RESEARCH ARTICLE

Biodegradation of Phenol by *Bacillus simplex*: Characterization and Kinetics Study

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Abstract: Phenol is one of the main pollutants that have a serious impact on the environment and can even be very critical to human health. The biodegradation of phenol can be considered an increasingly important pollution control process. In this study, the degradation of phenol by *Bacillus simplex* was investigated for the first time under different growth conditions. Six different initial concentrations of phenol were used as the primary substrate. Culture conditions had an important effect on these cells' ability to biodegrade phenol. The best growth of this organism and its highest biodegradation level of phenol were noticed at pH 7, temperature 28°C, and periods of 36 and 96 h, respectively. The GC-MS analysis of the bacterial culture sample revealed that further degradation of the catechol by 1,2-dioxygenase produce a *cis*, *cis*-mucconic acid via *ortho*-pathway and/or by 2,3-dioxygenase into 2-hydroxymucconic semialdehyde via *meta*-pathway. The highest biodegradation rate was perceived at 700 mg/L initial phenol concentration. Approximately 90% of the phenol (700 mg / L) was removed in less than 96 hours of incubation time. It was found that the Haldane model best fitted the relationship between the specific growth rate and the initial phenol concentration, whereas the phenol biodegradation profiles with time could be adequately described by the modified Gompertz model. The obtained parameters from the Haldane equation are: 1.05 h^{-1}, 9.14 ppm, and 329 ppm for Haldane's maximum specific growth rate, the half-saturation coefficient, and the Haldane's growth kinetics inhibition coefficient, respectively. The Haldane equation fitted the experimental data by minimizing the sum of squared error (SSR) to 1.36×10^{-3}.
Keywords: Bacillus, biodegradation, phenol, kinetics

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1 Introduction

All living things need to use chemicals as sources of carbon or energy. However, over the centuries, the adaptation of cells to consume these natural biochemicals on earth is the generally accepted narrative, but man is responsible for the environmental problem through the production of various organic species that are resistant or inability to mineralize by living organisms, especially microorganisms. Phenol is one of the main pollutants that have a serious impact on the environment and can even be very critical to human health. Phenol compounds and their substituting analogues are common starting materials. These are the waste products of processing industrial and agricultural materials (Khleifat et al., 2006; Wen et al., 2020). Phenol is toxic to some

aquatic organisms at concentrations of mg/liter and led to problems in drinking water, even at lower concentrations such as taste and odor (Khleifat, 2007a; Younis et al., 2020). Therefore, phenolic compounds due to their potential toxicity were included in the list of priority pollutants by the United States Environmental Protection Agency (US EPA) (Aisami et al., 2020). Direct inhalation and skin ingestion of phenol may lead to death orally due to penetration into the skin and rapid absorption, in addition to being a carcinogen (Abboud et al., 2007; Khleifat et al., 2006; Wen et al., 2020; Sewidan et al., 2020).

Abundant processes as chemical or physical techniques have been used for phenol removal such as chemical oxidation, ion exchange; liquid-liquid extraction, activated carbon adsorption, incineration and abiotic treatment procedure bear serious drawbacks such as their need for large efforts costly and the production of unpredictable harmful byproducts. Besides, most of these processes do not degrade phenol, but rather transform it into other compounds (secondary pollutants), which can be more dangerous by-products. On the other hand, removing phenol using live cells (biodegradation) is an alternative option and is more cost-effective and environmentally friendly. Thus, the biodegradation of phenol can be considered an increasingly important pollution control process (Abboud et al., 2010; El-Naas et al., 2010; Khleifat and Abboud, 2003; Khleifat et al., 2008; Liu et al., 2009). Biodegradation is generally considered more favorable because of reduced costs and complete mineralization. There is much debate regarding whether possible or not to use natural or genetically engineered microorganisms (GEM) in the biodegrading processes. Government agencies often unfavorable to unleash GEMs environmentally due to the possibility of unforeseen environmental impacts (Khleifat et al., 2010; Khleifat et al., 2008; Liu et al., 2009). There is still substantial interest in isolation of bacteria that are able to use high concentrations of aromatic compounds as growth substrate, (Khleifat, 2007a), as the phenol compound that has been studied here. It has been stated that the degradation mode of catechol, the availability of carbon and nitrogen sources, the toxins presence and some physical parameters such temperature may affect bacterial growth using phenol as sole carbon and energy sources (El-Naas et al., 2010).

Many plant-friendly bacteria can be utilized in agricultural crop production (Vílchez et al., 2016; Vilchez and Manzanera, 2011). The features of these microorganisms can be applied to fortify plants in various ways, such as maximizing nutrient bioavailability and bio-uptake, controlling pathogens, building materials that support plant growth and curbing the increase of harmful particles in the soil such as harmful compounds that can disrupt plant growth. Factory (Adesemoye et al., 2008; Imran et al., 2014). Among these microorganisms, bacteria, called plant growth-promoting bacteria (PGPB), are an important alternative source of both organic and inorganic fertilizers and at the same time as pesticides for genetically modified plants and pests. PGPB can play a role in mitigating the effects of unfavorable environmental stresses in soil. An example of such unfavorable conditions are high concentrations of salts and environmental pollutants such as heavy metals and some other organic or inorganic compounds as well as dehydration (Nadeem et al., 2010; Vílchez et al., 2016). Thus, it is possible that PGPB could acts as a catalyst promoting the restoration of farmland that was not suitable for forage or forage cultivation.

