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Tracking of phenol bioremediation by two marine eco-friendly *Aspergillus terreus* MHG30 (ON649683) and *A. terreus* MHG60 (ON649704) strains at optimum conditions

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ABSTRACT

The rapid population and industrial development worldwide have increased chemical pollutants reaching the marine environment. In the present study, marine fungi were isolated from two collected petroleum sites at the Gulf of Suez, Red Sea, Egypt, during spring (2021). Fungal isolates were purified and screened for their ability to bio-remediate phenol and ortho-cresol bioremediation. Out of twenty two, eight marine fungal isolates represented their potency for the bioremediation of these phenolic compounds. These selected fungi were identified morphologically and numbered according to the AUMC center as *Aspergillus flavus* 15524, *Aspergillus terreus* 15525, *Aspergillus flavus* 15526 and *Aspergillus terreus* 15527 for phenol bioremediation, and *Fusarium oxysporum* 15528, *Fusarium oxysporum* 15529, *Aspergillus terreus* 15530 and *Aspergillus terreus* 15531 for o-cresol bioremediation. The two most potent marine fungal isolates able to degrade phenols with high efficiency are strains number 15527 and 1553 which were identified by DNA sequence as *A. terreus* MHG30 (ON649683) and MHG60 (ON649704), respectively. The optimum condition for phenol degradation by *A. terreus* MHG30 were 1000 mg/l phenol, pH=8 at 34°C, and contact time 168 hrs., while *A. terreus* MHG60 showed optimum condition using 250 mg/l o-cresol, pH 6 at 31 C^0 after 168 hrs. Maximum degradation rates were recorded at 88.34 and 98.1 % for ON649683 and ON649704 strains, respectively. Both strains, ON649683 and ON649704 were acquired with 61 and 64 inhibitory % for oxidase with 81 and 78 % for peroxidase enzymes, respectively. The K_m/V_{max} of both isolates was achieved at 0.18, 0.19, 0.03, and 0.29 for the studied enzymes. In addition, the active compounds of GC-Mass (Hexadecanoic acid, methyl ester, and 6, 9, 12-octadecatrienoic acid) are used to determine the unique eco-friendly compounds that result from the bioremediation procedure for phenol and o-cresol.

INTRODUCTION

Phenol and its derivatives are among the most severe organic pollutants entering the marine environment (Hanafi & Sapawe, 2020). They derived from different industrialized activities and untreated effluent discharges from oil refineries, coal

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gasification, plastic industries, and coke manufacturers (Bera *et al.,* 2019; Barik *et al.,* 2021). Due to their persistence, acute toxicity, and carcinogenic qualities pose significant threats to human health and environmental degradation and are readily soluble in water (Acosta *et al.,* 2018). Besides, they are distinguished for genotoxicity and endocrine-disrupting compounds. Therefore, the Environmental Protection Agency of the United States classifies it as a "priority organic pollutant" (EPA) (Darbre, 2019; Spataro *et al.,* 2019). Among the several strategies applied for eliminating toxic and risky organic pollutants, such as adsorption, ionization, and photocatalysis bioremediation method has attracted more interest as a probable method for the complete mineralization of phenolic compounds into CO_2 and H_2O or harmless end products. Consequently, increasing environmental concerns make them biodegradable, eco-friendly, and practical achievability (Khalil *et al.,* 2021; Panigrahy *et al.,* 2022).

The efficient utilization/bioremediation of phenolic compounds depends on several factors, including the bioavailability and activity of some essential enzymes in the microbial cells. In addition, pollutants' physical properties, pH, temperature, agitation, and chemical structure play a vital role in bioremediation (Al Farraj *et al.,* 2019). As the most efficient users of several carbon sources via enzymatic mechanisms, fungi provide promising opportunities for metabolizing phenols and other aromatic compounds. Also, they can tolerate excessive concentrations of toxic compounds due to their potent extracellular oxidative enzymes exploited (Mohanty & Jena, 2017; Ezz El-Din, 2017; Kottb *et al.,* 2019). Fungi have abroad success in phenol bioremediation due to the active production of various enzymes and less sensitivity to inhibition. Several studies focused on bioremediation and characterization of phenolic and cresols compounds, as authenticated by (Bera *et al.,* 2019) which isolated microorganisms can utilize p-cresol at concentrations 500-600 mg/l, pH 7.0 and Temperature 37^0C.

The main goal of the present study is to obtain the efficient fungi for phenol bioremediation via isolation, ability screening, and identification of marine isolates collected from sediment of the Gulf of Suez, Red Sea, Egypt. Moreover, optimization studying of phenol degrading strains with inhibitory % examination by oxidase and peroxidase enzymes is going to be studied besides using the GC-Mass system to detect end products through the bioremediation process.

MATERIALS AND METHODS

2.1. Sampling:

Two sediment samples were collected from two different locations in the Gulf of Suez (El-Kabanon & Suez Petroleum Company "SPC"), Red Sea, Egypt, during the spring of 2021. The samples were collected in sterile bags, transferred to the laboratory in the ice box, and stored at 4^0C till analysis.

2.2. Preparation of stock solution and calibration curve:

The calibration curve was prepared for various highly purified phenol and its derivatives ortho-cresol (obtained from sigma Co.) concentrations (250, 500, and 1000 mg/l). The phenol concentration can be measured according to (Martin, 1949) using the UV/VIS spectrophotometer JANWAY Model 6800 at λ=510 nm.

2.3. Isolation, phenol degradation screening, and purification of fungal isolates:

The procedure of isolation from sediment was described by Chikere & Azubuike (2014). In addition, Czapek's broth medium was used for fungal isolation and purification (Hasanin & Hashem, 2020).

