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ISOLATION AND CHARACTERIZATION OF PHENOL DEGRADING BACTERIA FROM INDUSTRIAL WASTEWATER AND SEWAGE WATER

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ABSTRACT: This study examines the isolation and characterization of phenol-degrading-bacteria, Five isolates named C2, P4, M4, O3, and S3, from the effluent of ceramic factories (C2), petrochemical (P4), the pulp paper mill (M4), crude oil (O3) and sewage water (S3) have been investigated. These isolates were characterized depending on their morphological, and biochemical characteristics, only one isolate was characterized as a Gram-positive, strictly aerobic, nonmotile, and cocci-shaped bacterium might be *Micrococcus* sp. (M4), two isolates (*Pseudomonas* sp.: (P4&S3) were recogniced as a Gram-negative, strictly aerobic, motile and short rod-shaped bacterium and two isolates(*Bacillus* sp.: (C2& O3) were idenihied as a Gram-positive, strictly aerobic, motile and long rod- shape bacterium. The five bacterial isolates were able to utilize phenol as a sole carbon source. These isolates were checked for growth on a minimal salt medium amended with different concentrations of phenol. The five tolerant bacterial isolates were able to grow at the higher concentrations of phenol and they were investigated for their ability to grow and degrade phenol. Among the five higher phenol degrading isolates, two isolates can tolerate up to 1500 ppm .of phenol concentrations, grow and degrade 90% of phenol within 72 hrs. The optimum temperature and pH were 35 ^{0}C and 7, respectively. The yeast extract and ammonium chloride is the best nitrogen source for the growth and degradation of phenol, respectively. The isolate P4 was efficient in removing about 90% of the initial 1000 ppm phenol within 48 hrs., and had a tolerance of phenol concentration as high as 1500 ppm. These results indicated that presumptive *Pseudomonas* spp. possesses a promising potential in treating phenolic wastewater.

Key words: Phenol degrading-bacteria, sewage water, petrochemical effluent, crude oil effluent, paper mill factories.

INTRODUCTION

Phenol is highly a toxic for a compound living organisms. Nowadays, with increasing industrial growth aromatic compounds are environmental pollutants that exist in different niches such as freshwater, sea, and land **(Deng *et al.*, 2018)**. Phenol and phenolic compounds are hazardous when present in low concentration. The high and acute concentration of phenol can cause humans' disorders in the central nervous system, myocardial depression, irritation of eyes, swelling, corneal whitening, blindness, cardiovascular diseases and gastrointestinal damage **(Govindarajalu, 2003; Naresh *et al.*, 2012)**. The production and application of phenol and phenolic compounds in industrial activities make them major environmental pollutants in most wastewater, such as oil refineries, coking plants, pharmaceuticals, and plastic industries **(McCall *et al.*, 2009; Park *et al.*, 2012)** and it is highly toxic to all life grows in all concentrations (5-2000ppm)and considered as a priority pollutants **(ATSDR, 2003)**. Due to these adverse health effects of phenolics, as per the rules of the World Health Organization(WHO) the maximum permissible level for phenol in the environment is 0. 01 mg/L **(Nuhoglu and Yalcin, 2005; Saravanan *et al.*, 2008)** and in tap water to below 1–2 µg/L **(Gami *et al.*,**

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2014). A variety of treatment methods, such as adsorption (**Carmona *et al.*, 2006**), solvent extraction **(Lazarova and Boyadzhieva, 2004)**, Wet oxidation, and hydrogen peroxide. Fenton's reagent has been employed to eliminate phenol from the polluted samples **(Lin and Chuang, 1994),** chemical oxidation, and incineration **(Wu *et al.*, 2005).** But these methods are complex, high cost, and not environment friendly **(Yan *et al.*, 2006; Bai *et al.*, 2007; Zhai *et al.*, 2012)**.

Biodegradation is the best way to get rid of phenol since this chemical and physical biological process is cheap, environment-friendly, and easy to handle **(Tay *et al.*, 2005; Basha *et al.*, 2010)**. Bacteria oxidize phenol into CO_2 and H_2O during metabolic processes **(Loh and Chua, 2002)** and can utilize phenol for their growth through utilizing phenol as the sole source of carbon and energy **(Geng *et al.*, 2006; Tuah *et al.*, 2009; Nair *et al.*, 2008)**. Phenol degrading bacteria have been isolated like *Burkholderia cepacia* (**El-Sayed *et al.*, 2003)**, *Rhodococcus* **(Rehfuss and Urban, 2005)**, *Xanthobacter flavus* **(Lowry *et al.*, 2009)**, *Bacillus cereus* **(Banerjee and Ghoshal, 2010)**, *Staphylococcus epidermis* (**Mohite *et al.*, 2010**), *Acinetobacter* sp. **(Ahmad *et al.*, 2012)**, *Gulosibacter* sp. **(Zhai *et al.*, 2012),** *Pseudomonas* sp. **(Mahiudddin *et al.*, 2012; Ahmad *et al.*, 2014**) and *Acinetobacter calcoaceticus* **(Liu *et al.*, 2016)**. The microbial phenol degradation focusing on aerobic degradation using specifuc bacterial strains, different methods for improving the phenol degradation rate, effects of various physicochemical factors on degradation process, and mechanisms of degradation were reported (**Bhattacharya *et al.*, 2018**). The bacterial stains SP-4 and SP-8 from the pulp and paper mill effluent were capable of tolerating phenol up to a concentration of 1600 and 1800 ppm, respectively **(Sachan *et al.*, 2019)**. These strains were found to be efficient amongst the sixteen strains established by checking their capability of phenol tolerance with respect to the incubation time. These strains can be utilized in real-scale systems as identification of phylogenetically closely related species for phenol degradation is an important aspect. This will help in treatment of industrial wastes, and the utilization of such isolated microorganisms prove to be more economical and feasible. This will reduce the environmental burden with the development of technology as an effective and economical method therefore, This study investigates the isolation and characterization of a phenol-degrading bacteria from different sources such as the effluent of ceramic factories (C), effluent of petrochemical (P), effluent of the paper mill (M), effluents of crude oil (O) and sewage water (S). These isolates could be a useful in the biotreatment process of high-strength phenol-containing industrial wastewaters and in the *in-situ* bioremediation of phenol-contaminated soils and industrial wastewater.

MATERIALS AND METHODS

Chemicals, Media and Phenolic Wastewater Sample

All chemicals used were analytical reagents. The Minimal salt medium (MSM), and Luria-Bertani (LB) medium were used in the current study. The minimal salt medium (MSM) contained KH_2PO_4 0.5 g, K_2HPO_4 0.5 g, $CaCl_2$ 0.1 g, NaCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $MnSO_4 \cdot 7H_2O$ 0.01 g, $FeSO_4 \cdot 7H_2O$ 0.01 g and NH_4NO_3 1.0 g per liter. MP medium contained (in g\L): K_2HPO_4, 2.75; KH_2PO_4, 2.25; $(NH_4)_2\ SO_4$, 1.0; $MgCl_2 \cdot 6H_2O$, 0.2; NaCl,0.1; $FeCl_3 \cdot\ 6H_2O$, 0.02and $CaCl_2$, 0.01 **(Geng *et al.*, 2006).** Luria-Bertani (LB) medium composed of tryptone 10 g, yeast extract 5 g and NaCl 5 g per liter. Deionized distilled water was used for the experiments.

