

Degradation of Phenol by A New-Degradable Marine Halophilic Fungus *Fennellia Flavipes* Isolated From Mangrove Sediments

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Abstract: Phenol and its derivatives are one of the largest groups of environmental pollutants. Intensive efforts to screen species with high-degradation activity are needed to study their capabilities of degrading phenol and phenolic derivatives. Six different halophilic fungi were isolated from sediments along Suez Gulf and mangrove sediments in Red Sea coasts, Egypt. All tested fungal isolates exhibited the ability to grow and degrade phenol and phenolic derivatives as sole carbon source, these fungal isolates belonging to three genera (*Aspergillus*, *Penicillium* and *Fusarium*). The most potent isolate identified as *F. flavipes* exhibited the highest efficiency to phenol degradation. It could consume 1000 mg l⁻¹ in 96 hours only, with degradation rate 10.42 mg l⁻¹h⁻¹, reached to 1500 mg l⁻¹ phenol within 72 hours (degradation rate, 20.83 mg l⁻¹h⁻¹) after acclimation process. *F. flavipes* was also capable of degrading, m-cresol (9.26 mg l⁻¹h⁻¹), o-cresol (8.33 mg l⁻¹h⁻¹), 4-chlorophenol (4.56 mg l⁻¹h⁻¹) and 4-bromophenol (5.20 mg l⁻¹h⁻¹). *F. flavipes* exhibited optimum temperature for growth on phenol at 30°C. The initial pH of the medium that gave the highest rate of phenol degradation was pH 6. Supplementing the medium with yeast extract and glucose stimulated the degradation, especially at concentration 0.2 and 0.2%, respectively. The best nitrogen source was ammonium sulphate which yield highest rate of degradation (35.9 mg l⁻¹h⁻¹). Optimum concentration of sodium chloride was 6% (rate of phenol degradation, 41.67 mg l⁻¹h⁻¹) and its omission reduced the rate of degradation. Also, the presences of metal ions such as Cr²⁺, Cd²⁺ and Zn²⁺ at relatively high concentration (20 mg l⁻¹) slightly reduce the efficiency of phenol degradation while the lower concentration of Pb²⁺ and Hg²⁺ highly reduce rate of degradation. The *F. flavipes* showed a high tolerance and degradation capacity of phenol, it was able to register growth in the presence of 2000 mg l⁻¹ phenol.

[Aida E. Farag and Hanan M. Abd-Elnaby. **Degradation of Phenol by A New-Degradable Marine Halophilic Fungus *Fennellia Flavipes* Isolated From Mangrove Sediments.** *Life Sci J* 2014;11(9):836-845]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 126

Key words: Phenol, *Fennellia flavipes*, Optimization, Acclimation.

1. Introduction

The effluents of chemical and petroleum industries often contain aromatic organic compounds that are rather resistant to natural degradation and therefore persist in the environment. Phenolic compounds are among the most prevalent forms of chemical pollutants in the industrial wastewater. Environmental Protection Agency (1) defines phenols as priority pollutants (2,3). Most countries specify the maximum allowable concentration of phenols in the effluent streams to be less than 1 mg l⁻¹ (2, 4) Phenol is a very toxic compound, not only to humans but too many microorganisms. Due to their toxicity, persistence and common occurrence in the biosphere is considerable as one of the most important groups of ecotoxins.

The phenolic compounds are in a common use such as ingredient (components) and precursors of other chemicals such as organic polymers, solvents, dyes (amino phenols), explosives (nitrophenols), surfactants (alkphenols) or drugs (5). Phenols,

cresols, xylenols and polyaromatic compounds are by-products of petroleum and petrochemical industries. Also, a great variety of phenolic compounds found in plants is presumably released in the process of biological degradations of lignins, various biocides and/or synthetic detergents by soil microorganisms.

Phenols are easily soluble in water and hence, cause the carbolic odor in water. They cause toxic effects on aquatic flora and fauna and ultimately affect the ecological balance (6). Phenols inhibit respiration and bring about precipitation of proteins and they are responsible for cases of testicular cancer, breast tumors and decreased sperm counts (7). Phenol removal in water treatment and effluent treatment include super chlorination, ozonization and activated carbon adsorption (8). But these methods are impractical due to high costs and the production of other toxic end products (9). However, in recent years bioremediation is gaining more popularity in treating the industrial effluents over the physical and

chemical methods because of cost effective alternative, universally preferred, because of the possibility of complete mineralization of phenol (10, 11) which results in complete conversion of phenol to its inorganic mineral constituents (12).