2.4. Screening efficiency of fungal isolates:

Bio-removal experiments were conducted at different phenol concentrations (250, 500, and 1000 mg/l), pH (2 – 9), temperature (25.0 – 43.0 ^{0} C), contact time (12-168 hrs) and agitation speed (100 – 300 rpm). All previous tests were measured by UV/VIS spectrophotometer at λ=510 nm. The bio-removal efficiency of the isolates was then calculated according to the following equation:

$$\text{Phenol removal efficiency (\%)} = \frac{Ci - Cf}{Ci}\ x\ 100$$

Where, C_i and C_f: initial and final concentrations

2.5. Characterization and identification of the highly Phenols degrading fungi:

The most prominent isolates can downgrade phenolic compounds and their derivatives were morphologically identified (colour, shape, size, hyphae, and lactophenol cotton blue staining) and at molecular level using standard methods (Alsohaili & Bani-Hasan, 2018). The growing cultures were sent to the Molecular Biology Research Unit, Assiut University, for DNA extraction using Intron Biotechnology Company, Korea's Patho-gene-spin DNA/RNA extraction kit. The fungal DNA was then sent to SolGent Company, Daejeon, South Korea, for polymerase chain reaction (PCR) and rRNA gene sequencing. PCR was performed using ITS1 (forward) and ITS4 (reverse) primers incorporated into the reaction mixture. Primers have the following composition: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3') and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). The purified PCR product (amplicons) was sequenced with the same primers, incorporating ddNTPs in the reaction mixture. The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegaAlign (DNA Star) software version 5.05.

2.6. Fungal enzymatic activity for phenol bioremediation:

The inhibitory effect of phenolic compounds produced by fungal isolates was evaluated by enzyme and protein concentration determination. The enzymes include peroxidase and polyphenol oxidase, designed according to Miller 1951. The Lowry method determined the protein assay using bovine serum albumin as a standard curve for protein with a concentration range of 0.1 mg/l to 1 mg/l (Lowry, 1951). The specific activity of peroxidase and polyphenol oxidase was calculated according to Al-Agamy *et al.* (2021).

The mechanism of most potent strains producing peroxidase and oxidase inhibitors was maintained by the K_m and V_{max} parameters using substrate concentration from 0.1 to 1.2 %. The results were plotted according to the (Lineweaver & Burk, 1934) graphic method (Lineweaver & Burk, 1934; de Souza *et al.,* 2015).

2.7. GC-Mass analysis:

The procedure of residual phenol extraction was described by (APHA-AWWA-WPCF. 1981)**.** Bio-products and their concentrations obtained after phenol and o-cresol bioremediation by fungal isolates were detected by GC-MS System: (Thermo Scientific TRACE 1310 Gas Chromatograph attached with ISQ LT single quadrupole Mass Spectrometer); with specific conditions.

Column: DB5-MS, 30 m; 0.25 mm ID (J&W Scientific), Ionization mode: EI, Ionization voltage: 70ev, Temperature program: 40^0C (3 min) - 280 ^{0}C (5 min) at 5^0C /min. -290^0C (1 min) at 7.5 ^{0}C /min, Detector temperature: 300 ^{0}C, Injector temperature: 200 ^{0}C and Carrier gas: Helium; Flow rate 1 ml/min.

RESULTS AND DISCUSSION

The results pointed out that 22 fungal isolates were recorded, screened, and purified from sediment samples after being tested with phenol and o-cresol as substrates, 16 of them were isolated from St.2 (SPC), and the rest from St.1 (El-Kabanon). This was followed by 60 separated tests at pH 5 and 31°C. The obtained results showed that, higher concentration of phenol (1000 mg/l) recorded the highest bioremediation rate than the other concentrations using marine fungal isolates. On the other hand, o-cresol recorded the highest bioremediation rate by fungal isolates at low concentrations (250 mg/l) during 7 days of incubation. This result matches other finding by Farag & Abd-Elnaby, 2014 who detected that most polluted stations reflect high fungal numbers able to degrade phenol (Ibrahim & El-Gamdi, 2019).

3.1. Optimized conditions:

The effect of different parameters for phenol/ o-cresol degrdation, such as phenol/ o-cresol concentrations, pH, temperature, contact time, and agitation speed, were carried out to obtain the optimum condition for bioremediation as showed in Figs. (1 and 2).

3.1.A. Phenol and o-cresol concentrations:

Different concentrations were affected at pH 5 and temperature (31^{0}C). The results indicated that the highest concentration of phenol (1000 mg/l) has a high bioremediation rate which varied between 43.28, 45.26, 47.08, and 48.17% for (*A. flavus* 15526, *A. terreus* 15527, *A. terreus* 15525 and *A. flavus* 15524 sp., respectively) than the other concentrations. On the other hand, o-cresol recorded the highest bioremediation rate by fungi at low concentrations (250 mg/l), that it recorded at18.61, 22.64, 29.03, and 33.44 % for *F. oxysporum* 15529, *A. terreus 15530*, *A. terreus* 15531 and *F. oxysporum* 15528 sp., respectively (Figure 1). This result is matching with other

findings by Farag & Abd-Elnaby, 2014 who founding that fungal strains are acquired different bioremediation percentage using phenol.