Both LB and MS media were supplemented with specific amounts of phenol or other nitrogen sources up to a final concentration required. The pH of the media was adjusted to 6.8–7.0 and supplemented with varying amounts of phenol up to a final concentration of 500–2000 ppm. Phenol was added directly from the stock solution (50,000 mg l^{-1}phenol) through 0.45-µm syringe filters. Cells were grown in flasks on a rotary shaker at room temperature (25°C) and 120 rpm. Samples were withdrawn periodically for bacterial growth and phenol concentration or dlterminations were monitored.

An industrial wastewaters [*i.e.*, the effluent of ceramic factories (C), effluent of petrochemical compang (P), effluent of the pulp paper mill (M), effluents of crude oil (O) and

sewage water (S)] were collected due to standard procedures of sampling from Tenth of Ramadan City Industrial Water Works (El-Sharkia Governorate, Egypt).

Enrichment and Isolation of Phenol-Degrading Bacteria

Ten milliliters of each sample were aseptically added to shaking flasks containing 90 ml of MP medium supplemented with 500 mg/L phenol and was incubated on a rotary shaker (120 rpm) at room temperature **(Geng *et al.*, 2006)**. During incubation, the optical density at 600 nm (OD_{600}) of the culture broth was monitored. After an obvious OD increase had been observed (2–3 days), 10 ml of the culture broth was transferred to a new shaking flask containing 90 ml of fresh medium. This operation was repeated three times, and the final culture broth was serially diluted and spread on LB- agar plates supplemented with phenol (500 mg/L) as well as spread onto agar plates containing MP medium supplemented with 500 mg phenol. Colonies appeared on the agar plates after 3–4 days of incubation at 30°C were picked and subcultures several times to obtain pure cultures. Purity was confirmed by microscopic examination. The morphological properties of the isolated colonies were observed by optical microscopy for cell size and shape**,** motility and sporulation. The typical physiological and biochemical characteristics of the phenol-degrading bacteria isolates strains, such as Gram's staining, motility, **(Kloos, *et al.*, 1998)** were systematically performed according to Bergey's Manual of Determinative of Bacteriology **(Holt *et al.*, 1994)**. Indole test, methyl red test were also analyzed, as described previously **(Lanyl et *al.*, 1987)**. Isolates are giving name based on the source of isolate i.e**.,** effluent of ceramic factories (C-1 to 5), effluent of petrochemical Company (P-1 to 5), effluent of the paper mill (M-1 to 5), effluents of crude oil (O-1 to 5) and sewage water (S1- to 5) were preserved in nutrient agar slants at 4°C and nutrient broth supplemented with 20% sterilized glycerol at−20°C.

Phenol Biodegradation and Cell Growth Performance

To clarify the phenol degradation potentials of bacterial isolates, batch experiments were performed in duplicate. Prior to inoculation for each experiment, one loop of stock culture maintained on nutrient agar slants was transferred aseptically into 250-ml flasks with 100 ml of sterilized MPsupplemented with 500 mg/L phenol. Five milliliters of inoculum from the late exponential growth phase of each culture were then transferred aseptically to each flask with 95 ml of MS- medium **(Geng*, et al.*, 2006).** The bacterial isolates (C 1-5, P1-5, M1-5, O1-5 and S1-5) were tested in 500 mg/L phenol concentration then all the bacterial isolates were selected for phenol removal assay at different phenol concentrations (0.0, 500, 1000, 15000 and 2000 mg/L) by a modified colorimetric technique 4-aminoantepyrene method as described by **Klibanov *et al.*, (1980)** and according to standard methods reported by the **APHA (2005).**

Effect of Environmental Factors on Growth and Phenol-Degradation by Two Isolates *i.e.*, *Pseudomonas* sp. P4 and *Bacillus* sp. (C2)

Experimental procedure

To study the optimum functional pH, temperature, and nitrogen source for growth and degradation of phenol at constant concentration of phenol (1000 mg/L), in incubation temperature (35 °C) and neutral pH 7 in absence of carbon was carried out. Similarly, other parameters were kept constant, and pH was varied between 6 - 8. Assessment of growth was measured at 0, 24, 48, 72 and 96 hrs. and phenol degradation were determined after an incubation period of 96 hrs **(Geng *et al.*, 2006)**. All the results were given as a mean with Standard Deviation (±SD). All experiments were carried out in trireplicates and the mean value considered.

Effect of temperature on the growth and phenol degradation

The bacterial isolate M5 was grown in MS - medium with 1000 mg/L of phenol at different temperature values (25°C, 30°C, 35°C and 40°C) at pH 7 using 250ml Erlenmeyer flasks. The cultures were placed on a shaker (120 rpm) at the above-mentioned temperatures. The growth was measured at 0, 24, 48, 72 and 96 hrs. and phenol degradation were measured at time 96 hrs **(Geng *et al.*, 2006)**.

Effect of pH on the growth and phenol degradation

The effect of pH (6-8) on growth and phenol degradation were tested. Cells were grown as shake cultures at 35°C in MS- medium supplemented with 1000 mg/L phenol and inoculum size 5 %v/v in 250 mL flask. The growth was measured at 0, 24, 48, 72 and 96 hrs. and phenol degradation were measured at time 96 hr. **(Geng *et al.*, 2006)**.

Effect of nitrogen sources on the growth and phenol degradation

Effect of different nitrogen sources on growth and phenol degradation were determined. Each culture was grown as shake cultures at 35°C in MS medium supplemented with 1000 mg/L phenol and in the source of nitrogen was replaced with peptone, yeast extract, urea extract, $NaNO_3$, KNO_3 and NH_4Cl at concentration of 2 g/L. The used positive control was NH_4Cl. The growth was measured at 0, 24, 48, 72 and 96 hrs. and phenol degradation were measured at time 96 hrs. **(Geng *et al.*, 2006)**.

RESULTS AND DISCUSSION

Screening of Bacterial Isolates from Different Sources

Twenty five bacterial cultures (C1 to C5, P1 to P5, M1 to M5, O1 to O5 and S1to S5) were screened for growth on different phenol concentrations (Table 1). Out of 25 bacterial isolates which isolated from effluents of ceramic factories (C), sewage water (S), petrochemical soil (P), paper mill factories(M) and crude oil (O), only five isolates were more active on growth on varied initial phenol concentrations of 500 - 2000 mg/L phenol. The study was carried out at temperature of 35 °C and pH 7. Growth of bacterial isolates on treated media and control was taken and expressed by streaking growth degree as follows: no growth (-), moderate growth (+), good growth (++), very good growth (+++) and excellent growth (++++). All cultures showed fast growth and activity at 500 mg/L at 35°C and pH 7 as indicated by plate growth and turbidity. However, only five isolates named C2, P4, M4, O3 and S3 showed fast growth in up to 2000 mg/L phenol concentration. Bacterial isolate M4 showed only moderate growth at 1500 mg/L of phenol concentration.