Microbes play an important role in degrading synthetic chemicals in soil (13). They have the capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source. The aerobic biodegradation of phenol by microorganisms has long been an object of intense research globally. Many attempts have been made to isolate prokaryotic and eukaryotic microorganisms capable of degrading phenolic and other aromatic hydrocarbons. Among the eubacteria, representatives of families Micrococcaceae, Mycobacteriaceae, Bacteroidaceae, Bacillaceae and Pseudomonadaceae (14- 16), other microorganisms that have been reported to degrade phenol include fungal species. Fungi are capable to effective degradation of phenols due to the activity of these lignolytic enzymes. In recent years, researchers have developed the use of fungal species such as *Phanerochaete chrysosporium*, *Hormodendrum bergeri*, *Fusarium oxysporium* and *Asperigillus flavus* (17). *Aspergillus niger* (18), *Penicillium chrysogenum* (19), yeast species such as *Candida tropicalis* (20) and *Aureobasidium pullulans* FE13 (21).

Due to the widespread of phenol and phenolic compounds in water, it is important to isolate efficient phenol- degrading microbes for use in bioremediation. The present work aimed to isolate and screen the ability of different isolates of marine halophilic fungi to degrade phenol. Moreover, to study the acclimation and optimization of phenol degradation using new halophilic *Fennellia flavipes* isolated from marine sediment of mangrove ecosystem.

2. Materials and Methods

Collection of samples

Twelve sediment samples were collected from different locations of Suez Gulf and mangrove ecosystem along the Red Sea Coasts, Egypt, during September 2013. The samples were collected in sterile bags, transferred to laboratory in ice box, and stored at 4°C till analysis (Figure1).

Growth media

The biodegradation of phenol was carried out in Czapek's Dox medium (deficient, without carbon source) (DCD), which contained (g l^{-1}): $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; K_2HPO_4 , 1.0; NaCl , 50.0; NaNO_3 , 3.0; KCl , 0.5 and FeSO_4 , 0.01. The initial pH was adjusted to 5.0 and phenol as the sole carbon and energy source was added at 1000 mg l^{-1} . To prepare

the DCD/phenol, medium base and phenol solution were mixed after sterilization at 121°C for 20 minutes (22, 23). The DCD/phenol medium was solidified by addition of 2% agar.

Screening and selection of phenol degrading fungi

Ten grams from each sediment sample were suspended in 90ml aliquots of normal saline with vigorous shaking for 20 minutes. Serial dilutions from each sample were done. One ml of each dilution was spread on DCD/phenol agar plates; incubated for 5 days at 30°C. The morphologically different molds were selected, isolated and purified by streaking on DCD/phenol agar. After purification the isolates were transferred into slants of the DCD/ phenol medium for preservation at 4°C. Based on colony morphological, six characteristics different mold isolates were obtained. The molds were identified mainly on the basis of cultural and morphological characteristics (24- 26), which revealed that they belong to three genera (*Aspergillus*, *Penicillium* and *Fusarium*). The identification of fungal isolates was confirmed by Mycological Center, Faculty of Science, Assiut University, Egypt.

Cultivation of different fungal isolates for phenol degradation was carried out in 250 ml Erlenmeyer flasks each containing 50 ml of the DCD/phenol medium (1000 mg l^{-1}). Each flask was inoculated with 2 ml of spore suspension (5×10^6 spore per ml) of different isolates prepared from a 5-day culture. The flasks were incubated on a rotary incubator shaker (120 rpm) at 30°C at different time intervals. Samples from the culture media were collected for estimation of residual phenol concentration and dry weight of the cells. Triplicates were used for each.

Estimation of biomass

The growth of the isolated fungi were monitored by increase in mycelia biomass measured as dry weight. The mycelia were separated from the media by centrifugation at $6000 \times \text{g}$ per min, washed twice with distilled water and dried in an air oven at 60°C until a constant weight.

Phenol assay

Phenol and other phenolic compounds analysis was carried out by measurement the absorbance at a wavelength of 492nm using Unico UV-2000 spectrophotometer. The method described by Martin (27) was used. It is based on rapid condensation with 4-aminoantipyrine followed by oxidation with potassium ferricyanide under alkaline conditions to give a red- coloured product which is immediately measured. The standard calibration curve was previously constructed using phenol as a standard.

Degradation of some phenolic compounds

The fungal isolates were incubated with various phenolic compounds phenol, m-cresol, o-cresol, 4-

chlorophenol and 4-bromophenol at different concentrations 250, 500 and 1000 mg l⁻¹ and the rate of degradation was estimated at different time intervals.