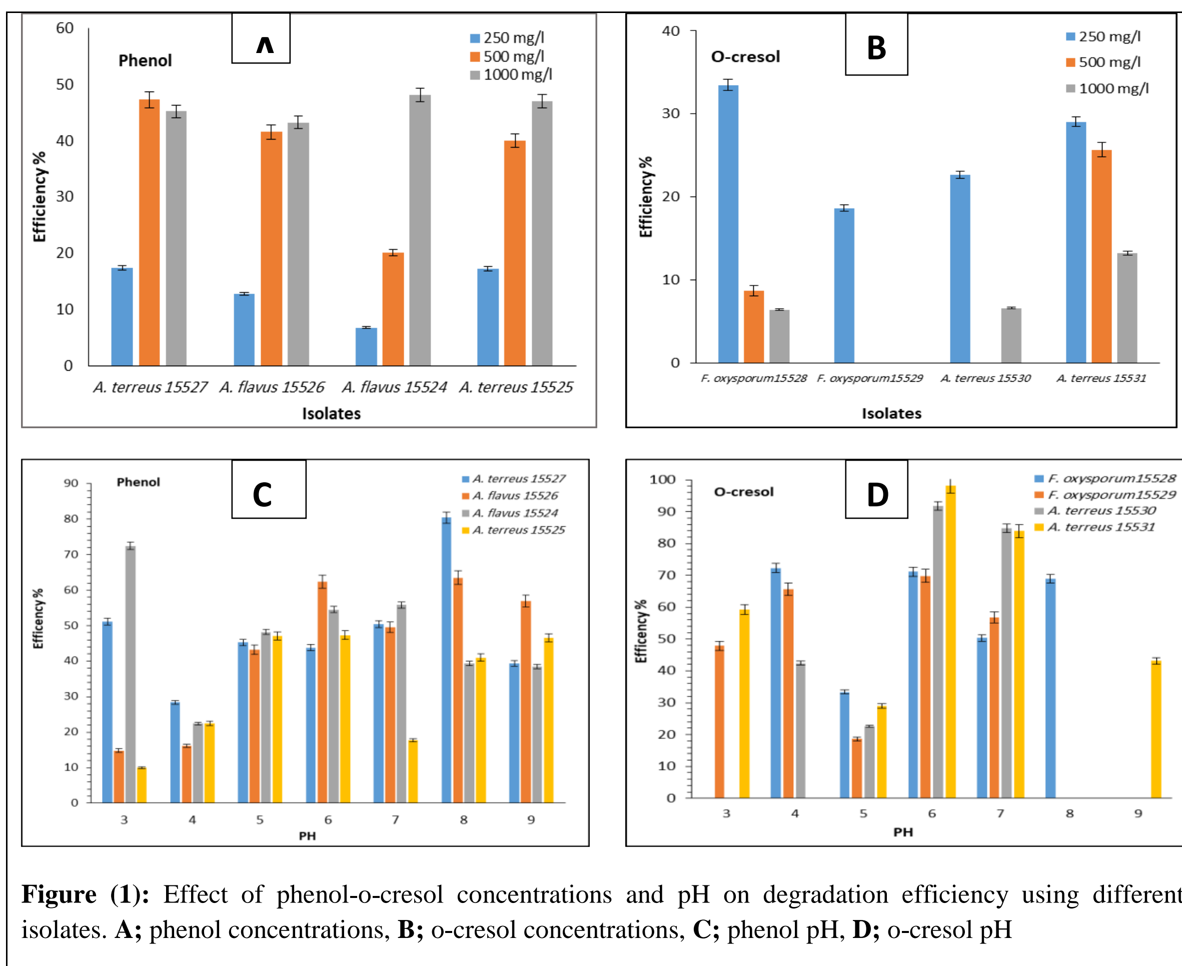

Figure (1): Effect of phenol-o-cresol concentrations and pH on degradation efficiency using different isolates. **A;** phenol concentrations, **B;** o-cresol concentrations, **C;** phenol pH, **D;** o-cresol pH

3.1.B. Effect of pH:

The pH significantly affects the reactions required for phenol and o-cresol bioremediation. The results revealed that pH (8) is the most effective for (*A. terreus* 15527) with bioremediation efficiency of 80.41% using (1000 mg/l) phenol concentration. The pH (6) appears suitable for the growth of *A. terreus* 15530 and *A. terreus* 15531 on o-cresol (250 mg/l) with high bioremediation efficiency 91.76 and 98.20 %, respectively during incubation time as shown in (Figure 1). This pH range serves to be the optimum for proper growth of microorganisms (Jacob & Alsohaili, 2010).

3.1.C. Effect of temperature

The temperature Play a crucial role than other parameters in the bioremediation of these organic pollutants (Margesin & Schinner, 1997). To determine the optimum

temperature for fungal growth, isolates were incubated with (1000 and 250 mg/l) for phenol and o-cresol at different temperatures from 25 to 43^{0}C. It was noted that 34^{0}C is the best temperature for the maximum activity of *A. terreus* 15527 for phenol bioremediation (88.73%) at pH 8. For o-cresol, the most effective temperature for growth and bioremediation rates (98.2 %) are 31^{0}C for *A. terreus* 15531 at pH 6, as shown in Figure (2). Microorganisms have a limited range of optimum temperatures supporting growth and yield a high rate of phenol bioremediation (Sharma & Gupta, 2012; Srivastava & Sharma, 2014)**.**

3.1.D. Effect of contact time:

The results of optimum pH and temperature for *A. flavus* 15526, *A. terreus* 15527, *A. terreus* 15530, *and A. terreus* 15531 were introduced for the contact time effect test. The results showed that, the highest bioremediation rate occurred after 7 days of incubation using maximum concentrations of phenol, and o-cresol. the highest bioremediation rate was achieved at 88.34% for *A. terreus* 15527 using phenol and (98.1%) for *A. terreus* 15531 using o-cresol with optimum conditions as shown in figure (2). The bioremediation time was found to be less than other previously recorded data by Sharma & Gupta, (2012) and Ibrahim & El-Gamdi, (2019) who found that recorded the maximum bioremediation rate for phenol recorded after 15 days.