Phenol and its derivatives have shown surprising capability in phenol elimination with bacteria having fast reproduction after acclimatization **(Sun *et al.*, 2012)**. So, isolation, purification, and growth of species, which have a high capability of phenol removal, can be utilized in areas with wastewaters containing high phenol concentrations. However, logically it can be inferred that the increase in the phenolic concentration causes the selection and utilization of the phenol-degrading microorganisms with less diversity. **Hussain *et al.* (2008, 2009, 2010)** conducted a study using membrane bioreactor in the treatment of phenolic wastewater. They found that the various significant features regarding diversity, physiology, and function of *Pseudomonas* population that is found in industrial phenol-degrading bioreactors. However, significant physiological heterogeneity in the tolerance limit of bacterial isolates has been observed in the treatment of phenolic wastewater **(Whiteley *et al.*, 2001; Whiteley Mark, 2000)**. In the present study, five bacterial isolates C2, P4, M4, O3, and S3 showed luxuriant growth on phenol-amended minimal salt medium (MSM) in the presence of 1% glucose (w/v), whereas the growth was absent in the absence of glucose. Four isolates showed fast and luxuriant growth at phenol concentration of 0–2000 mg/L as shown in Table 1. The results obtained from the present study pertaining the growth studies indicate that the M4 isolate is capable to tolerate the phenol up to a phenol concentration of 1500 mg/L. Also, the bacterial isolates C2, P4, O3 and S3 can tolerate the phenol up to a phenol concentration of 2000 mg/L. However, no growth has been observed in other bacterial isolates (the rest of the 25 isolates) at a phenol concentration of 1500 and 2000 mg/L (Table 1). A similar study being conducted by **Yang and Lee (2007)** reported that phenol has a potent inhibitory effect on cell growth based on the fact that the high phenol concentration of up to 2000 mg/L causes substrate inhibition. It is also found that the next disclosure to the increasing phenol concentration from 0 to 2000 mg/L on isolated microorganisms can degrade if effectively and efficiently. Therefore, from the present study, it can be inferred that the

Table 1. Capability of bacterial isolates collected from different sources used to grow on different concentrations of phenol

Source of Isolates	Concentrations of phenol mg/L				
	Control	500	1000	1500	2000
C1	++++	++	+	-	-
C2	++++	+++	+++	+++	+++
C3	++++	++	+	-	-
C4	++++	++	-	-	-
C5	++++	++	+	-	-
P1	++++	++	-	-	-
P2	++++	+	-	-	-
P3	++++	-	-	-	-
P4	++++	+++	+++	+++	+++
P5	++++	-	-	-	-
M1	++++	++	+	-	-
M2	++++	-	-	-	-
M3	++++	+	-	-	-
M4	++++	+++	+++	+	+
M5	++++	++	+	-	-
O1	++++	++	+	-	-
O2	++++	+	+	-	-
O3	++++	+++	+++	++	++
O4	++++	+	+	-	-
O5	++++	+	+	-	-
S1	++++	++	+	-	-
S2	++++	++	-	-	-
S3	++++	+++	+++	++	++
S4	++++	+	+	-	-
S5	++++	+	-	-	-

Excellent (++++) Very good growth (+++) Good growth (++) Moderate growth (+) No growth (-)

industrial wastewater containing a phenol concentration of 1500 mg/L can be effectively treated, and thus, phenol can be removed by using the efficient degrading bacteria isolation recovered from some original source.

Identification and Characterization of Phenol-degrading Bacterial isolates

The wastewater and sludge samples collected from an effluent of ceramic factories (C), effluent of petrochemical (P), effluent of the paper mill (M), effluents of crude oil (O) and sewage water (S) were inoculated in the MP-medium containing phenol for the enrichment and isolation of phenol-degrading bacteria. Phenol degrading bacteria were monitored and only 5 prevalent bacteria were selected for more study. All these isolates utilized phenol as the sole carbon source and energy, and 5 of the 20 isolates exhibited more growth in phenol-containing media than the others. The outstanding isolate was named as phenol-

degrading isolates C2, P4, M5, O3 and S3 and they were applied in the current study. After three weeks of enrichment and one week of bacterial isolation, a total of 25 isolates were obtained after 24 hrs., growth on the LB- agar plates with 100 µL of a 10^3–10^6-fold dilution of enrichment culture. Identification of the isolates based on their morphology, microscopic and biochemical characteristics are shown in Table 2. The enrichment method for isolation has been used in the present study in order to obtain specific bacteria amongst diverse natural population. However, a total of 25 different types of bacterial isolates were isolated from the collected effluent samples. To obtain pure culture, nutrient agar medium was used. The cultures were repeatedly streaked on nutrient agar medium and incubated at 30°C for 24 hrs. Pure culture of all five bacterial isolates was developed and categorized as C2, P4, M4, O3 and S3. These different bacteria were subjected to the primary tests, *i.e.*, Gram staining, oxidase, catalase, oxidative fermentative, motility, production of acid from glucose and growth under aerobic conditions, it was observed that two were Gram-negative rods, motile, oxidase and catalase positive, grew aerobically and were positive for production of acid from glucose. The isolates P4 & S3 were Gram-negative and short rod-shaped bacteria with a cell size approximately 1.2 µM in length and 0.85 µM in diameter under the microscope. The isolates C2 & O3 were Gram-positive and long rod-shaped bacteria with a spore forming under the microscope. The isolate M4 was Gram-positive and cocci-shaped bacterium. These isolates could grow at temperatures range of 25°C to 40 °C and a wide range of pH 5 to 9. The optimum growth was at the condition of 30 to 35°C and pH 7.0. Phenol-degrading enrichment cultures were readily established with all three sediments **(Hassanshahian *et al.*, 2012; Singh *et al.*, 2013)**. The five bacterial isolates are all able to use phenol as a sole source of carbon and energy under the current study. As the characteristics listed in Table 2 show, isolate C2 is quite similar to isolate O3, while isolate P4 and S3 are quite similar however, all the tested isolates can be distinguished from the other isolate M4 by a number of characteristics. For example, isolate M4 is more sensitive to phenol than the other isolates, initiating growth only at phenol concentrations below 1500 mg/L. The isolates were identified, two isolates were presumptive *Pseudomonas* sp. (P4 & S3), two isolates were *Bacillus* sp. (C2 & O3) and only one isolate might be *Micrococcus sp*. (M4) (Table 2). These isolates were further checked for growth on MSM-broth medium amended with different concentrations of phenol. Growth has been found to be negligible on applying higher concentration of phenol (2000 mg/L). This may be due to the sensitivity of these bacteria towards higher concentration of phenol, or they may require acclimatization on phenol prior to its degradation **(Abd-El-Haleem *et al.*, 2002)**. It has also been observed that with respect to time and acclimatization, the growth of bacteria appears in the phenol medium, indicating that the bacteria gradually adapt themselves to the compound. A large number of phenol tolerant bacterial and fungal species have been isolated from phenol contaminated sites **(Bhushan *et al.*, 2000; Sun *et al.*, 2012). Arutchelvan *et al.* (2005)** reported isolation of competent bacterial cells from the wastewater of industry manufacturing phenol–formaldehyde resins.

Phenol Biodegradation by *Bacillus* sp. C2 and *Pseudomonas* sp. P4

In the present study, a total of 5 higher phenol degrading bacterial isolates were isolated from various environmental samples. Bacterial isolate C2 and P4 showed maximum degradation of phenol within 72 hrs., of incubation followed by bacterial isolate S3, O3 and M4. Thus, isolates C2 and P4 was selected for further study as it exhibits the highest degradation of phenol. **Ghaima *et al.* (2017)** found that *Pseudomonas aeruginosa* KBM13 from contaminated soil with diesel fuel, and it was the most active in phenol degradation and efficient in removing 92 % of the initial concentration 500 mg/L phenol within 48 hrs., and had a tolerance of phenol concentration as high as 1400 mg/L. Thus, the phenol-degradation characteristics and optical density of *Bacillus* sp. C2 and *Pseudomonas* sp P4 at 1000 mg/L of phenol concentration were determined by monitoring phenol concentration and cell growth at OD_{600} periodically. The maximum cell growth and degradation of phenol were observed at pH 7, 35^0C and yeast extract as

Table 2. Morphological and biochemical characteristics of selected bacterial isolates