Acclimatization of the isolate

The acclimatization of the selected fungal isolate was carried out in a phenol containing media. In the beginning, the isolate was cultivated in medium with 200 mg l⁻¹ phenol. Thereafter, cell mass was centrifugated after complete degradation of phenol and sub-cultured into a fresh medium supplemented with 400 mg l⁻¹ phenol. Incremental additions of different concentrations of phenol to the medium (range from 200 to 2500 mg l⁻¹), and the isolate was grown in a shaking incubator at 120 rpm and 30°C. Samples from the culture were withdrawn at time intervals and analyzed for phenol degradation (28). All tests were done in triplicate and the results are the means ± standard deviation.

Optimization of different parameters for degradation of phenol

Effect of agitation, temperature, pH, and different concentrations of yeast extract, different types of sugars, nitrogen sources and sodium chloride concentrations were observed for the best degradation of phenol.

Effect of agitation

The experiments were performed to know the effect of agitation at 30°C and pH 5 in shaking incubator. Different shaking speeds maintained from 80 to 180 rpm. Samples were collected at different incubation period, analyzed for phenol degradation and growth.

Effect of different temperature and pH

To determine the optimum temperature for the best phenol degradation, the inoculated DCD/phenol medium was incubated at different temperatures (from 20 to 50 °C) at 120 rpm. Residual phenol concentration was measured by 4-aminoantipyrine method at different time interval. To optimize the best pH for the highest degradation of phenol by the fungal isolate, the initial pH of the medium was varied from 4.0 to 9.0, inoculated and incubated in shaking incubator at 120 rpm, 30°C and different incubation period. After incubation, residual phenol was measured by 4-aminoantipyrine method.

Effect of different concentrations of yeast extract

Different concentrations of yeast extract (from 0.1 to 1.0%) be tested in the DCD/phenol medium inoculated by spore suspension of the fungus, incubated in an orbital incubator shaker (120 rpm) at 30°C and pH 6.0 to study its affect on the degradation rate of phenol.

Effect of different carbon and nitrogen sources

The DCD medium containing 1500 mg l⁻¹ phenol was supplemented with different carbon sources (at

equivalent carbon) such as glucose, lactose, maltose, sucrose, fructose and dextran. All the media were inoculated with 2ml of the fungal spores (5×10⁶ spores per ml) and incubated in an orbital incubator shaker (120 rpm) at 30°C, pH 6.0 and different time intervals. The rate of phenol degradation and growth was measured. The DCD was supplemented with the selected carbon source and different sources of nitrogen as ammonium sulphate, ammonium nitrate, sodium nitrate, potassium nitrate, casein, peptone and phenylalanine (at equivalent nitrogen). Inoculated and incubated at appropriate conditions. Rate of phenol degradation was estimated (29).

Effect of different concentrations of sodium chloride (salinity)

The effect of different concentrations of sodium chloride (0.0 to 10 %) on the rate of phenol degradation by *F. flavipes* was investigated using the above optimized conditions. After incubation at different times, the rate of degradation and dry weight were estimated.

Effect of some heavy metals

The optimized biodegradation medium was supplemented with different concentrations (10 and 20 mg l⁻¹) of some heavy metals such as Cr²⁺, Cd²⁺, Zn²⁺, Cu²⁺, pb²⁺ and Hg²⁺. After incubation at optimum conditions, the degraded phenol was assayed.

3. Results and Discussion

Screening of phenol degrading fungi

Microorganisms have the ability to adapt to a variety of environmental conditions. In the present work, the preliminary objective was to isolate a fungal strain that could grow on phenol as the sole carbon and energy source. The results presented in Table 1 indicated that all fungal isolates were able to grow and degrade phenol. A comparison of the six fungal isolates, showed that the isolates *Aspergillus niger* and *Aspergillus terreus* (isolated from Ras-Gharib, Suez Gulf) degraded phenol in 108 hours with a rate of degradation 9.26 mg l⁻¹h⁻¹, while *Aspergillus flavus* (isolated from Ras Shukheir, Suez Gulf), take 120 hours for the complete phenol degradation with a rate of 8.33 mg l⁻¹h⁻¹ and *Fusarium oxysporium*, (isolated from mangrove ecosystem in Safaga, Red Sea) need 144 hours for complete degradation of 1000 mg l⁻¹ phenol with rate of degradation 6.94 mg l⁻¹h⁻¹. The lowest rate of phenol degradation (6.41 mg l⁻¹h⁻¹) was observed by *Penicillium globrum (wehumer) westling*, (isolated from sediment in mangrove ecosystem in Abo-Aoson, South of Mersa-Alam,,Red Sea) after 156 hours. On the other hand, the most potent phenol degrader fungal isolate was *Fennellia flavipes* (Anamorph: *Aspergillus flavipes* (Bain. & Sart.)