In contrast, utilization of phenol or other aromatic compounds by *B. simplex* as growth substrates has not been reported. The bacterium, *B. simplex* used in the current study was isolated from the Al-Ghweiler Agricultural Station, Karak, Jordan. It was identified by means of 16S rRNA techniques. The nucleotide accession number in GenBank was MN083287. It is Gram-positive, motile, non-spore-forming rods (Rai et al., 2021; Schwartz et al., 2013). It was found to be positive for nitrogen fixation and phosphate solubilisation. In this study, the degradation of phenol by *B. simplex* was investigated for the first time under different growth conditions, including substrate concentration, pH and incubation temperature. In addition, mathematical modeling was used for studying the kinetics of bacterial growth on phenol besides the biodegradation kinetics of phenol by the same bacteria.

2 Materials and Methods

2.1 Bacterial strain

The bacterium used in the current study was *B. simplex* strain, which was isolated from the Al-Ghweiler Agricultural Station, Al-Karak, Jordan. It was identified by using 16S rRNA techniques through Anna Rosa Sprocati, Rome, Italy. The nucleotide accession number in GenBank was (MN083287). The morphological characteristics were always being microscopically checked and its biochemical identity was reverified using the REMEL kit (RapIDONE and RapID NF plus systems) procedure.

2.2 Plant Growth-Promoting Characteristics

Isolate of *R. nepotum* was tested for ability to produce indole-3-acetic acid (IAA), using a positive reaction to Salkowski's reagent with pink or red development as an indicator. Salkowski's reagent in contact with IAA forms a pink complex; a positive reaction indicates the ability of bacteria to metabolize L-tryptophan into IAA or into similar compounds (Patten and Glick, 2002). IAA is a carboxylic acid belonging to the group of auxins, which are known to promote plant growth by increasing branching and root development. The solubilization of phosphate was estimated using Pikovskaya (PKO) medium for inorganic phosphorous at 30°C and monitored every day for 7 days. When a transparent halo appears around the colonies, it indicates the ability to solubilize phosphate. The production of siderophore was uncovered using chrome azurol S (CAS) agar plates, and the change of color of surroundings of colonies from blue

to orange denotes the presence of siderophores (Nithyapriya et al., 2021). The ability of *R. nepotum* to fix N was tested using a nitrogen-free media. An overnight grown *R. nepotum* cultures in Trypticase Soy Broth (TSB) medium was resuspended in phosphate-buffered saline (PBS), pH 7.4, after washing twice. Bacterial concentration was acclimatized for OD_{600} of 0.5. 10 ml vials harboring 4 ml of NFb medium (semi-solid New Fabian broth) (Cueva-Yesquén et al., 2020; Al-kafaween et al., 2020) was inoculated with a bacterial suspension (30 μl) and incubated at 28°C (Mohammad et al., 2020; Cueva-Yesquén et al., 2020). The bacterial growth was proven by making a sub-surface pellicle after 72 h of incubation on the culture medium. Consecutive re-growing in NFb medium evidenced the potential of nitrogen fixation. In all cases, the experiments were performed in triplicate.

2.3 Preparation of mineral medium with phenol

In order, to prepare this medium; three solutions were prepared separately: (a) Phenol solution was prepared by dissolving 5 g in 200 ml de-ionized water (the final concentration was 25,000 ppm). To avoid any possible temperature effect on phenol, this solution was filter sterilized. (b) Mineral media; It was prepared by dissolve the following ingredients in 1000 ml Erlenmeyer flask: 1.0 g K_2HPO_4, 1.0 g NH_4NO_3, 0.5 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4$, 0.5 g KH_2PO_4, 0.5 g NaCl, 0.02 g $CaCl_2$, 0.02 g $FeSO_4$. The media was then distributed in 125 ml Erlenmeyer flasks containing the proper volume of mineral medium according to the phenol concentration needed and then autoclaving was made. (c) Wolfe's mineral solution, it was prepared by dissolving 1.5 g of nitrilotriacetic acid in 500 ml deionised water in 1000 ml Erlenmeyer flask, the pH was adjusted by KOH to 6.5 to achieve the best solubility. Then the followings were added: 3 g $MgSO_4$·$7H_2O$, 1 g NaCl, 0.5 g $MnSO_4$·H_2O, 1 g $FeSO_4$·$7H_2O$, 0.1 g $CoCl_2$·$6H_2O$, 0.1 g $CaCl_2$, 0.1 g $ZnSO_4$·$7H_2O$, 0.01 g $CuSO_4$·$5H_2O$, 0.01 g $AlK(SO_4)_2$·$12H_2O$, 0.01 g H_3BO_3, 0.01 g Na_2MoO_4·$2H_2O$. The volume was then completed to the mark with distilled water and shake vigorously followed by sterilization using filter. Finally, the mineral media with phenol was prepared by adding 0.5 ml Wolfe's and proper phenol volume to each flask based on the wanted phenol concentration. For example, to achieve mineral media with 200 ppm phenol concentration, 0.5 ml wolfe's solution and 0.4 ml phenol solution were added to the 49.1 ml mineral media. Moreover, for the 700 ppm; 0.5 ml Wolfe's solution and 1.4 ml phenol solution were added to the 48.1 ml mineral media.