Characteristics	Bacterial isolates				
	C2	P4	O3	M4	S3
Colonial	Circular, white	Circular, white	Circular, white	Circular, white	White milk
Morphological					
Gram's reaction	+	-	+	+	-
Shape	Bacilli	Rod	Bacilli	Cocci	Rod
Spore staining	+	-	+	-	-
Motility test	+	+	+	-	+
Biochemical of					
Indole	-	-	-	-	-
Methyl red	-	-	-	-	-
Voges-Proskauer	-	-	-	-	+
Citrate utilization	+	-	+	-	+
Catalase	+	-	+	+	+
Oxidase	+	-	+	+	-
Nitrate reduction	+	-	+	-	+
Urease test	-	-	-	-	+
fermentation of Glucose	+	+	+	-	+
Lactose	+	+	+	-	+
Maltose	-	+	-		+
Sucrose	+	+	+	-	+

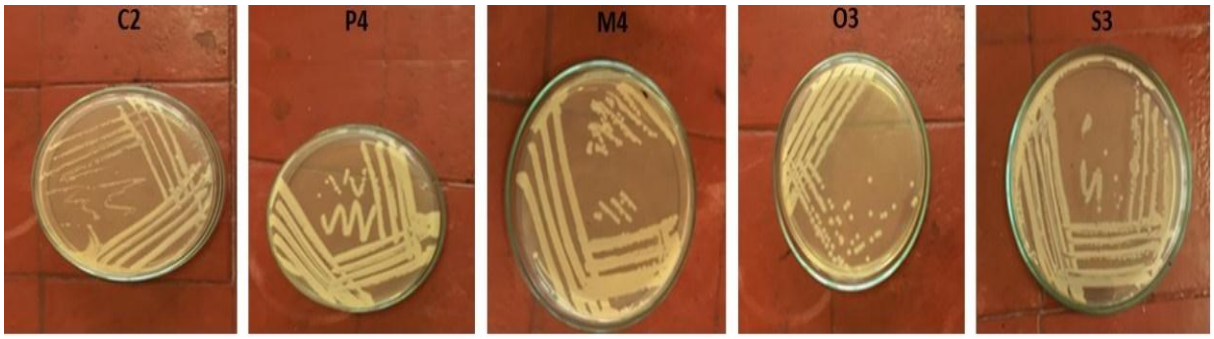

Fig. 1. Morphological growth of the selected bacterial on nutrient agar

source of nitrogen (Figs. 2, 5 and 7). An inhibitory effect showed that the bacterial growth and the degradation of phenol were declined at pH 6 and 8; 25 and 40 ^{0}C and $NaNO_3$and NH_4Cl as source of nitrogen. The removal rates of phenol were above 90% at the initial phenol concentration 1000 mg/L, yeast extract as source of nitrogen, at pH 7 and incubation temperature 35 ^{0}C. While there were no phenol-degradation by selected bacteria when the initial phenol concentration was higher than 2000 mg/L. The isolates could grow on phenol up to a concentration of 1500 mg/L with the low degradation rate. The effects of factors such pH values and temperature on the degradation were investigated **(**Figs. 3 and 4). The bacterial isolate could grow within a range of pH 6–8, and the degradation of phenol was ranged between 33-90 % in the range of pH 6–8. The optimum pH for phenol degradation was 7.0. These results showed that the bacterial growth of isolates C2 and P4, the degradation of phenol (about 40 to 90%) were favored at temperatures of 35°C (Fig. 3).

The cell growth and phenol degradation reached the maximal values at a temperature of 35°C, and tested parameter showed only a slight change when the temperature was changed to 35°C. On the contrary, the phenol degradation declined sharply when the temperature reached 40°C. Therefore, the optimal temperature for the growth of isolates C2 and P4 was 35°C. Temperature exerts an important regulatory influence on the rate of growth. The bacterial growth and degradation of phenol of isolates C2 and P4 are the maximal value at about 35°C by isolate P4 (90.33%). On the contrary, the phenol degradation declined sharply when the temperature reached 25°C and above 35 °C. It is believed that sudden exposure to temperatures higher than 35°C may have a detrimental effect on the bacterial enzymes that are usually responsible for the benzene ring cleavage, which is the main step in the biological degradation process. On the other hand, exposure to lower temperatures is expected to slow down the bacterial activity. Also, one of the studies demonstrated that the strain *Pseudomonas aeuriginosa* MTCC 4997 isolated from effluents collected from petrochemical industries exhibited a complete degradation of phenol at a wide temperature from 15°C to 45°C with an optimum of 37°C **(Kotresha and Vidyasagar, 2014). Ghaima *et al.* (2017)** found that the maximum phenol degradation of *P. aeruginosa* KBM13 was observed at 40˚C.

The effect of pH value on the growth of C2 and P4 was investigated. The bacterial isolates C2 and P4 grow within a range of pH 6–8 (Fig. 4). The results showed that the optimum growth of the isolate was observed at pH 7. The growth decreased as the pH decreased. At pH 8 the growth is less. The degradation rate of C2 and P4 at different pH values is shown in Fig. 5 and the degradation rate of phenol was the highest 91.2% by P4 isolate at only pH 7.0 when cultured for 72hrs., and the degradation of phenol at pH 6.0 was the least (33.0%). From the obtained result it was observed that pH of the medium has a significant effect on the growth of the C2 and P4 isolates and ultimately the degradation of the phenol. Also, the optimum degradation rate was at pH 7.0. Many previous studies indicated that the optimum pH for the growth and phenol degradation of *Pseudomonas* sp. was in the range 7-10 and it's dependent on the bacterial origin **(Sarnaik and Kanekar, 1995; Shahriari *et al.*, 2016)**. **Ghaima *et al.* (2017)** showed that the maximum rate of phenol degradation by *P. aeruginosa* was at pH 8. Studies have shown each strain shows the best phenol biodegradation at certain pH values. For instance, the best pH range for the biodegradation of phenol by *Klebsiella oxytoca* was 6.8 **(Khleifat, 2006)**. The effect of pH in phenol degradation which may be because of its effects on transportation, stimulating the enzymatic activities and nutrient solubility **(Lin *et al.*, 2010).** The pH significantly affects the biochemical reactions required for phenol degradation. Reports indicate that the pH affects the surface charge of the cells of the activated sludge biomass **(Aksu and Gonen, 2004)**.

The effect of three organic nitrogen sources; peptone, yeast extract, and urea, and three inorganic nitrogen sources namely, sodium nitrate, potassium nitrate, and ammonium chloride on the growth and degradation of phenol by C2 and P4 isolates was tested . The nitrogen sources were supplemented individually at 2% g/L to MS-medium containing 1000 mg/L phenol and incubated at 35°C in a temperature and pH 7 for 96 hrs. in trireplicates and the results are shown in Figs. 6 and 7. It was observed that the growth of C2 and P4 isolates and phenol degradation affected clearly by the type of nitrogen source used . Hence, among the organic nitrogen sources tested, yeast extract was the best source for maximum growth and phenol degradation by these isolates (C2 and P4).