Thom & Church) taking 96 hours to completely degrade 1000 mgL⁻¹ phenol with a rate of 10.42 mgL⁻¹h⁻¹. From these results, the fungal isolate *F. flavipes* was chosen for further experiments. *Fennellia flavipes* isolated from mangrove ecosystem in Safaga, Red Sea coast. Many reports in the literature showed that *Penicillium*, *Fusarium* and *Aspergillus* can metabolize phenols (17,18).

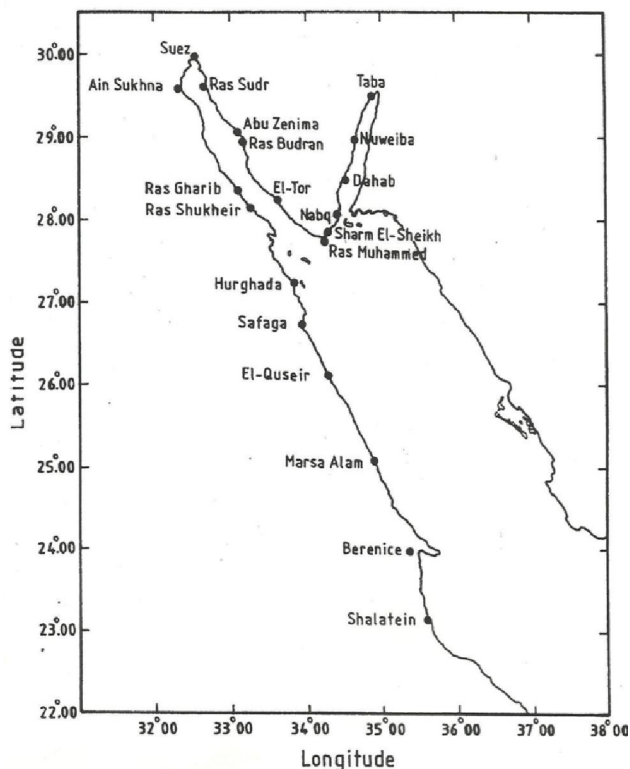


Figure 1: The map of Red Sea and Suez Gulf (The locations of samples)

Degradation of different phenolic compounds

The ability to degrade various phenolic compounds by different fungal isolates showed that *F. flavipes* was actively degrading phenol with highest rate (10.42 mgL⁻¹h⁻¹) followed by m-cresol (9.26 mgL⁻¹h⁻¹) and o-cresol (8.33 mgL⁻¹h⁻¹) (Table 2). Much lower activity was recorded upon using 4-chlorophenol and 4-bromophenol as a substrate (4.56 and 5.20 mgL⁻¹h⁻¹, respectively). All the other fungal isolates can degrade phenol, m-cresol and o-cresol at different concentrations but with lower rate of degradation than *F. flavipes*, and could not degrade both 4-chlorophenol and 4-bromophenol at high concentration (1000 mgL⁻¹). So the fungal isolate *F. flavipes* was the most efficient organism that can use different phenolic compounds as a sole carbon and energy source and has the ability to degrade them effectively. Many microorganisms which are able to

degrade phenol, also can degrade other phenolic compounds (3,30,31)

Acclimatization process

The acclimatization of *F. flavipes* was carried out as shown in Figure 2. The fungal isolate was able to grow and degrade 1000 mgL⁻¹ phenol within 4 days (96 hours) before acclimatization. During the acclimation, the fungal isolate became adapted to 800 and 1000 mgL⁻¹ phenol within 60 h (rate of degradation 13.33 and 16.67 mgL⁻¹h⁻¹, respectively) and 1200 and 1500 mgL⁻¹ phenol within 72 h (rate of degradation was 16.67 and 20.83 mgL⁻¹h⁻¹, respectively) with no signs of cell lyses. On the other hand, the rate of phenol degradation was decreased when the phenol level increased up to 2000 mgL⁻¹. Such reduction may be referred to the toxic effect of phenol when used at high concentrations (32). These results are in agreement with other workers (28). In Contrast to our results, Gonzales et al (33) used only two adaptation steps, and the phenol biodegradation capability of *Pseudomonas putida* ATCC 17484 decreased with increasing phenol concentration. The adapted microorganisms to the toxic chemicals are much useful and can be improved the biodegradation of industrial wastes. This is much more important when dealing with toxic compounds like phenol (34).