2.4 Growth assessment and Phenol degradation

B. simplex ability to grow on phenol (as a sole of carbon source) was studied by growing *B. simplex* in TSB to the middle of exponential phase (OD at 600 nm $\approx$ 0.50). The bacterial cells were harvested at 4000 rpm for 15 min by centrifuge. The OD was adjusted to 0.2 at 600 nm to be used later as an inoculum by washing and suspend the cells with mineral media. Mineral media with 700 ppm phenol concentration was prepared and inoculated with *B. simplex*. The bacterial growth behavior was monitored spectrophotometry at 600 nm for 12, 24, 36, 48, 60, 72, 84, and 96 hours. Same procedure was carried out for the control (mineral media with 700 ppm phenol and without bacterial inoculation). The average degradation rates of phenol were quantified by dividing the amount of phenol that was diminished from the culture media for 24 hours, because during this time period, much of cells stopped from further deterioration of phenol, or this exemplified the elapsed time for all the experiments that were performed. The average degradation was calculated by this method as suggested by Loh and Wang (1997) to avoid any possible wrong procedure resulting from the difference in the lengths of the lag phases and thus the difficulty of ascertaining the times needed to complete the degradation or even when the degradation stop (Loh and Wang, 1997).

2.5 Phenol assay procedure

The 4-aminoantipyrine colorimetric method was used to determine phenol concentration (Der and Humphrey, 1975). Ammonium hydroxide (0.5 N) was supplemented and stirred well. The adjustment of pH 7.9 $\pm$ 0.1 was by made by adding potassium phosphate buffer, pH of 6.8 pursued by adding 2 % (w/v) 4-aminoantipyrine and 8 % (w/v) potassium ferricyanide. Specimens were left for 15 min at room temperature to achieve the completion of reaction and measuring the absorbance at 510 nm was conducted by using single beam of UV-Vis Spectrophotometer. Standard curve was calibrated to elicit the concentration of phenol using congruent recorded absorbance through using spectrophotometer.

2.6 Effect of pH and temperature on phenol degradation

The ability of *B. simplex* to degrade phenol was examined in 700 ppm phenol concentration which, prepared in mineral media *B. simplex* were cultured under different parameters using shaking incubator in the mineral media. Different pH (5.5, 7, 8, and 9) was used to assess the effect of pH on the degradation of phenol by *B. simplex*. Moreover, the effect of different temperatures (25, 28, 33 and 37°C) upon phenol degradation by *B. simplex* was investigated.

2.7 Mathematical Modeling

The logistic equation is widely used to describe the growth profile of biomass in a batch system with both exponential and stationary phases. The differential form of the logistic equation is given as follows:

$$\frac{dX}{dt} = \mu_m X \left(1 - \frac{X}{X_m}\right) \quad (1)$$

where μ_m is the maximum specific growth rate (hr^{-1}) in a given environment and X_m is the maximum cell concentration achievable in that environment (OD_{600}). An integration of the logistic equation yields the following equation for the cell concentration:

$$X = \frac{X_o e^{\mu_m t}}{1 - \left(\frac{X_o}{X_m}\right)(1 - e^{\mu_m t})} \quad (2)$$

where X_o is the initial cell concentration (OD_{600}).

Several kinetics models have been developed to describe the relationship between the specific growth rate μ and substrate concentration S. At high concentrations of substrate, and in case that the growth is affected by inhibitor concentration, Haldane equation is the most widely used inhibition expression:

$$\mu = \frac{\mu_{\max} S}{K_S + S + \frac{S^2}{K_I}} \quad (3)$$

where $\mu_{\max}$ is Haldane's maximum specific growth rate (hr^{-1}), K_I is Haldane's growth kinetics inhibition coefficient (ppm) and K_S is the half saturation coefficient (ppm). The experimental biomass growth profiles at different initial phenol concentrations were fitted to the logistic equation by means of nonlinear regression technique. Minimization of the sum of squared error (SSR) by using the Solver add-in in Microsoft Excel 2007 were carried out to obtain the model fitting parameters. Figure 1 shows the logistic model profiles along with the experimental data at different initial phenol concentrations.

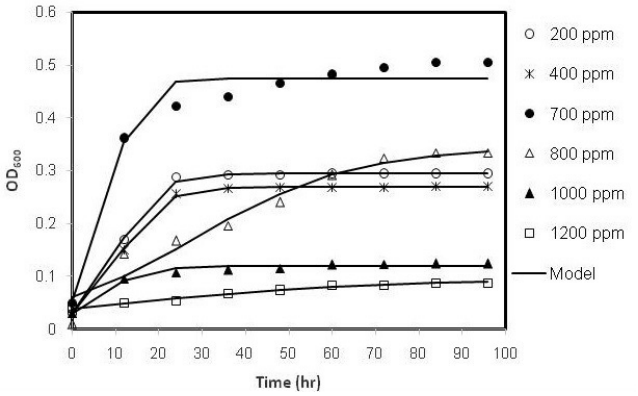

Figure 1. Growth profile of the isolate *B. simplex* different initial concentrations of phenol.

3 Results and Discussions

3.1 Characterization of PGPB

Bacillus simplex was investigated for four growth-promoting features, which were, phosphate solubilizing ability, synthesis of indole-acetic-acid, nitrogen fixation, and siderophore production. It was characterized by having three of four features studied, including nitrogen fixation, siderophore production and phosphate solubilisation (Table 1). Nitrogen-fixing reaction regulated by nitrogenase enzyme, which depends in its formation on the *nif* gene (Dobereiner et al., 1976; Mirza and Rodrigues, 2012; Vílchez et al., 2016; Vilchez and Manzanera, 2011). Rhizosphere microorganisms produce siderophores, which promoting plant growth by sequestering Fe, and increasing its availability roots zone (Nithyapriya et al., 2021). Siderophores are a small molecule assemble rapidly through short and well-defined metabolic pathways and include side chains and functional groups that give the ligands a high affinity for ferric ion (Fe^{3+}) and chelate it by forming multiple bonds with the ferric ion. Siderophores classified according to their chemical nature of the iron binding sites based on the competition for Fe between the ferric complex of the CAS and the siderophores produced by the bacteria. The high affinity of the siderophores for Fe^{3+}, removes the iron from the CAS and makes it turn from blue to yellow-orange. The CAS medium was prepared without nutrients and poured directly onto the bacteria growth plates (TSA) according to Prez-Miranda (Pérez et al., 2007) and Schwyn and Neilands (Schwyn and Neilands, 1987).