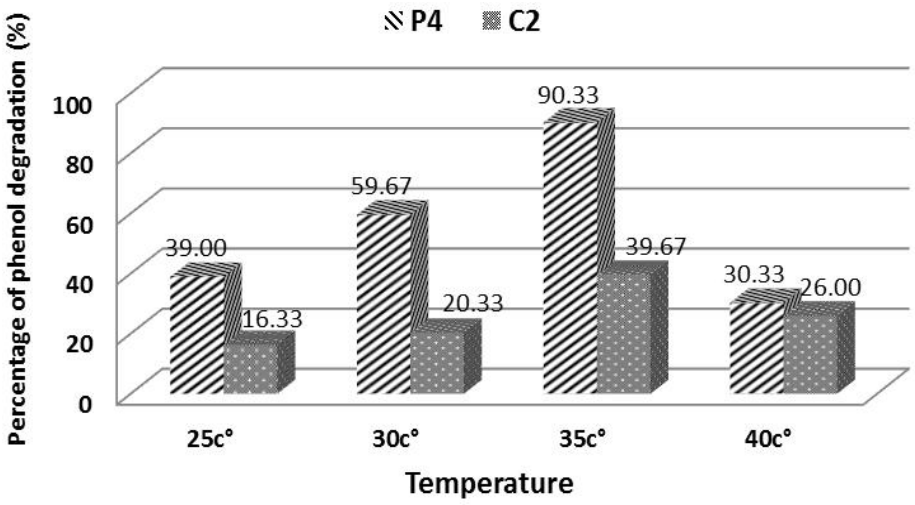

Fig. 2. Effect of temperature on phenol degradation by P4 and C2 isolates

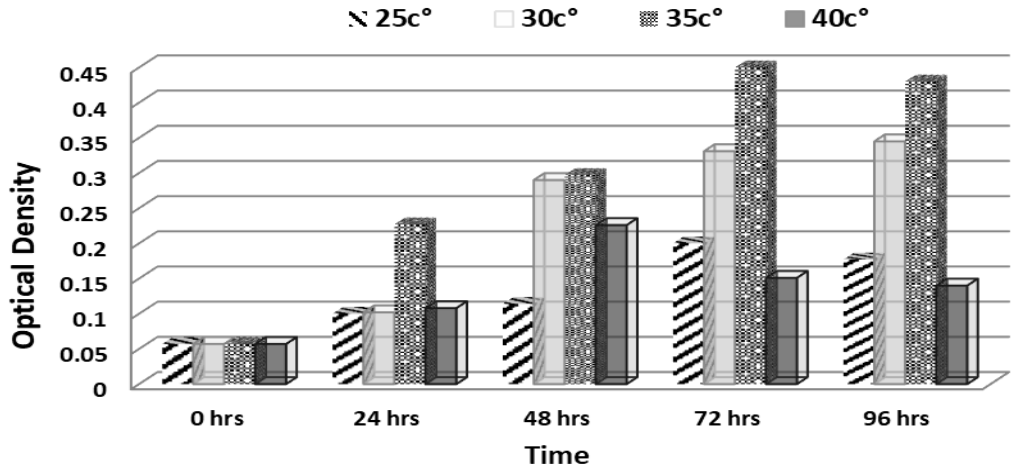

Fig. 3. Growth profile of P4 bacteria at different degrees of temperature

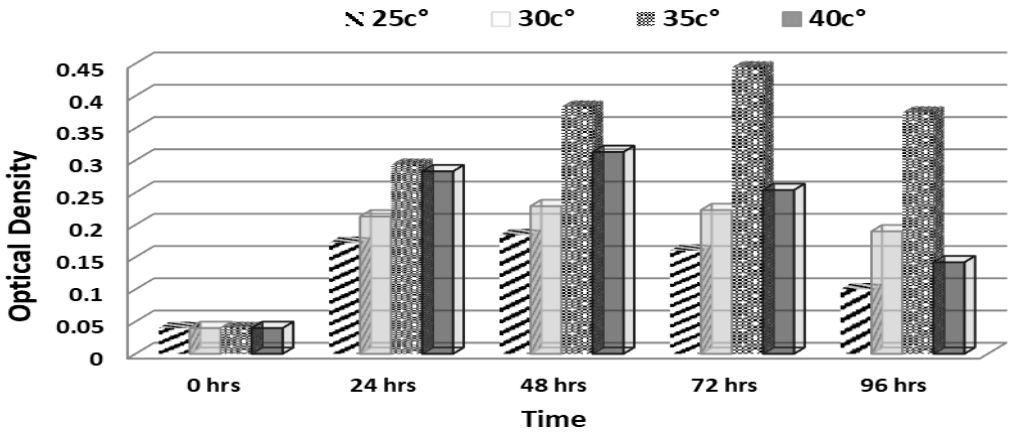

Fig. 4. Growht profiles of C2 bacteria at different degrees of temperature

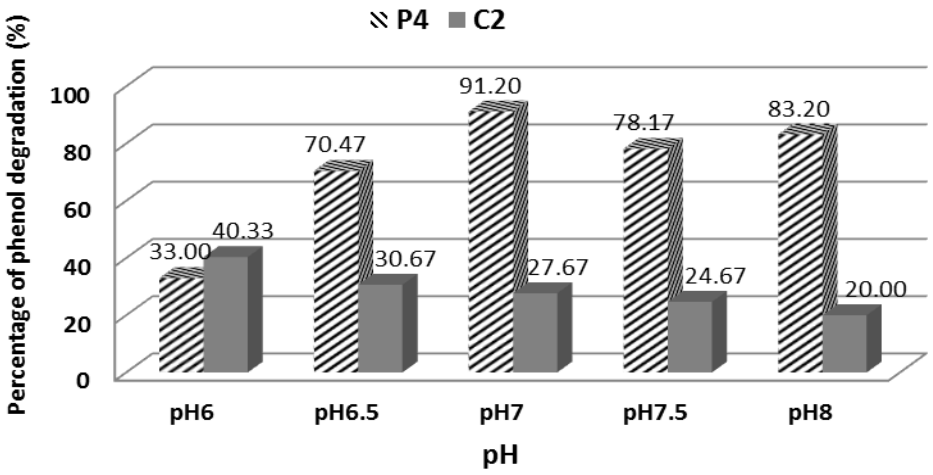

Fig. 5. Effect of different pH on phenol degradation by P4 and C2 isolates

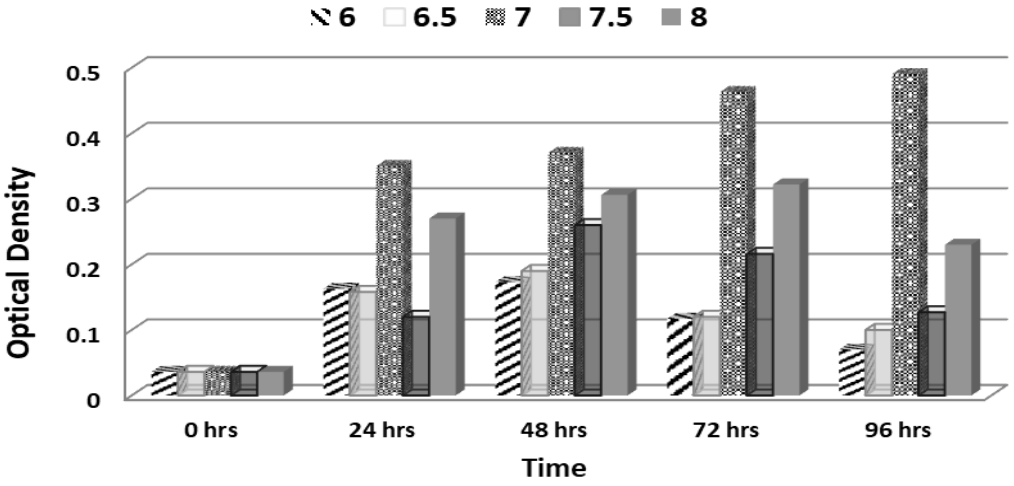

Fig. 6. Effect of different pH on growth of P4 isolate

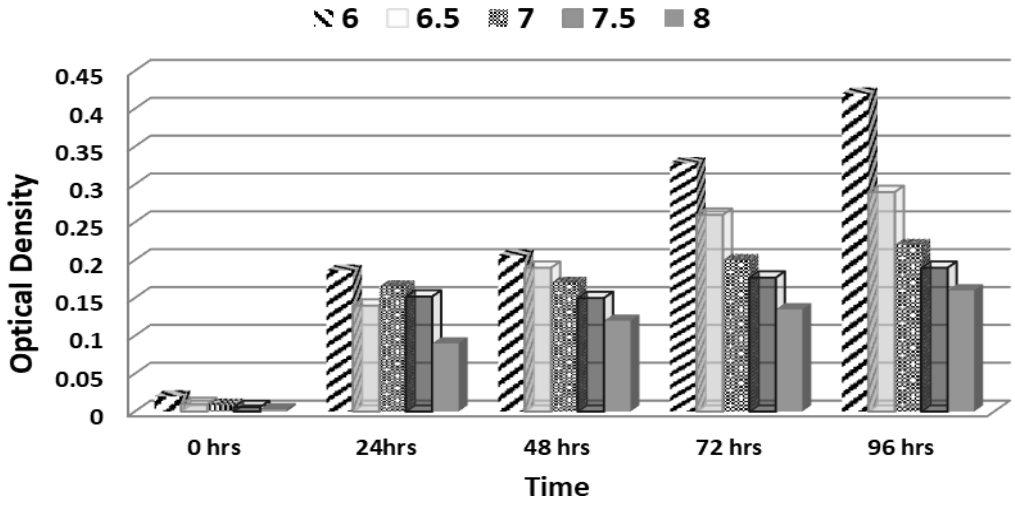

Fig. 7. Effect of different pH on growth of C2 isolate

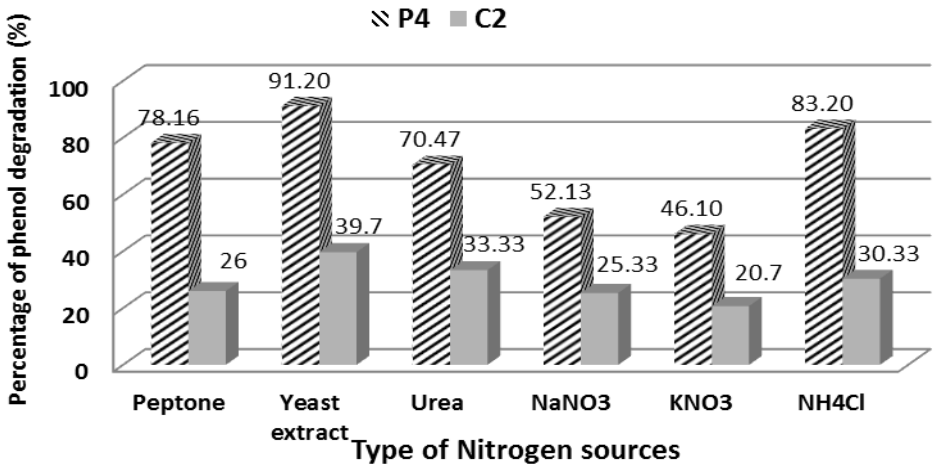

Fig. 9. Effect of nitrogen sources on phenol degradation by P4 and C2 isolates

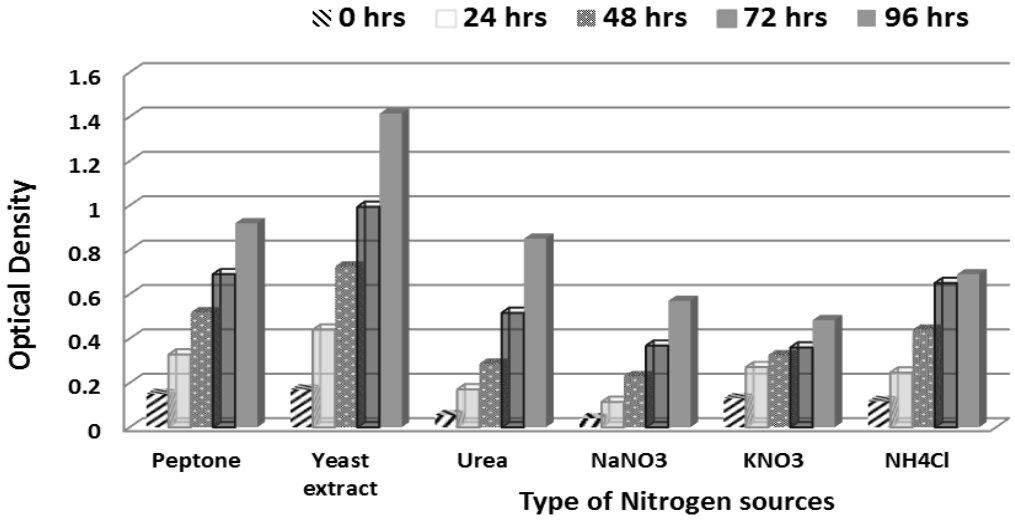

Fig. 9. Growth assessment of isolated P4 bactteria on the different nitogen source

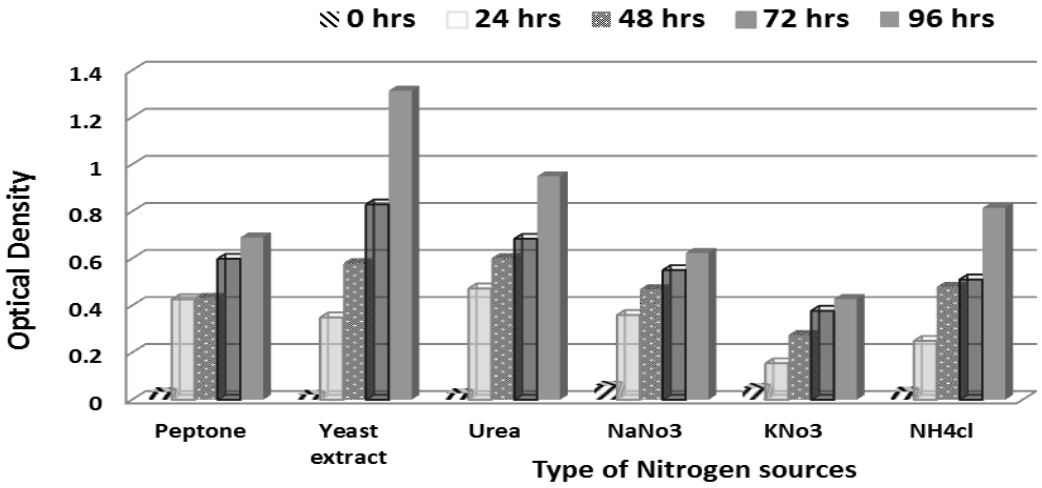

Fig. 10.Growth assessment of isolated C2 bacteria on the different nitrogen source

Conclusions

In conclusion, bacterial isolates capable of degrading phenol were isolated from an effluent of ceramic factories (C2), the effluent of petrochemical company (P4), the effluent of the paper mill (M4), effluent of crude oil (O3) and sewage water (S3) in EL-Sharkia Governorate, and they were identified as two isolates of *Pseudomonas Sp.* (P4 & S3), two isolates of *Bacillus Sp.* (C2 & O3) and only one isolate of *Micrococcus* sp. (M4) based on the morphological and biochemical analyses. These bacterial isolates have the ability to grow in a liquid medium with phenol at different concentrations as the sole carbon and energy source. The isolate P4 was able to degrade more than 90 % of the initial 1000 mg/L phenol and grow at the phenol concentration of as high as 1500 mg/L. The optimal growth conditions for phenol degradation of the strain were at 35°C and pH 7.0. Regarding that native microbial species were more adaptive than non-indigenous microorganisms in polluted environments, their predominance facilitated the bioremediation of the phenol-contaminated environments. *Pseudomonas* sp P4 isolated may be used for the bioremediation of the phenol-contaminated environments.