3.1.E. Agitation speed:

It was observed that no response was recorded by agitation speed in the shaking incubator through different shaking speeds (100, 150, and 200 rpm) for phenol and o-cresol bioremediation using these fungal isolates. This result agrees with (Ibrahim & El-Gamdi, 2019)**.** From the achieved previous results, it is evident that; the rate of o-cresol bioremediation was found more effective than the rate of phenol bioremediation, which may be attributed to the role of the methylation, which decreases the solubility of phenol as the methyl position increases from ortho through meta to para, thus translation the phenol less available for fungal attack than o-cresol (Zhou & Nemati, 2018)**.**

3.3. Characterization and identification of the highly degrading fungi:

According to the first screening for phenolic compounds collapsed using fungi, eight isolates were chosen for morphological identification at Assiut university mycological center (AUMC). Samples 21th, 23 th, 26 th, 30 th, 50 th, 54 th, 55 th and 60 th were named *via* center by *Aspergillus flavus* (15524), *Aspergillus terreus* (15525), *Aspergillus flavus* (15526), *Aspergillus terreus* (15527), *Fusarium oxysporum* (15528), *Fusarium oxysporum* (15529), *Aspergillus terreus* (15530) *and Aspergillus terreus* (15531), respectively (Table 2S). Further, *A. terreus* (15527) and *A. terreus* (15531) were selected for molecular identification. The sequence data of these samples were ranged with closely related sequences accessed from the GenBank. *A. terreus* AUMC15527 and *A. terreus* AUMC 15531 showed 100% identity and 99%-100% coverage with several *A.*

terreus. Samples were certified in the world gene bank with accession number ON649683 for *A. terreus* MHG30 and ON649704 for *A. terreus* MHG60 with the phylogenetic tree for two samples. These results are aligned with earlier studies by El-Zaher *et al.* (2011) and Fouda *et al.* (2015), who used fungi to break down phenolic compounds (Fig. S1).

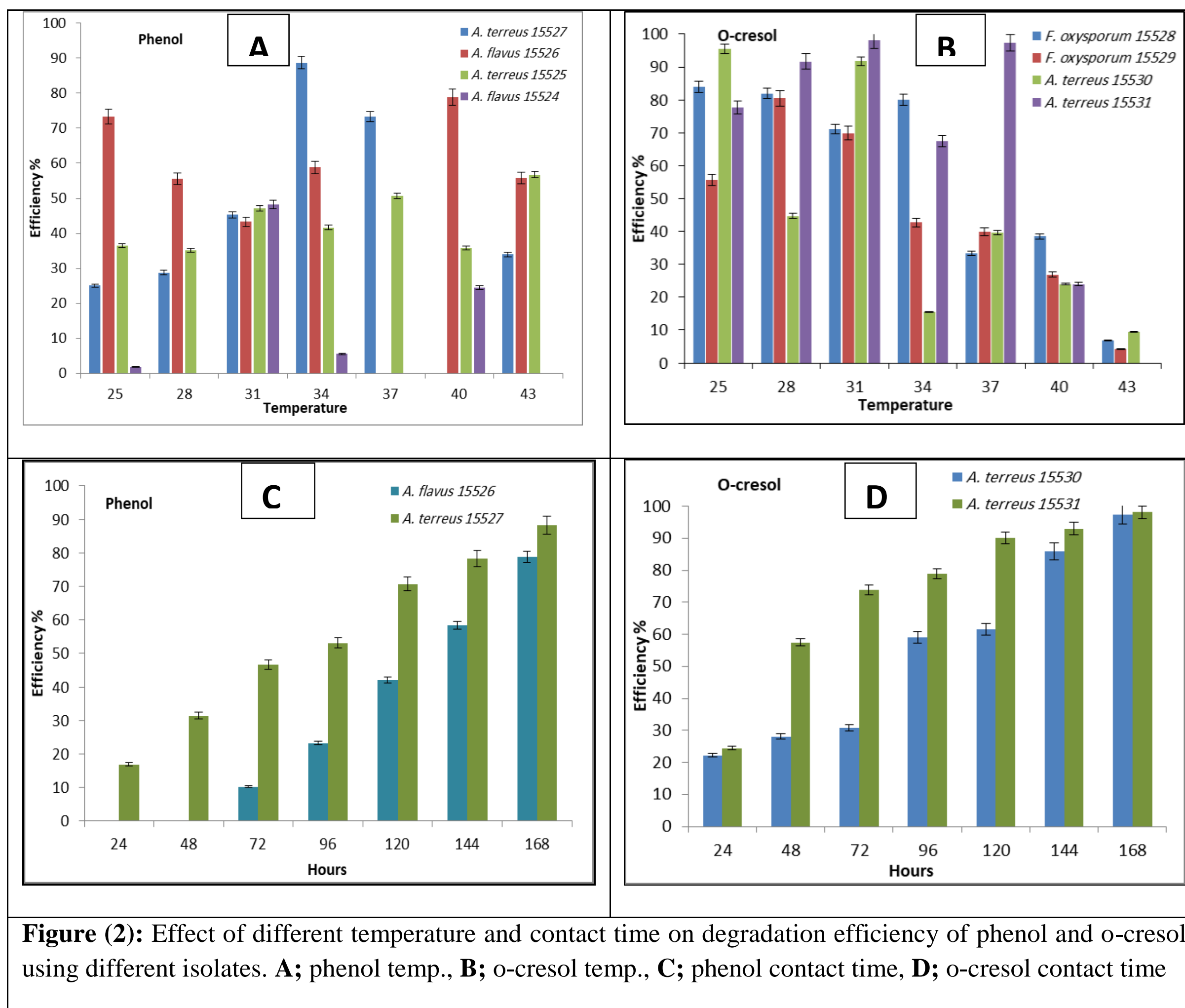

Figure (2): Effect of different temperature and contact time on degradation efficiency of phenol and o-cresol using different isolates. **A;** phenol temp., **B;** o-cresol temp., **C;** phenol contact time, **D;** o-cresol contact time

3.4. Enzyme inhibition:

The most potent isolates that can enzymatically degrade phenols and o-cresol compounds are proven for peroxidase and polyphenol oxidase determination with the calculation of specific activity, as illustrated in tables 1 & 2. *A. terreus* AUMC 15527 and *A. terreus* AUMC 15531 were found the highest inhibitory % across phenol-derivatives compounds. Many processes involving phenols and its derivatives can result in response known as enzymatic bioremediation of phenolic chemicals. The browning reaction requires the presence of oxygen, phenolic compounds, and oxidative enzymes. Thus, antioxidant compounds with similar potential to those in this study inhibit enzymatic browning (Mohammadi *et al.,* 2018).