Many essential nutrients including phosphorus are present in the soil in an unavailable form. Therefore, it is important to elevate the mobility of phosphorus in the soil for plant growth. According to the Nautiyal protocol (1999), the isolated strains were tested for their ability to mobilize phosphorus by using agar and modified Pikovskaya medium (PVK) with the addition of bromo phenol blue (BPB), which emphasizes and makes the formation more visible ring. Ef-

Table 1. Plant Growth Promoting traits of the isolate *Bacillus simplex*

Phylogenetic affiliation with similarity(16S rDNA sequencing)	GENBANK ACCESSION NUMBER	Plant Growth Promoting traits			
		Nitrogen fixation	Siderophore production	Phosphate solubilisation	Auxin production
Bacillus simplex 99%	MN083287	+	++	+/-	-

ficiency of phosphorus mobilization by bacterial strains are evaluated by selecting bacteria producing clear areas on the agar plate due to the production and release of organic acids (Nautiyal, 1999).

Plant growth-promoting bacteria (PGPB) could be used as an alternative to organic and inorganic fertilizers, pesticides and genetically modified plants. These bacteria are an important alternative source of both organic and inorganic fertilizers and at the same time as pesticides for genetically modified plants and pests. PGPB can play a role in mitigating the effects of unfavorable environmental stresses in the soil. An example of these unfavorable conditions is high concentrations of salts and environmental pollutants such as heavy metals and some other inorganic or organic compounds in addition to desiccation (Nadeem et al., 2010; Vílchez et al., 2016). Thus, it is possible that PGPB acts as a catalyst in restoring agricultural lands that were not suitable for cultivation with fodder or nourishment.

3.2 Biodegradation of phenol versus growth of *B. simplex*

The main method for removing phenols from the environment include its biotransformation by bacteria in water and soil (Krijgsheld and Van der Gen, 1986). The mainstream emphasis on investigation using pure bacterial cultures, with fewer studies dealing with plant growth promoting bacteria (Orhan, 2016). In the current investigation, phenol degrading bacterium *B. simplex* is isolated from Al-Ghweiler Agricultural Station, Jordan and used for the biodegradation of phenol. It was identified by 16S rDNA. To guarantee the using of phenol as carbon and energy sources for this bacterium, the phenol minimal medium was implied via having this compound as only carbon and energy sources. Thus the formation of any bacterial biomass will be a function of exhaustion of these substrates (Khleifat, 2007a, 2007b; Khleifat et al., 2006; Khleifat et al., 2006; Khleifat et al., 2006; Loh and Wang, 1997). Two different negative controls were used to test the biodegradability of phenol, the uninoculated phenol-containing culture and the heat-killed suspensions. There was no biodegradation activity shown verifying the biodegrading activity made by *B. simplex*. Our results showed that *B. simplex* could use phenol compound as carbon and energy sources with different extents (Table S1).

3.3 Growth Kinetics of the Isolated Strain for Phenol Biodegradation

Six different concentrations of phenol were used as the primary substrate (Figure 1). The experimental biomass growth profiles at different initial phenol concentrations were fitted to the logistic equation by means of nonlinear regression technique. Minimization of the sum of squared error (SSR) by using the Solver add-in in Microsoft Excel 2007 were carried out to obtain the model fitting parameters. Figure 1 shows the logistic model profiles along with the experimental data at different initial phenol concentrations. It can be noticed that at initial phenol concentration of 700 ppm, the biomass growth approached the maximum stationary population size. On the other hand, at initial phenol concentration of 200 ppm, the biomass possessed the highest specific growth rate (μ_m). At initial phenol concentration of 1200 ppm, biomass growth was characterized by slow growth rate and low stationary population size (Table 2).

Table 2. Logistic growth kinetic parameters for phenol biodegradation by *B. simplex*

Phenol Concentration (ppm)	μ_m (hr^{-1})	X_m (OD_{600})	SSR
200	0.212	0.294	$1.06x10^{-4}$
400	0.195	0.270	$4.44x10^{-5}$
700	0.267	0.475	$5.72x10^{-3}$
800	0.054	0.344	$5.05x10^{-3}$
1000	0.193	0.119	$2.19x10^{-4}$
1200	0.035	0.094	$4.23x10^{-5}$

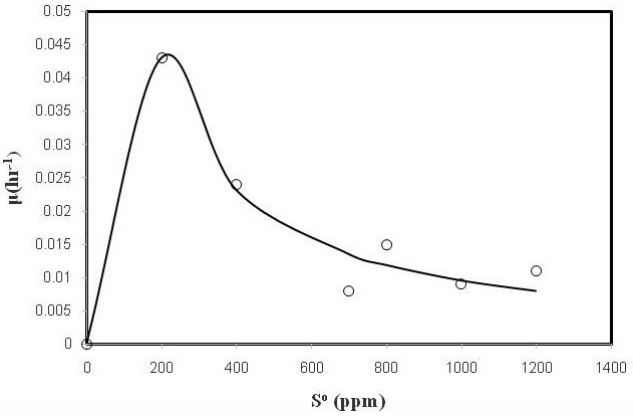

Figure 2. Haldane growth kinetic model fitted to experimental batch growth data of the strain *B. simplex*.