Effect of agitation on phenol-degradation

The effect of trembling on the phenol-degrading ability of locally isolated *F. flavipes* was investigated by trying different shaking speeds ranging from 80 to 180 rpm at 30°C under pH 5.0 for 72 hours. In laboratory conditions, the incubator shaker can provide the aerobic state for fungal growth and phenol degradation. Since aerobic phenol degradation is preferred aerobically, so the dissolved oxygen concentration may be a limiting factor of phenol degradation process. A speed of 120 rpm was institute to be the best for high phenol degradation rate (20.83 mgL⁻¹h⁻¹), (Figure 3). The biodegradation rate might be suitable to sufficient lofty mass shift consequently more oxygen to be dissolved and become accessible for the metabolism of microorganism (35, 36). Lower or higher speed decreases the degradation rate of the phenol. On the other hand, other investigators used different shaking speed range from 50 to 200 rpm for the highest degradation of phenol by different microorganisms (18,37)

Effect of temperature and pH on phenol-degradation

Temperature might play an equivalent or larger role than nutrient availability in the biodegradation of organic pollutants (38). To determine the optimum temperature for fungal growth, *F. flavipes* was incubated with 1500 mgL⁻¹ phenol at different temperatures from 20 to 50°C. The maximum activity

of phenol degradation ($20.83 \text{ mg l}^{-1} \text{ h}^{-1}$) by *F. flavipes* was recorded at 30°C (Figure 4). So the optimum temperature is 30°C , this is a parentage especially when the microorganism is to be used in a commercial form to cater for a wide range of geographical areas (38). The increase of temperature from 20 to 30°C induced the rate of degradation yielding a 1.39-fold increase in the culture. However, lower or higher temperature reduced greatly the rate of degradation. Most laboratory studies of phenol degradation have been carried out at the optimum temperature of 30°C , and the extent and the rate of degradation was shown to be relatively sensitive to deviation outside the optimal range (39). Microorganisms have a limited range of optimum temperature supporting growth and yield high rate of phenol degradation (18, 28, 40, 41).

The pH significantly affects the reactions required for phenol degradation. The pH range of 5 to 7 seems appropriate for growth of *F. flavipes* on phenol with high degradation efficiency. However, pH 6 was the optimum for maximum degradation efficiency of 120.02% (Figure 5). This pH range serves to be the optimum for proper growth of microorganisms (37). On the other hand, some fungi preferred acidic pH for the best degradation of phenol (18,40).

Effect of different concentrations of yeast extract on phenol-degradation

The presence of yeast extract was reported to enhance the affinity of some microorganisms for phenol degradation (28,42). The results in Figure 6 indicated that *F. flavipes* showed a high tolerance and degradation capacity of phenol ($27.78 \text{ mg l}^{-1} \text{ h}^{-1}$), when the medium supplemented with 0.2 % yeast extract, higher yeast extract concentrations showed an adverse effect on rate of degradation. Such effect may be attributed to a better growth and supplementation of rich source of vitamins, amino acids and mineral salts which could promote cell mass and enzymatic activities (28).

Effect of different carbon and nitrogen sources on phenol-degradation

DCD medium was supplemented with auxiliary carbon sources, the best results of phenol degradation of around 113 % was achieved in media supplemented with 0.2% glucose (Figure 7). Many studies on the role of sugars revealed that glucose supports growth and rate of phenol degradation (43, 29, 44). The addition of non-toxic compounds such as sugars may stimulate the viability of cells and enhance degradation. On the other hand, the addition of other sugars such as lactose, maltose, sucrose, fructose and dextrane suppressed the degradation capability of the organism (20.83 , 15.63 , 12.5 , 11.36 and $11.36 \text{ mg l}^{-1} \text{ h}^{-1}$, respectively). It was proposed that

the presence of a more metabolizable carbon permitted more growth and the rate of degradation was suppressed due to quicken biomass acclimation to glucose as the alternate carbon source (45).

On an equivalent nitrogen basis, NaNO_3 in the culture basal medium supplemented with a phenol at a concentration of 1500 mg l^{-1} was replaced by different nitrogen sources. The data in Figure 8 indicate that the growth and the rate of phenol degradation were differently affected by the nature of the nitrogen source. Ammonium sulphate, was the most preferable nitrogen source yielding the highest growth ($480 \text{ mg}/100 \text{ ml}$) as well as maximum rate of degradation ($35.90 \text{ mg l}^{-1} \text{ h}^{-1}$). On the other hand, the lowest rate of degradation was obtained in the presence of phenylalanine ($11.5 \text{ mg l}^{-1} \text{ h}^{-1}$). The absence of nitrogen source from the medium highly reduced the efficiency of phenol degradation (28.08%). These results indicate the importance of supplementing the medium with nitrogen to improve the degradation. Many investigators showed the importance of nitrogen source to improve the degradation. These results are in good agreement with other workers who used $(\text{NH}_4)_2\text{SO}_4$ for best phenol degradation by many fungi (37, 46, 47).