Table (1): Screening of peroxidase activity with specific activity using different fungal strains.

Fungal strains	Average of replicate measurements per 100 ml of sample			
	Peroxidase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)	Inhibitory %
Aspergillus flavus **(15524)**	0.34±0.011	0.64±0.013	0.53±0.007	53±0.7
Aspergillus terreus **(15525)**	0.34±0.012	0.59±0.012	0.57±0.008	57±0.8
Aspergillus flavus **(15526)**	0.33±0.01	0.63±0.013	0.52±0.007	52±0.7
Aspergillus terreus **(15527)**	0.36±0.012	0.59±0.012	0.61±0.008	61±0.8
Fusarium oxysporum **(15528)**	0.38±0.012	0.64±0.013	0.59±0.008	59±0.8
Fusarium oxysporum **(15529)**	0.35±0.011	0.62±0.013	0.56±0.007	56±0.7
Aspergillus terreus **(15530)**	0.31±0.01	0.59±0.012	0.52±0.007	52±0.69
Aspergillus terreus **(15531)**	0.37±0.012	0.64±0.013	0.64±0.04	64±1.15

Table (2): Screening of peroxidase activity with specific activity using different fungal strains.

Fungal strains	Average of replicate measurements per 100ml of sample			
	Peroxidase activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)	Inhibitory %
Aspergillus flavus **(15524)**	0.49±0.015	0.66±0.014	0.74±0.009	74±0.89
Aspergillus terreus **(15525)**	0.44±0.014	0.62±0.013	0.7±0.008	70±0.85
Aspergillus flavus **(15526)**	0.47±0.012	0.64±0.012	0.73±0.007	73±0.74
Aspergillus terreus **(15527)**	0.53±0.017	0.65±0.013	0.81±0.009	81±0.98
Fusarium oxysporum **(15528)**	0.47±0.016	0.67±0.013	0.701±0.009	70±0.93
Fusarium oxysporum **(15529)**	0.42±0.014	0.62±0.013	0.68±0.008	68±0.89
Aspergillus terreus **(15530)**	0.36±0.015	0.61±0.013	0.59±0.009	59±0.92
Aspergillus terreus **(15531)**	0.51±0.017	0.65±0.013	0.78±0.01	78±1.15

The inhibitory percentage of *Aspergillus flavus* (15524)*, Aspergillus terreus* (15525)*, Aspergillus flavus* (15526)*, Aspergillus terreus* (15527)*, Fusarium oxysporum* (15528)*, Fusarium oxysporum* (15529)*, Aspergillus terreus* (15530) *and Aspergillus terreus* (15531) were taped with 53, 57, 52, 61, 59, 56, 52 and 64 %, respectively (figure S2). Growing cultures or harvested mycelia *of A. terreus* were found to efficiently degrade the phenolics such as catechol, 4-methyl catechol, tyrosol, caffeic acid, etc. (Jain, 2020; Al-Agamy *et al.,* 2021).

According to peroxidase analysis, fungal isolates recorded inhibitory activity with 74, 70, 73, 81, 70, 68, 59, and 78 for *A. flavus* (15524)*, A. terreus* (15525)*, A. flavus* (15526)*, A. terreus* (15527)*, F. oxysporum* (15528)*, F. oxysporum* (15529)*, A. terreus* (15530) *and A. terreus* (15531), respectively (Table S3). This heterogeneous activity of the different isolates showed the ability extent of hyphae-forming fungi to use substrate and break it down by the enzymes secreted internally (Prakash *et al.,* 2019).

Kinetic mechanism of oxidase and peroxidase:

The K_m and V_{max} of *A. terreus* 15527 and 15531 were determined using different concentrations of the substrate with the determined volume of oxidase and peroxidase (Figure 3). Fungal mycelia free supernatant of fungal isolates showed an uncompetitive inhibition, where the value of km and Vmax were found low with the tested inhibitor concentration. Such performance for *A. terreus* 15527 differed from other strains, which displayed competitive inhibitory behaviour, where the km/Vmax values lowered with the inhibitor addition. This action indicates a competitive inhibition for most strains; therefore, the phenolic compounds are similar to the preferred enzyme substrate. The findings of this study showed that the extract of filamentous fungi led to an increase in the digestive phenol-derivatives compound, which has a lower antioxidant activity to induce peroxidase enzyme action. Furthermore, these compounds can be converted into other compounds of interest, such as vanillin (Demarche *et al.,* 2012).