REFERENCES

Abd -El-Haleem, D., H. Moawad, E.A. Zaki and S. Zaki (2002). Molecular characterization of phenol- degrading bacteria isolated from different Egyptian ecosystems. Micro- Ecol., 43: 217–224.

Ahmad, S.A., N.A. Shamaan, N.M. Arif, G.B. Koon, M.Y.A. Shukor and M.A. Syed (2012). Enhanced phenol degradation by immobilized *Acinetobacter* sp. strain AQ5NOL 1. World, Microbiol. Biotechnol., 28:347–352.

Ahmad, N., I. Ahmed, A. Shahzad, N. Khalid, F. Mehboob, K. Ahad and G.M. Ali (2014). Molecular identification and characterization of *Pseudomonas sp*. NCCP-407 for phenol degradation isolated from industrial waste. J. Korean Soc. Appl. Biol. Chem., 57: 341-346.

Aksu, Z. and F. Gonen (2004). Biosorption of phenol by immobilized activated sludge in a continuous packed bed: prediction of breakthrough curves, Process Biochem., 39: 599–613.

APHA (2005). American Public Health Association. Standard Methods for the Examination of Water and Wastewater; American Public Health Association, Ame. Water Works Assoc. and Water Environ. Federation: Washington, DC, USA,

Arutchelvan, V., V. Kanakasabai, S. Nagarajan, and V. Muralikrishnan (2005). Isolation and identification of novel high strength phenol degrading bacterial strains from phenol – formaldehyde resin manufac-turing industrial wastewater. J. Hazard-ous Materials, 127: 238-243.

ATSDR (2003). Agency for toxic substances and disedse registry. Medical management guidelinesforphenolAvailabtefrom:http⁄⁄ www. astdr.cdc.gov/MHM./mmg115.html.

Banergee, A. and A.K. Ghoshal (2010). Phenol degradation by Bacillus cereus:Pathway and kinetic modeling. Bioresour. Technol., 101: 5501-507.

Basha, K.M., A. Rajendran and V. Thangavelu (2010). Recent advances in the biodegradation of phenol: A review. Asian J. Exp. Biol. Sci, 1:219- 234.

Bai, J., J. Wen, H. Li and Y. Jiang (2007). Kinetic modeling of growth and biodegradation of phenol and mcresol using alcaligenes faecalis. Proc Biochem., 42:510-517.

Bhattacharya, A; Gupta, A., Kaur, A., and Malik, D. (2018). mediation of phenol using microorganisms: Sustainable way to tackle the chemical pollution menace. Currt. Organic Chem., 22(4): 370–385.

Bhusan, B., A. Chauhan, S.S. Samanta and R.K. Jain (2000). Kinetics of biodegradation of p-nitrophenol. Biochem. Biophy Res. Commun., 274:626–630

Carmona, M., A.D. Lucas, J.L. Valverde, B. Velasco and J.F. Rodriguez (2006). Combined adsorption and ion exchange equilibrium of pgenol on Amberlite IRA-420 Chem Eng. J., 117: 155-160.

Deng, T., W. Hongyu and Y. Kai (2018). Phenol biodegradation by isolated *Citrobacter* strain under hypersaline conditions. Water Sci. Technol., 77 (1-2): 504-510.

El-Sayed, W.A., M.K. Ibrahim, M. Abu-Shady, F. El-Beih, N. Ohmura, H. Saiki and A. Ando (2003). Isolation and characterization of phenol-catabolizing bacteria from a coking plant. Biosci. Biotechnol. Biochem., 67 (9): 2062-2029.

Gami, A.A., M.Y. Shukor, K.A. Khalil, F.A. Dahalan, A. Khalid and S.A. Ahmad (2014). Phenol and its toxicity. J. Environ. Microbiol. Toxicol., 2: 11–24.

Geng, A., A. Soh, C. Lim and L. Loke (2006). Isolation and characterization of a phenol degrading bacterium from an industrial activated sludge. Appl. Microbiol. Biotechnol., 71: 728–735.

Ghaima, K.K., B.S. Raha and M.M. Mohamed (2017). Biodegradation of phenol by *Pseudomonas aeruginosa* isolated from soil contaminated with diesel fuel. Biosci. Res., 14 (4): 713-720.

Govindarajalu, K. (2003). Industrial effluent and health status a case study of noyyal river basin. Proc. 3rd Ed. Int. Conf. Environ. and Health, Chennai, India, 150-157.

Hassanshahian, M., G. Emtiazi and S. Cappello (2012). Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea, Marine Polletin, 64 (1): 7-12.

Holt, S.G., N.R. Kriey, P.H.A. Sneath, J.T. Staley and S.T. Williams (1994). Bergy's Manual of Determinative for Bacteriology; Williams and Wilkins: New York, NY, USA, 1998.

Hussain, A., P. Kumar and I. Mehrotra (2008). Treatment of phenolic wastewater in UASB reactor: effect of Nitrogen and Phosphorous. Biores. Technol., 99: 8497–8503.

Hussain, A., P. Kumar and I. Mehrotra (2010). Anaerobic treatment of phenolic wastewater: effect of phosphorous limitation. Desalination Water Treat., 20:189–196.

Hussain, A., T. Parveen, P. Kumar and I. Mehrotra (2009). Phenolic wastewater: effect of F/M on anaerobic degradation. Desalination Water Treat., 2:254–259.

Khleifat, K.M. (2006). Biodegradation of phenol by *Ewingella americana*: Effect of carbon starvation and some growth conditions. Process Bioch., 41(9):2010-2016.

Klibanov, A.M., B.N. Alberti, E.D. Morris and L.M. Felsin (1980). Enzymatic removal of toxic phenols and anilines from wastewaters. J. Appl. Biochem., 2: 414-421.

Kloss, W.E., D.N. Ballard, C.G. Geroge, J.A. Webster, R.J. Hubner and W. Ludwig (1998). Delimiting thegenus Staphylococcus through description of Macrococcus caseolyticus gen.nov., comb. Nov. and Macrococcus equiperciucus sp Nov. and Macrococcus bovicus sp. Macrococcus carouselicus sp. Nov. Int. J. Syst. Bacteriol., 48: 859-877.

Kotreshha, D. and G.M. Vidyasagar (2014). Degradation of phenol by novel strain Pseudomonas aeruginosa MTCC4997 isolated from petrochemical industrial effluent. Int. J. Microb. Res. Technol., 2 (3): 7-15.

Lanyl, B. (1987). Classical and rapid identification methods for medically important bacteria. Method. Microbiol., 19: 1–67.

Lazarova, Z. and S. Boyadzhieva (2004). Treatment of phenol- containing aqueous solutions by membrane based solvent extraction in coupled ultrafiltration modules. Chem. Eng. J., 100 : 129-138.

Lin, L.X., H. Liu and N.Y. Zhou (2010). MhbR, a LysR-type regulator involved in 3-hydroxybenzoate catabolism via gentisate in *Klebsiella pneumoniae* M5a1 Microbiol. Res., 165: 66-74.

Lin, S.H. and T.S. Chuang (1994). Combined treatment of phenolic wastewater by wet air oxidation and activated sludge. Toxicol. Environ. Chem., 44: 243–258.

Liu, Z., W. Xie, D. Li, Y. Peng, Z. Li and S. Liu (2016). Biodegradation of phenol by bacteria strain *Acinetobacter calcoaceticus* PA isolated from phenolic wastewater. Int. J.