Effect of different concentrations of sodium chloride

The effect of varying concentrations of sodium chloride on the efficiency of degradation of the tested organisms was investigated. The data registered in Table 3, showed that the presence of NaCl in the medium was very essential for the growth of *F. flavipes* and phenol degradation in the cultures. The absence of this salt affects negatively on both growth ($143 \text{ mg}/100 \text{ ml}$) and rate of phenol degradation ($8.93 \text{ mg l}^{-1} \text{ h}^{-1}$) of *F. flavipes*, indicating the importance of presence this salt for the growth and efficiency of the marine microorganisms (48). The highest rate of phenol degradation ($41.67 \text{ mg l}^{-1} \text{ h}^{-1}$) was obtained when 60 g l^{-1} of NaCl added into the medium. Phenol degradation rate was decreased at lower and higher concentration of NaCl than 60 g l^{-1} , though significant inhibition on growth was observed. This can be therefore used for treatment phenol with high concentration of NaCl in the industrial wastewater released in the marine environment (48,49).

Effect of some heavy metals on phenol-degradation

Under optimum conditions, microbial capability of phenol biodegradation in the presence of different concentrations of heavy metals was studied. *Fennellia flavipes* exhibited inhibition of phenol degradation to about 7.5, 10.6, 10.5, 19.9, 31.7, 69.2 and 69.9% in the presence of 20 mg l^{-1} of each Cr, Cd, Zn, Cu, pb or Hg, respectively (Table 4). The suppressed of phenol degradation by these metals

was observed by many workers (50,51). The presence of Cr^{2+} and Cd^{2+} did not reduce the rate of degradation even at a concentration of 10 and 20 mg l^{-1} . These results indicate that the presence of Cr^{2+} and Cd^{2+} at relatively high concentration in the environment will have an slightly effect on the

capability of phenol, while the presence of Cu^{2+} , Zn^{2+} , pb^{2+} and Hg^{2+} in the environment at relatively low concentration will reduce the efficiency of degradation of the isolate by inhibiting its growth or the activity of the enzymes responsible for phenol degradation (52-55).

Table 1: Growth and degradation rate of phenol by different fungal isolates

Fungal isolate	Time of phenol consumption (h)	Phenol degradation rate ($\text{mg l}^{-1}\text{h}^{-1}$)*	Dry weight (g/100ml)
<i>Pencillium glabrum</i> .	156	6.41±0.21	0.095
<i>Fusarium oxysporium</i>	144	6.94±0.25	0.123
<i>Aspergillus flavus</i>	120	8.33±0.48	0.255
<i>Aspergillus terrus</i>	108	9.26±0.50	0.268
<i>Aspergillus niger</i>	108	9.26±0.54	0.274
<i>Fennellia flavipes</i>	96	10.42±0.64	0.288

*Values are mean ±standard deviation (n=3).

Table 2: Degradation of different phenolic compounds by different fungal isolates

Component	Conc. (mg l^{-1})	Rate of dedegradation ($\text{mg l}^{-1}\text{h}^{-1}$)					
		<i>Pencillium glabrum</i>	<i>Fusarium oxysporium</i>	<i>Aspergillus flavius</i>	<i>Aspergillus s terrus</i>	<i>Aspergillus s niger</i>	<i>Fennellia flavipes</i>
Phenol	250	3.47	4.17	5.21	6.94	6.94	6.94
	500	6.94	7.25	7.25	8.33	8.33	9.26
	1000	6.41	6.94	8.33	9.26	9.26	10.42
m-cresol	250	2.61	3.47	5.21	6.94	5.21	6.94
	500	5.21	5.95	5.95	6.94	6.41	6.94
	1000	6.41	5.95	8.33	9.26	9.26	9.26
o-cresol	250	5.20	5.20	2.98	6.94	5.21	6.94
	500	6.94	8.33	4.17	8.33	6.41	10.42
	1000	5.55	6.94	7.57	8.33	9.26	8.33
4-Chorophenol	250	3.47	2.60	4.17	4.17	5.21	5.21
	500	2.98	2.78	3.79	4.16	4.16	5.21
	1000	0.0	0.0	0.0	0.0	0.0	4.56
4-Bromophenol	250	2.31	5.42	5.21	6.94	6.94	6.94
	500	2.71	3.47	4.17	4.16	4.63	5.20
	1000	0.0	0.0	0.0	0.0	0.0	5.20

Table 3: Effect of sodium chloride concentration on phenol degradation by *Fennellia flavipes*

NaCl (g l^{-1})	Rate of phenol degradation ($\text{mg l}^{-1}\text{h}^{-1}$)	Efficiency of degradation (%)	Dry weight (g/100ml)
0	8.93	24.87	143
10	10.41	28.99	256
20	15.63	43.58	299
30	20.83	58.02	322
40	27.78	77.38	392
50	35.90	100.00	393
60	41.67	116.72	367
70	27.78	77.38	289
80	20.83	58.02	211
90	17.86	49.75	176
100	12.50	34.82	154

