g fresh wt) under effect of input and output of phenolic compounds degradation at different concentrations by *A.niger* and *P.chrysogenum*.

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Phenol sources	Concentrations	Treatments		
		Control	<i>A.niger</i>	<i>P.chrysogenum</i>
hydroxy-benzene	0.4	4.65±0.14	6.77 [*] ±0.23	5.53 [*] ±0.10
	0.6	5.00±0.00	5.87 [*] ±0.04	7.64 [*] ±0.22
	0.8	5.34±0.06	12.05 [*] ±0.15	10.23 [*] ±0.07
2-Naphthol	0.4	4.87±0.22	4.64±0.07	4.86±0.15
	0.6	5.94±0.26	7.47 [*] ±0.14	6.49 [*] ±0.066
	0.8	3.84±0.11	9.14 [*] ±0.04	12.83 [*] ±0.22
4-Nitrophenol	0.4	3.75±0.08	4.09 [*] ±0.04	4.35 [*] ±0.08

	0.6	6.84±0.051	5.62*±0.04	6.81±0.12
	0.8	6.25±0.10	6.87*±0.13	8.64*±0.06
1,3dihydroxy benzene	0.4	4.33±0.04	4.56*±0.06	4.65*±0.07
	0.6	4.76±0.13	6.11*±0.03	5.22*±0.08
	0.8	5.9±0.05	4.84*±0.09	12.84*±0.17

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*. The mean difference is significant at the 0.05 level.