The determination of the specific growth rate (μ) for the different initial phenol concentrations were obtained by plotting the biomass growth data during the exponential phase. The specific growth rate (μ) equals the slope obtained by plotting $\frac{\ln X}{X_o}$ versus time. Haldane equation gave an excellent representation of the relationship between the specific growth rate and the initial phenol concentration (Figure 2). The estimation of Haldane's parameters required the use of a non-linear regression technique based on minimization of SSR. The obtained parameters from the Haldane equation are: 1.05 h^{-1}, 9.14 ppm, and 329 ppm for Haldane's maximum specific growth rate, the half-saturation coefficient, and the Haldane's growth kinetics inhibition coefficient, respectively (Table 3). The Haldane equation fitted the experimental data with SSR of 1.36×10^{-3}. Figure 2 shows a typical trend for the growth kinetics data of an inhibitory substrate. The results indicate that the value of the specific growth rate increases

Table 3. Haldane's model parameters for the biodegradation of phenol by different bacteria

Microbial Strain	S_o (ppm)	μ_{max} (hr-1)	K_s (ppm)	K_I (ppm)	Reference
B. simplex	200-1200	1.05	9.14	329	This study
Pseudomonas putida	300-1000	0.031	63.9	450	Bakhshi et al., 2011
Pseudomonas sp.	100-800	0.464	113.5	376.7	Saravanan et al., 2011
Mixed	0-800	0.26	25.4	173	Pawlowsky and Howell, 1973
Acinetobacter calcoaceticus	60-500	0.542	36.2	145	Kumaran and Paruchuri, 1997
Pseudomonas WUST-C1	0-1600	2.5	48.7	100.6	Liu et al., 2013
Pseudomonas putida	25-800	0.9	6.93	284.3	Wang and Loh, 1999

with increasing phenol concentration up to approximately 200 ppm, then it starts decreasing with the increase in the initial phenol concentration. The Haldane's maximum specific growth rate reported in this study is comparable to those values reported in literature for phenol biodegradation in several bacterial media. The half-saturation coefficient (9.14 ppm) as obtained by the Haldane model indicates that phenol concentration must be low to get the specific growth rate equals half the maximum specific growth rate. The Haldane's growth kinetics inhibition coefficient represents a measure of biomass sensitivity to inhibition by inhibitory substances. The value of inhibition constant (329 ppm) indicates that phenol has an appreciated inhibition effect on biomass growth. Phenol biodegradation profiles were fitted very well into the modified Gombertz model (Wen et al., 2020).

$$S = S_o \left\{ 1 - exp\left\{ -exp\left[\frac{R_m}{S_o} e\,(\lambda - t) + 1 \right] \right\} \right\} \quad (4)$$

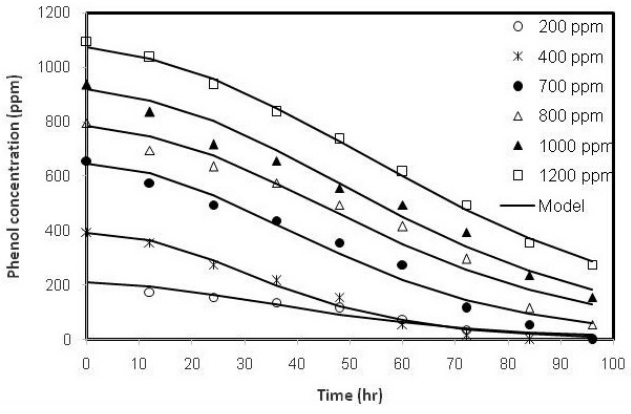

Figure 3. Fitting of *B. simplex* growth at different concentrations (mg/L) of phenol using the modified Gompertz model.

where S_o is the initial phenol concentration (ppm), R_m is the maximum phenol degradation rate (ppm/h), and λ is the lag phase time (hr). The fitting parameters of the model are shown in Table 4. At lower initial phenol concentrations, the rate of phenol biodegradation increases with increasing the initial concentration. The maximum phenol biodegradation rate (10.626 ppm/h) was obtained at an initial phenol concentration of 700 ppm. However, at higher initial phenol concentrations, the rate of phenol biodegradation tends to be constant. Therefore, based on growth profile and percentage of phenol removal, the 700 ppm was taken for further experiments (Figure 1 and Figure 3). The experiments included the effect of substrate concentration, incubation temperature and pH of growth media on the biodegradation of phenol by *B. simplex*.