Table 4: Effect of the presence of some heavy metals on phenol degradation by *Fennellia flavipes*

Heavy metals	% Phenol degradation	
	10 mg ^l ⁻¹	20 mg ^l ⁻¹
Control	99.0± 0.9	99.10 ±0.90
Cr ²⁺	99.0 ±0.80	92.50± 0.60
Cd ²⁺	97.6 ±0.50	89.44 ±0.65
Zn ²⁺	83.4 ±1.06	80.11 ±1.08
Cu ²⁺	79.5± 0.96	68.30 ±0.40
Pb ²⁺	47.5± 0.70	30.88 ±0.55
Hg ²⁺	38.4 ±0.30	30.11 ±0.06

Values are mean ±standard deviation (n=3).

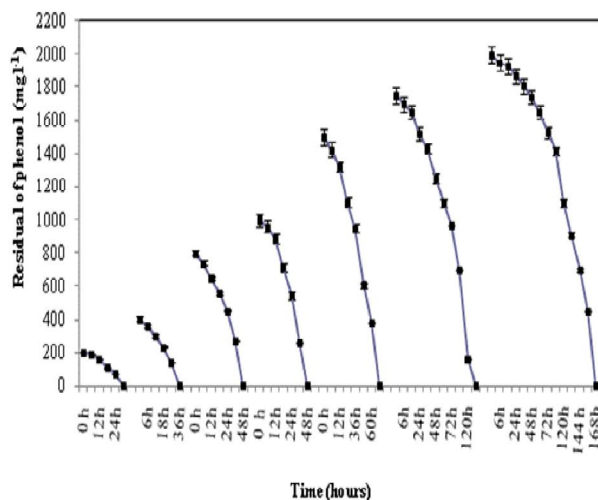


Figure 2: The growth of *Fennellia flavipes* and the phenol degradation in the DCD/phenol medium containing initial 200 mg^l⁻¹ followed by 400, 800, 1000, 1500, 1750 and 2000 mg^l⁻¹

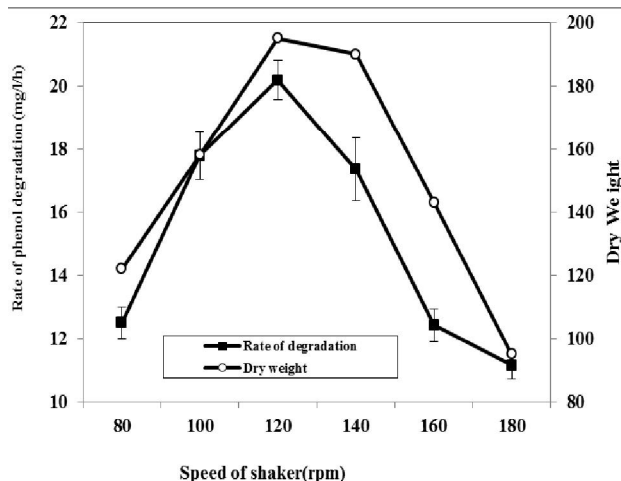


Figure 3: Effect of different speed of shaker on the growth and phenol (1500 mg^l⁻¹) degradation by *Fennellia flavipes*

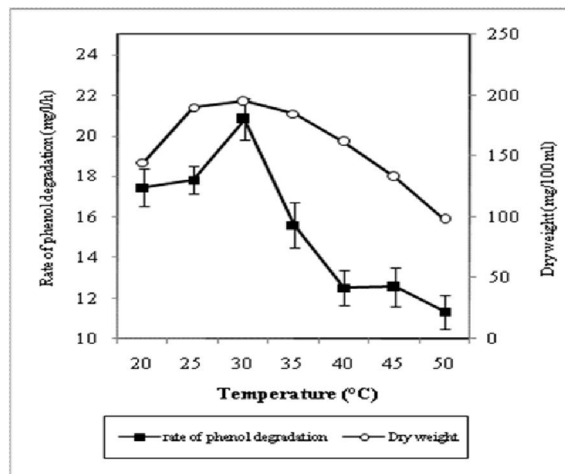


Figure 4: Effect of different temperatures on the growth and phenol (1500 mg^l⁻¹) degradation by *Fennellia flavipes*