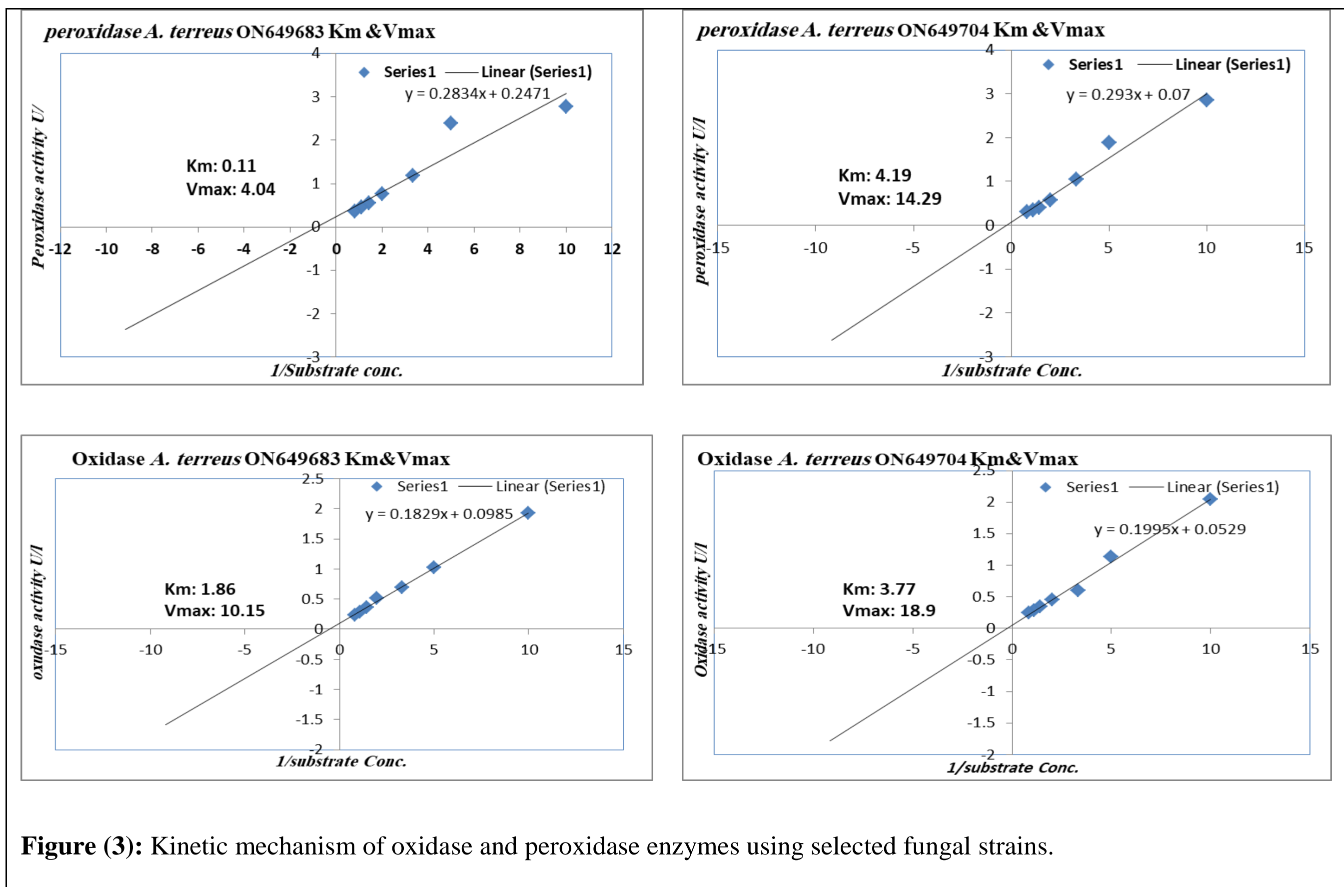

Figure (3): Kinetic mechanism of oxidase and peroxidase enzymes using selected fungal strains.

Fungi rely heavily on oxidation and hydrolysis pathways for the bioremediation of phenolic compounds. Secretion of peroxidase and oxidase by different fungal strains in the presence of phenol as a substrate might lead to oxidation. According to Torres & Ayala, (2010**),** peroxidase can increase the stability and solubility of phenols in three distinct steps. First, when the peroxidase enzyme consumes the hydrogen in a phenol

molecule, the resulting bi-phenols, 2,2'-biphenol and 4,4'-biphenol, are polymerization-ready compounds. The same enzyme catalyzes the conversion of a subset of these chemicals to 4,4'-biphenoquinone (Torres & Ayala, 2010). Contrarily, polyphenols can be oxidized by oxidase enzymes into catechol, oxidizing to o-quinones (Raymundo-Pereira *et al*., 2020)**,** as shown in Fig. 4.

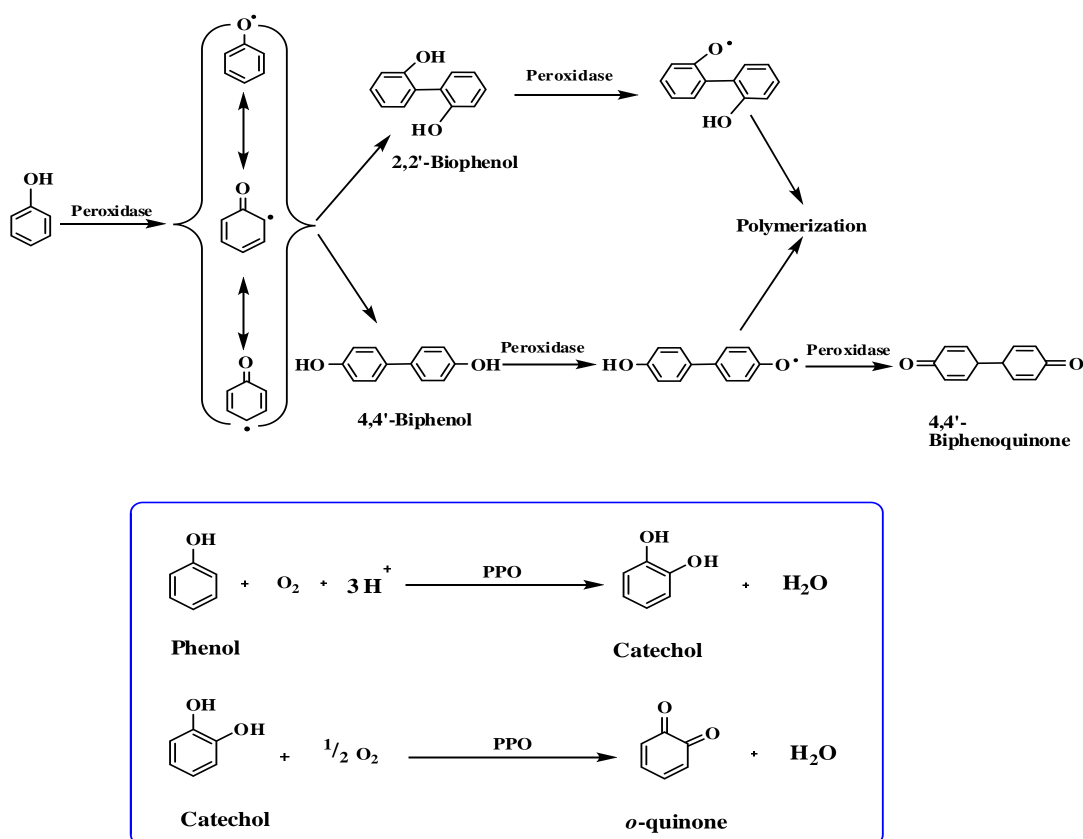

Figure (4): Mechanism of phenol oxidation and polymerization by peroxidase-catalyzed reaction and polyphenol oxidases (PPO) Pathway.