Table 4. Parameters using the Gompertz model on the different concentrations of phenol substrate

Phenol Concentration (ppm)	R_m (mg/L.hr)	λ (hr)
200	3.1	8.1
400	7.8	10.8
700	9.5	11.5
800	9.6	13
1000	10.4	13
1200	10.7	13.6

3.4 Effect of Incubation Temperature

Four different incubation temperatures (25, 28, 33 and 37°C) were established to estimate their outcome on phenol biodegradation. Experimental results showed a significant difference in the level of phenol degradation, depending on the variation in incubation temperatures (Table S2). It is evident that the temperature factor was characterized by being vital when it was less than 28°C, and that any additional rise in temperature above 33°C led to a decrease in growth at 700 ppm phenol. The incubation temperature was found to have a clear effect on the level of cellular mass formation, as is also evident on the phenol degradation rate. Thus, it turns out that phenol degradation biologically appears to occur at room temperature, where it becomes 28°C is the optimum temperature for *B. simplex* cells. It was mentioned that temperature exhibited a physiologically strong influence on the fate of the phenol compound (Al-kafaween et al., 2020;

Aljundi et al., 2020; Althunibat et al.,2016; Ereqat et al., 2018; Khleifat et al., 2010; Tarawneh et al., 2009; Ying et al., 2007). As the average temperature (28°C) turned out to be the best environmental factor for phenol degradation, or this may be especially due to the influence of temperature on the activities of the enzyme(s) responsible for it (Levén and Schnürer, 2005). It has been confirmed that temperature may play an equal or even greater function than the availability of nutrients during the degradation of organic compounds including phenol (Margesin and Schinner, 1997; Onysko et al., 2000).

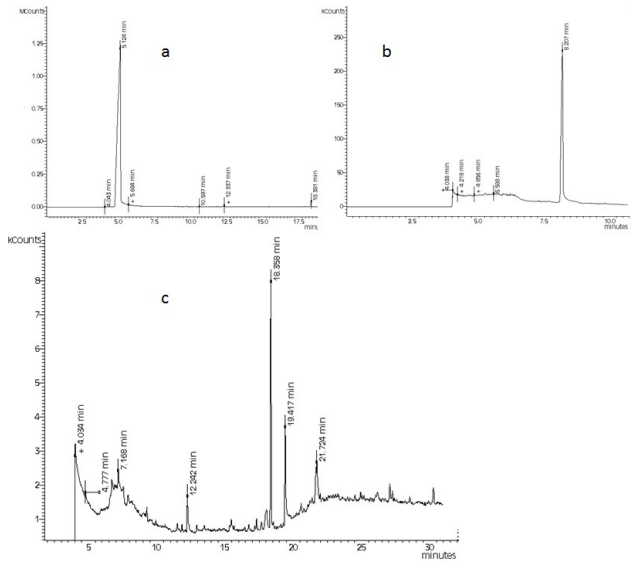

Figure 4. GC-MS chromatograms for the sample (a) control (phenol) , (b) catechol (standard) and (c) sample

3.5 Effect of pH on Biodegradation of phenol

When studying the pH conditions, the uninoculated culture was used as a biological control to ensure that the phenol drop was caused by a chemical reaction or because of something else. The pH factor, when tested, was found to have an effect on the amount of phenol within the uninoculated culture. *B. simplex* cells performed preferably for biological breakdown of phenol and growth biomass at pH 7.0. This was supported also by the results of best degradation rate at pH of 7 (Table S2). Therefore, it turns out in these data that the optimum pH for phenol biodegradation is 7.0 by this organism. Certainly, the enzymes have a role so that they are responsible for this degradation by *B. simplex* cells and therefore this optimal activity is at pH 7.0. It has been mentioned that the optimum pH plays a fundamental role in the biological decomposition of these different aromatic compounds, which vary according to the type of bacteria (Ahmad et al., 2017). For example, Arthrobacter optimally biodegrade 4-CBA at optimum pH of 6.8 (Marks et al., 1984), and *Halomonas campisalis* biodegrade phenol and catechol at pHs range between 8 and 11 (Alva and Peyton, 2003) and

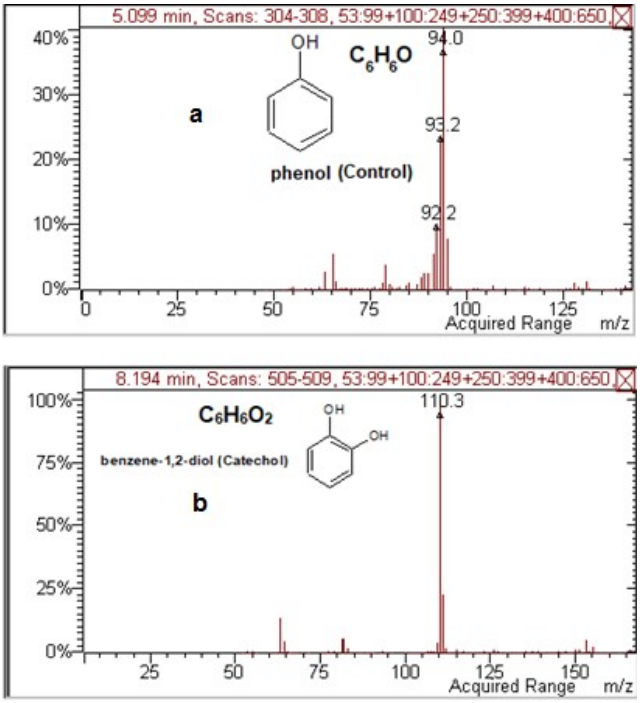

Figure 5. The fragmentation pattern of the significant peaks detected in the GC-MS analysis; (a) phenol(control) and (b) catechol(standard)

those for biodegradation of phenol from before *Klebsiella oxytoca*, it was 6.8 (Khleifat, 2007a, 2007b; Khleifat et al., 2015; Shawabkeh et al., 2007).