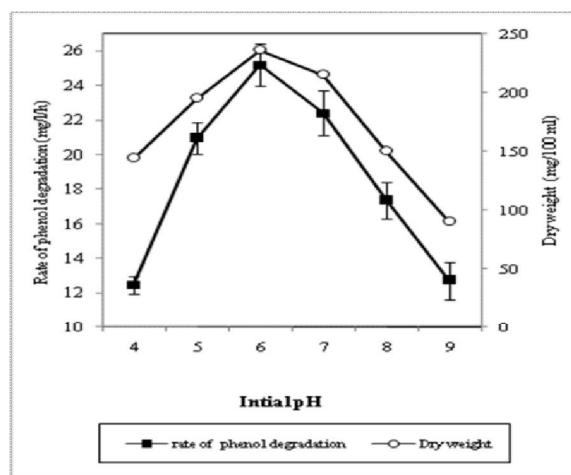


Figure 5: Effect of different initial pH on the growth and phenol (1500 mg^l⁻¹) degradation by *Fennellia flavipes*

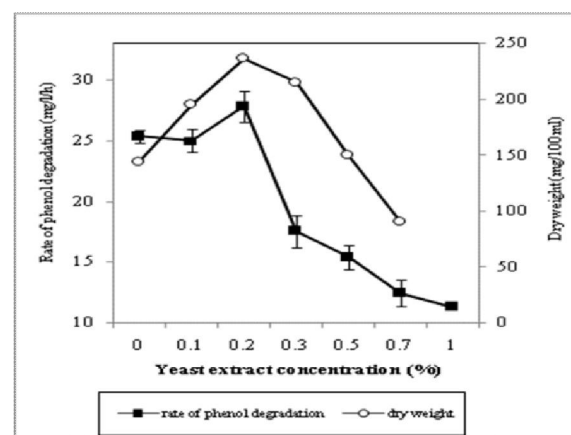
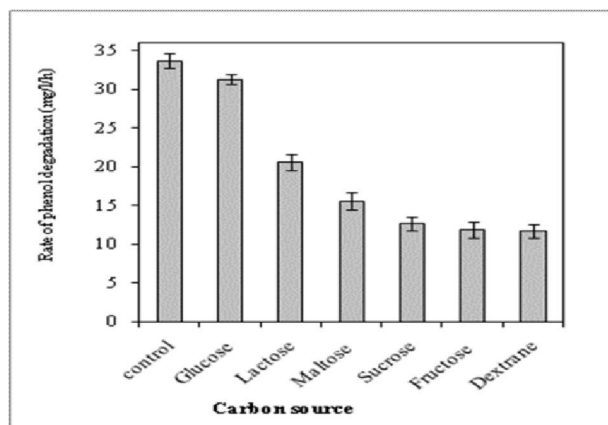
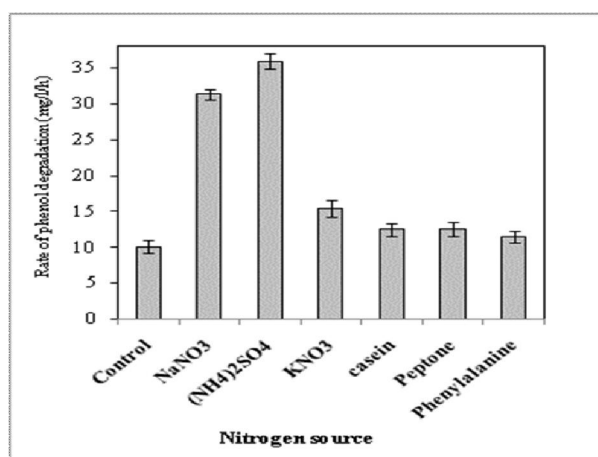


Figure 6: Effect of using different concentrations of yeast extract on the growth and phenol (1500 mg^l⁻¹) degradation by *Fennellia flavipes*



*Control= media without sugar

Figure 7: Effect of adding different sugars on the phenol (1500 mg l^{-1}) degradation by *Fennellia flavipes*



*Control = media without nitrogen source

Figure 8: Effect of different nitrogen source on the phenol (1500 mg l^{-1}) degradation by *Fennellia flavipes*

Conclusion

The fungi (*Penicillium*, *Fusarium* and *Aspergillus*) isolated from sediment at different sites of Suez Gulf and mangrove ecosystem of Red Sea Coasts, Egypt, can use phenol and phenolic derivatives as a sole source of carbon and energy. The most potent fungal isolate was *Fennellia flavipes* which isolated from the sediment of the mangrove from Savage in the Red Sea Coast, showed a highest rate of phenol degradation and the capability to degrade different phenolic compounds. This strain has not been previously shown to grow and degrade phenolic compounds. Acclimation of the isolate and optimization of some important cultural conditions as agitation, temperature, pH, addition of yeast extract, carbon and nitrogen sources, and sodium chloride were tested for highest phenol degradation. The

acclimation and optimization processes increased the efficiency of phenol biodegradation. Hence this strain has remarkable potential for application in bioremediation and wastewater treatment.

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