Environ. Res. and Public Health, 13 (3): 300-308.

Loh, K.-C. and S.-S. Chua (2002). Ortho pathway of benzoate degradation in *Pseudomonas putida*: induction of meta pathway at high substrate concentrations. Enz. Microbial Technol., 30:620-626.

Lowry, M., A. Nagamani, K. Sreenivasulu and R. Soligalla (2009). Isolation and characterization of phenol degrading soil bacterium *Xanthobacter flavus*. Bioremed. J., 13: 1-6.

Mahiuddin, M., A.N.M. Fakhruddin and A. AlMahin. (2012).Degradation of phenol via meta cleavage pathway by pseudomonas fluorescens PUI.ISRN. Microbiol., 1: 1-6.

McCall, I.C., A. Betanzos, D.A. Weber, P. Nava, G.W Miller and C.A. Parkos (2009). Effects of phenol on barrier function of a human intestinal epithelial cell line correlate with altere tight junction protein localization. Toxicol. Appl. Pharmacol., 241: 61–70.

Mohite, B., R. Jalgaonwala, S. Pawar and A. Morankar (2010). Isolation and characterization of phenol degrading bacteria from oil contaminated soil. Inn Roman Food Biotechnol., 7: 61-65.

Nair, C.I., K. Jayachandran and S. Shashidhar (2008).Biodegradatio of phenol. Afr. J. Biotechnol., 7 (25): 4951-495.

Nuhoglu, N. and Yalcin, B. (2005). Modeling of phenol removal in a batch reactor. Process Biochem., 40 (3-4): 1233-1239.

Park, J.S., M.T. Brown and T. Han (2012); Phenol toxicity to the aquatic macrophyte *Lemna paucicostata*. Aquat. Toxicol., 106: 182–188.

Rehfuss, M. and J. Urban (2005). *Rhodococcus phenolicus* sp. nov., a novel *bioprocessor* isolated actinomycete with the ability to degrade chlorobenzene, dichlorobenzene and phenol as sole carbon sources. Syst. Appl. Microbiol., 28:695-701.

Sachan, P., S. Madan and A. Hussain (2019). Isolation and screening of phenol-degrading bacteria from pulp and paper mill effluent. Appl. Water Sci., 9: 100.

Saravanan, P., K. Pakshirajan and P. Saha (2008). Growth kinetics of an indigenous mixed microbial- consortium during phenol degradation in a batch reactor. Bioresource Technol., 99: 205–209.

Sarnaik, S. and P. Kanekar (1995). Bioremediation of color of methyl violet and phenol from dye industry waste effluent using *Pseudomonas* spp. Isolated from factory soil. Appl. Bacteriol., 79: 459-469.

Shahriari, M.M., N. Safaei and G.H. Ebrahimipour (2016). Optimization of phenol biodegradation by efficient bacteria isolated from petrochemical effluents. Global J. Environ. Sci. Manag., 2 (3): 249-256.

Singh, A., V. Kumar and J.N. Srivastava (2013). Assessment of bioremediation of oil and phenol contents in refinery waste water via bacterial consortium. Pet. Environ. Biotechnol., 4 (3):1–4.

Sun, J.Q., L. Xu, Y.Q. Tang, F.M. Chen and X.L. Wu (2012). Simultaneous degradation of phenol and n-hexadecane by *Acinetobacter* strains. Bioresource Technol., 123: 664–668.

Tay, S.L., B.P. Moy, A. Maszenan and J.H. Tay (2005). Comparing activated sludge and aerobic granules as microbial inocula for phenol biodegradation. Appl. Microbiol. Biotechnol., 67: 708– 713.

Tuah, P.M., N.A.A. Rashid and M.M. Salleh (2009). Degradation pathway of phenol through ortho-cleavage by *Candida tropicalis* Retl-Cr1. Borneo Sci., 24:1-8.

Whiteley, A. and J. Mark (2000). Bacterial Community structure and physiological state within an industrial phenol bioremediation system. Appl. Environ. Microbiol., 66: 2401–2407.

Whiteley, A.S., S. Wiles, K. Lilley, J. Philip and M.J. Babailey (2001). Ecological and physiological analyses of Pseudomonad species within a phenol remediation system. J. Microbiol. Methods, 44:79–88.

Wu, Z.G., Y.M. Wang, Z.H. Xing, X.W. Wu and S.S. Song (2005). Study on degrading phenol by immobilized *Ralstonia metallidurans* CH34. Microbiol., 32: 31–36.

Yan, J., W. Jianping, B. Jing, W. Daoquan and H. Zongding (2006). Phenol biodegradation by the yeast *Candida tropicalis* in the presence of m-cresol. Biochem. Eng. J., 29: 227–234.

Yang, C.F. and C.M. Lee (2007). Enrichment, isolation, and characterization of phenol-degrading *Pseudo-monas resinovorans* strain P-1 and *Brevibacillus* sp. strain P-6. Int. Biodeterioration and Biodegradation, 59: 206-210.

Zhai, Z., H. Wang, S. Yan and J. Yao (2012). Biodegradation of phenol at high concentration by a novel bacterium: *Gulosibacter sp*. YZ4. J. Chem. Technol. Biotechnol., 87: 105-111.

عزل وتوصيف البكتيريا المحللة للفينول من مياه الصرف الصناعي ومخلفات الصـــرف الصحى

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فى هذه الدراسة تم عزل وتوصيف البكتيريا المحللة للفينول ،حيث تم عزل خمس عزلات تسمى C2 وP4وO3 و M4 وS3 ، من النفايات السائلة لمصانع السيراميك (C2) ، ومخلفات البتروكيماويات (P4) ، والنفايات السائلة لمصنع الورق (M4)، ومخلفات النفط الخام (O3) ومياه الصرف الصحي.(S3) تم التفريق بين هذه الخمس عزلات بالاعتماد على الخصائص المورفولوجية والفسيولوجية والبيوكيمائية ، وتم تصنيف العزلات الخمسةوكانت عزلة واحدة فقط على أنها بكتيريا موجبة لصبغة جرام ، هوائية وغير متحركة ، وهي على شكل مكورات ، وقد تكون (M4) .*Micrococcus* sp ، تم وصف اثنين من العزلات (*Pseudomonas* sp.: P4& S3) على أنها بكتيريا سالبة لجرام ، هوائية اجبارا ، متحركة على شكل عصوي قصير ، وعزلتان (*Bacillus* sp.: C2 & O3) وصفت بأنها بكتريا موجبة الجرام هوائية ، متحركة وعصوية طويلة الشكل. كانت العزلات البكتيرية الخمس قادرة على استخدام الفينول كمصدر وحيد للكربون والطاقة. تم فحص هذه العزلات على وسط منخفض من المغذيات وبمعدّل تركيزات مختلفة من الفينول بواسطة تقنية القارورة المهتزه. تم فحص العزلات البكتيرية الخمسة المتحملة لتركيزات عالية من الفينول واتضحت قدرتها على النمو وتحلل الفينول ومن بين العزلات الخمسة الأعلى تحلل للفينول، كانت العزلة P4 فعالة في إزالة أكثر من 90% من 1000 مجم/ لتر من الفينول في بداية التجربة خلال 48 ساعة، وكان تحمل تركيز الفينول يصل إلى 1500 ملغم/لتر، أشارت هذه النتائج إلى أن *Pseudomonas* sp تمتلك إمكانات واعدة في معالجة مياه الصرف الصحي ذات التركيزات المرتفعة من المركبات الفينولية.

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