3.5. GC-Mass analysis:

The GC-MS chromatograms detected bioremediation of the phenol sample by *A. terreus* 15527. Understudy, it was found that phenol compound biodegrades nearly 100% at intervals of seven days due to rapid use by fungi as a sole of carbon substrate and appears more than eight peaks at different retention times of new compounds. Table (3) and Fig. (5) show the identification and characterization of the prominent peaks using the NIST mass spectral data.

The profiles of bioremediation clarify that; the ortho enzymatic pathway during bioremediation and condensation with phenol form the Succinic acid 2, 3-dimethyl phenyl dodecyl ester intermediate compounds. Also, it reveals that this type of biomolecule is considered as tremendous potential as a chemical platform for many industrial chemicals such: as plastics, textiles, and pharmaceuticals.

Table (3): Compounds detected in GCMS analysis of phenol biodegraded by *A. terreus* 15527.

No.	RT (min.)	Name of compound	Formula	MW	Peak Area %
1	26.47	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	3.1
2	28.18	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	27.45
3	29.59	Methyl 9-cis, 11-trans-octadecadienoate	$C_{18}H_{31}O_2$	294.0	7.57
4	29.71	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.4	15.26
5	30.18	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.0	2.49
6	30.54	6,9,12-octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.0	0.79
7	31.21	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282.0	31.5
8	37.0	Succinic acid, 2,3-dimethylphenyl dodecyl ester	$C_{24}H3_8O_4$	390.0	0.34

When cells release molecules like hexadecanoic acid or other octadecanoic acids into the media or partially remove them from the cells, those molecules are products of biological synthesis that have their origins in cellular metabolism. These lipids from biomolecule fractions of oleaginous fungus are used as a potential feedstock for biodiesel production (Bardhan *et al.,* 2019). These findings show the potential of oleaginous *A. terreus* as a feedstock for further bioactive and biodiesel compounds with desirable properties and reveal the significant ability of fungi to consume phenol, making them seen as an essential tool for the bioremediation of wastewater effluents. Furthermore, the final bioactive and biodiesel compounds should be considered novel eco-friendly bi-products resulting from the bioremediation process of organic pollutants (Grace *et al.,* 2020).

Table (4): Compounds detected in GC-MS analysis of o-cresol biodegraded by *A. terreus* 15531.

No.	RT (min.)	Name of compound	Formula	MW	Peak Area %
1	26.47	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	6.19
2	27.96	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	9.86
3	29.58	9-Octadecenoic acid, methyl ester, (E)-	$C_{18}H_31O_2$	294.0	10.91
4	29.69	Methyl 9-cis, 11-trans-octadecadienoate	$C_{19}H_{36}O_2$	296.4	26.44
5	29.81	9-Octadecenoic acid (Z)-,methyl ester	$C_{19}H_{38}O_2$	296.0	4.37
6	30.18	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.0	5.12
7	30.54	6,9,12-octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.0	1.33
8	32.73	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354.5	0.3