3.6 GC analysis

The GC-MS chromatograms for the biodegradation phenol sample for *B. simplex* under study, Figure 4 shows more than eight peaks at different retention times. The GC-MS analysis in Figure 5, for those main peaks shows the degradation and condensation products of the catechol and/or phenol, which identified by using the NIST mass spectral data base as the following: the condensation of the acetaldehyde with phenol and/or catechol produced a 4-(1-hydroxyethyl) phenol (III) at (RT=7.301 min, and *m/z* = 138.1), and 4-(1-hydroxyethyl)benzene-1,2-diol (II) at(RT= 6.712 min, and *m/z* = 154.1), as shown in Figure 6.

The following product of the degradation sample produced a propanal (IV) at (RT= 12.243 min, and *m/z* = 58.2) and the condensation of the propanal with phenol and/or catechol produced a 3,3'-(1-hydroxypropane-1,1-diyl)di(benzene-1,2-diol) (V) at (RT=18.319 min and *m/z* =277.3), and other the degradation products such as, [1^1,2^1:2^4,3^1-terphenyl]-1^3,1^4,3^4-triol (VI) at (RT=19.394 min and *m/z* = 279.5), (3E)-4-(3,4-dihydroxyphenyl)-2-oxopent-3-enoic acid (VII) at (RT=21.765 min and *m/z* =220.4), and 4-hydroxy-2-oxopentanoic acid (VIII) at (RT= 27.072 min, and *m/z* = 129.2), all those products from degradation and condensation confirm the catechol cleavage through *meta* pathway

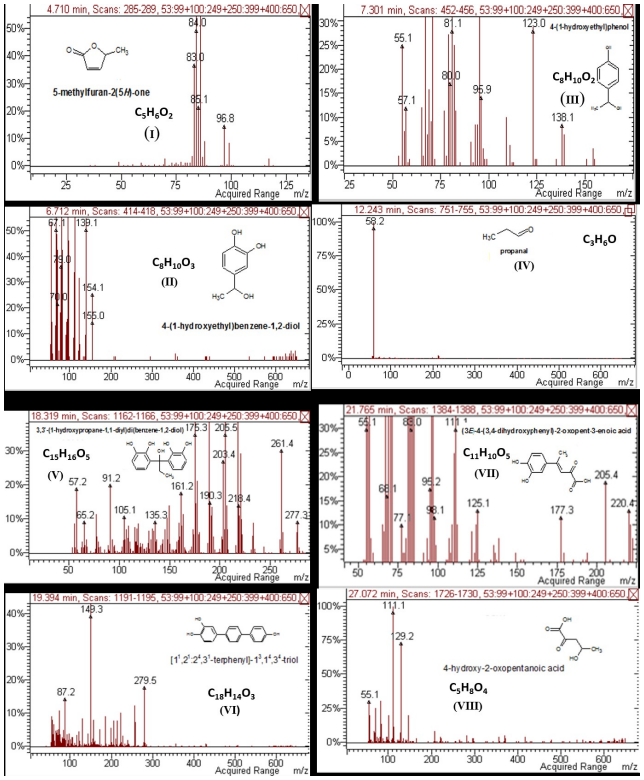

Figure 6. The fragmentation pattern of the significant peaks detected in the GC-MS analysis, and the putative chemical structure for predicted compounds (I-VIII).

(Catechol-2,3-dioxygenase). On other hand, the characterization of the degradation product 5-methylfuran-2(5H)-one (I) at (RT= 4.71 min and *m/z* =96.8) indicate that catechol degraded through *ortho*-pathway (Catechol-1,2-dioxygenase), as shown in Figure 6.

The GC-MS analysis in Figure 5, phenol control sample at (RT = 5.099 min, and *m/z* = 94.0), and standard sample of catechol at (RT = 8.194 min and *m/z* = 110.3). On other hand, the GC-MS analysis Figure 6, of the extractable products from bacterial culture sample revealed that phenol was converted to catechol by hydroxylase enzyme uses a molecular oxygen to produce catechol in the first step (Das et al., 2016), further degradation of the catechol by 1,2-dioxygenase produce a *cis*, *cis*-mucconic acid via *ortho*-pathway and/or by 2,3-dioxygenase into 2-hydroxymucconic semialdehyde via *meta*-pathway. Also, all the intermediate products produced from two degradation pathways undergo further multi-steps oxidations which take place to produce β-ketoadipate in *ortho*-pathway and/or acetaldehyde and pyruvate in *meta*-pathway, which they decomposed to carbon dioxide, and water. However, in our study, it was not possible to detect such compounds due to their rapid utilization by the bacteria. The degradation pathway of phenol as shown in Figure 7, revealed that catechol and acetaldehyde, propanal, 5-methylfuran-2(5H)-one, 4-hydroxy-2-oxopentanoic acid ,and [1^1,2^1:2^4,3^1-terphenyl]-1^3,1^4,3^4-triol could be formed from the degradation of the phenol substrate, which could be confirmed from products of the condensation reactions of those fragments with phenol and/or catechol and detected by GS-MS.