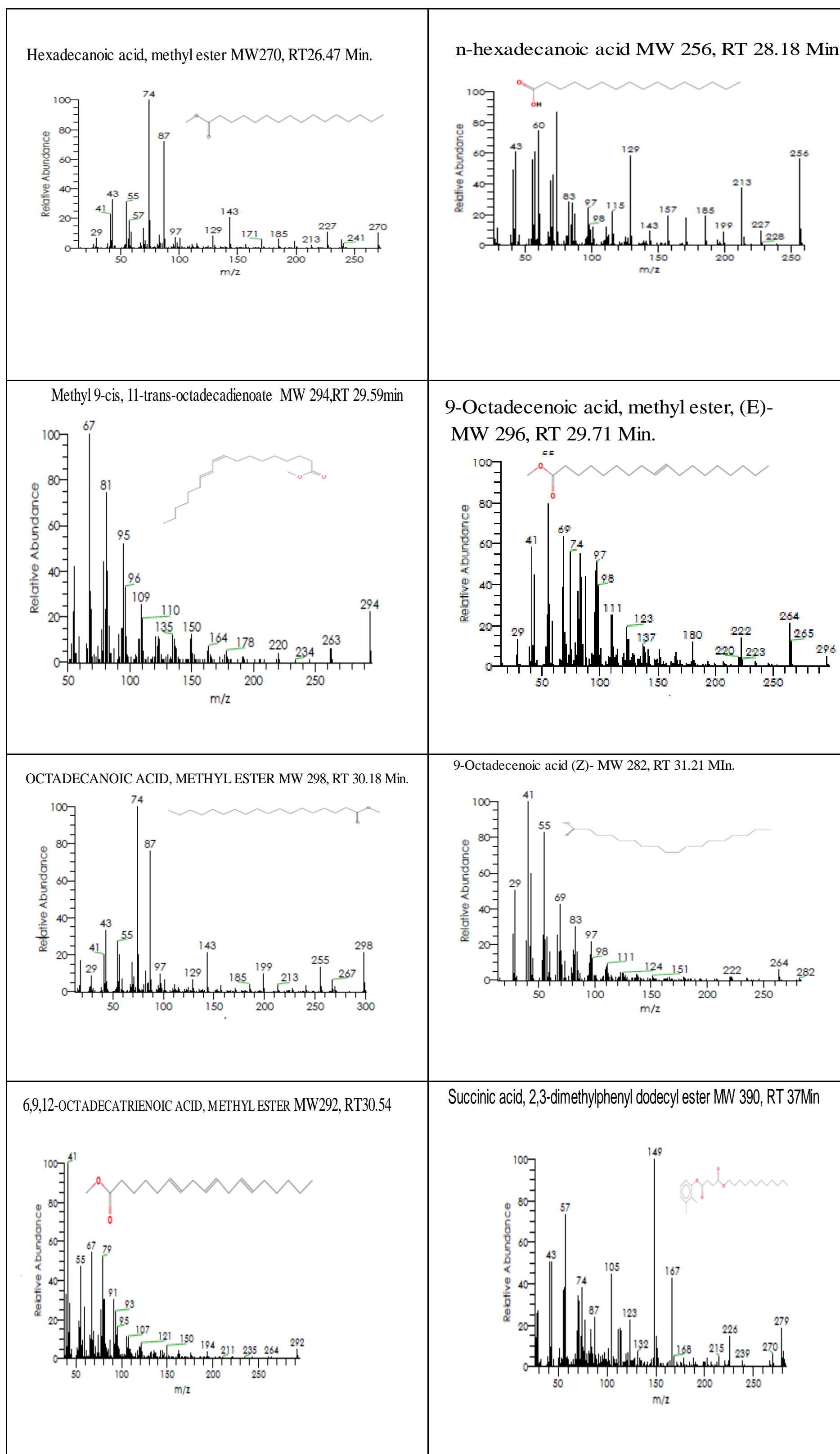

Figure (5): Ion chromatogram detected GC-MS analysis of phenol/ o-cresol biodegraded by *A. terreus* 15527 and *A. terreus* 15531.

Table (4) and Fig. (5) show the GC-MS analysis of bioremediation of o-cresol as derivatives of phenol by fungi. There is no significant difference in bioremediation behaviour as in phenol compound.

CONCLUSION

Aspergillus terreus MHG30 (ON649683) and *A. terreus* MHG60 (ON649704) were considered as the most efficient isolates used to degrade phenol and o-cresol with potential efficiency 88.34 and 98.1 %, respectively, by different optimum conditions each separate. The enzymatic assessment of oxidase and peroxidase is regarding to the different structures of each substrate with different Michaelis Menten (V_{max}) constants and maximal velocity. Also, the results of GC-Mass pointed out the occurrence of bioactive and biodiesel compounds (Hexadecanoic acid, methyl ester, and 6,9,12-octadecatrienoic acid) novel end products through bioremediation process of phenol and o-cresol, that it could be considered as an eco-friendly bio-products from bioremediation of phenolic compounds. Also, they are a safe alternative for the bioremediation process in marine environments. The fungal isolates can further used for production of eco-friendly biodiesel compounds.

Abbreviations

MIC: minimum inhibition concentration; µg/ml: microgram/mill; GC-MS: gas chromatography-mass spectrometry; FTIR: Fourier-transform infrared spectroscopy; IC50: half-maximal inhibitory concentration; HepG2: human liver cancer cell; HCT 116: Human colon cancer cell; DPPH: diphenyl-picrylhydrazyl; CHCl3: chloroform; NIOF: National institute of oceanography and fisheries; TCBS: Thiosulfate Citrate Bile Salt Sucrose; O/129: diamino-6,7-diisopropylpteridine phosphatase; 16SrRNA: 16 subunit ribosomal nucleotide analysis; QI: Qiagen international; BHI: Brain heart infusion; cfu/ml: colony forming unit/mill; MTT assay: dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO: dimethyl sulfoxide; GC1310-ISQ: Gass chromatography 1310-international single quadrupole; TG–5MS: Trace gold-5mass spectroscopy; AS1300: automatic sampler 1300; m/z 40–1000: mass resolving power 400-1000: 70 eV; 70 electron ionization: OC; degree celsius: rpm; rotation per minute: RTs: retention time per second; NIST 11: National Institute of Standards and Technology 11; KBr: potassium promide; IR index: index insulin resistance; DMEM: Dulbecco's modified Eagle's medium; HEPES buffer: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ODt/ODc: test optical density/control optical dwnsity; w/v: weight/volume; UV: ultra violet ; nm: nano meter; PI: inhibition percentage; PT: Prothrombin time; PPT: partial prothrombin time; mol/l: mol/liter; TT: thrombin time.

Supplementary files

Additional file 1: Figure (1S): Phylogenetic tree based on ITS sequences of rDNA of the fungal samples *A. terreus* AUMC15527 & *A. terreus AUMC 15531* with closely related sequences alignments (*A.* = *Aspergills,* T. = Type material), **Figure (S2):** Oxidase and peroxidase enzyme activity and its inhibitory % on phenol-derivatives compounds, **Figure (S3):** GC-MS chromatogram of phenol biodegrading by *A. terreus* 15527, **Figure (S4):** GC-MS chromatogram of o-cresol biodegrading by *A. terreus* 15531.

Table (S1): Fungal isolates were recorded, screened and purified from sediment samples after being tested with different concentrations of phenol and o-cresol. **Table (S2):** Showed morphological identification of selected fungal isolates at AUMC, **Table (S3):** Kinetic mechanism (Km and Vmax) of oxidase and peroxidase enzymes using two *A. terreus* strains (15527, 15531).

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors acknowledge that the research and its steps were conducted without coordinating commercial or financial relationships that could be interpreted as potential competing interests.

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Availability of data and material

The raw data supporting the conclusions of this manuscript would be available by the authors, without undue reservation, to any qualified researcher.

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