The results of *B. simplex* were similar to that of *Pseudomonas sp.* BZD-33 (Ke et al., 2019), *Pseudomonas aeruginosa* PDM (Al-Asoufi et al., 2017; Khleifat et al., 2019), *Rhodococcus* UKMP-5M (Suhaila et al., 2019). It has been observed that many of the oxygenase enzymes and the bacteria that harbor them are able to biodegrade different types of aromatic compounds as a substrate (Aravind et al., 2020; Khleifat et al., 2019; Mohite, 2015; Zou et al., 2018). It should be noted that many previous studies emphasized the necessity of providing an optimal amount of carbon and nitrogen sources such as yeast extract, to obtain the maximal rate of biodegradation of phenol or any other organic compounds. The reason for reasonable high degradation rate of phenol by *B. simplex* cells can be attributed to the fact that phenol is the only carbon source and possibly the amount of phenol-degrading enzymes is sufficiently large. Besides, the bacterium is capable of devouring this substrate readily, so the potential usual attenuation of phenol toxicity is efficiently enhanced (Abboud et al., 2007; Khleifat, 2007a, 2007b; Rughöft et al., 2020; Samadi et al., 2020; Wen et al., 2020). On other hand, the GC-MS analysis of the bacterial culture sample revealed that further degradation of the catechol by 1,2-dioxygenase produce a *cis*, *cis*-mucconic acid via *ortho*-pathway and/or by 2,3-dioxygenase into 2-hydroxymucconic semialdehyde via *meta*-pathway.

4 Conclusion

This study focused on the biodegradation of gradually increasing concentrations of phenol by using *S. simplex* in which the degradation kinetics of phenol was modeled. GC-MS analysis of the bacterial culture sample showed that further hydrolysis of catechol by 1,2-dioxygenase produces *cis*, *cis*-muconic acid via the *ortho*-pathway and/or by 2,3-dioxygenase to 2-hydroxymuconic semialdehyde via the *meta*-pathway. Therefore, there is a need in the later stage for additional studies related to the behavior of phenol as a model by studying and diagnosing specific genes and their regulation in relation to the degradation of different phenol concentrations so that we can explain the persistence of phenol at low concentrations by understanding functional gene expression.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author Contributions

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References

Abboud, M.M., Aljundi, I.H., Khleifat, K.M., Dmour, S.,2010. Biodegradation kinetics and modeling of whey lactose by bacterial hemoglobin VHb-expressing Escherichia coli strain. *Biochemical Engineering Journal*, 48(2), 166-172. https://doi.org/10.1016/j.bej.2009.09.006

Abboud, M.M., Khleifat, K.M., Batarseh, M., Tarawneh, K. A., Al-Mustafa, A., Al-Madadhah, M., 2007. Different optimization conditions required for enhancing the biodegradation of linear alkylbenzosulfonate and sodium dodecyl sulfate surfactants by novel consortium of Acinetobacter calcoaceticus and Pantoea agglomerans. *Enzyme and Microbial Technology*, 41(4), 432-439. https://doi.org/10.1016/j.enzmictec.2007.03.011

Adesemoye, A., Torbert, H. and Kloepper, J., 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology*, 54(10), 876-886. https://doi.org/10.1139/W08-081

Ahmad, S., Shamaan, N., Syed, M., Dahalan, F., Khalil, K. A., Ab Rahman, N., Shukor, M., 2017. Phenol degradation by Acinetobacter sp in the presence of heavy metals. *Journal of the National Science Foundation of Sri Lanka*, 45(3), 247253. https://doi.org/10.4038/jnsfsr.v45i3.8189

Aisami, A., Yasid, N.A. and Abd Shukor, M.Y., 2020. Optimization of Cultural and Physical Parameters for Phenol Biodegradation by Newly Identified Pseudomonas sp. AQ5-04. *Journal of Tropical Life Science*, 10(3), 223-233. https://doi.org/10.11594/jtls.10.03.06

Al-Asoufi, A., Khlaifat, A., Tarawneh, A., Alsharafa, K., Al-Limoun, M., Khleifat, K., 2017. Bacterial Quality of Urinary Tract Infections in Diabetic and Non Diabetics of the Population of Ma'an Province, Jordan. *Pakistan Journal of Biological Sciences: PJBS*, 20(4), 179-188. https://doi.org/10.3923/pjbs.2017.179.188

Al-kafaween, M.A., Hilmi, A.B. M., Al-Jamal, H.A.N., Elsahoryi, N.A., Jaffar, N., Zahri, M.K., 2020. Pseudomonas Aeruginosa and Streptococcus Pyogenes Exposed to Malaysian Trigona Honey In Vitro Demonstrated Downregulation of Virulence Factor. *Iranian Journal of Biotechnology*, 18(4), 115-123.

Al-kafaween, M.A., Hilmi, A.B.M., Jaffar, N., Al-Jamal, H.A.N., Zahri, M.K., Jibril, F.I., 2020. Antibacterial and Antibiofilm activities of Malaysian Trigona honey against Pseudomonas aeruginosa ATCC 10145 and Streptococcus pyogenes ATCC 19615. *Jordan Journal of Biological Sciences*, 13(1), 69 - 76.

Aljundi, I.H., Khleifat, K.M., Khlaifat, A.M., Ibrahim, A.M., Tarawneh, K.A., Tarawneh, S.A., 2010. Biodegradation of 2-chlorobenzoic acid by Klebsiella oxytoca: mathematical modeling and effect of some growth conditions. *Industrial & Engineering Chemistry Research*, 49(16), 7159-7167. https://doi.org/10.1021/ie100964f

Althunibat, O.Y., Qaralleh, H., Al-Dalin, S.Y.A., Abboud, M., Khleifat, K., Majali, I.S., Jaafraa, A., 2016. Effect of thymol and carvacrol, the major components of Thymus capitatus on the growth of Pseudomonas aeruginosa. *Journal of Pure and Applied Microbiology*, 10(1), 367-374.

Alva, V.A. and Peyton, B.M., 2003. Phenol and catechol biodegradation by the haloalkaliphile Halomonas campisalis: influence of pH and salinity. *Environmental Science & Technology*, 37(19), 4397-4402. https://doi.org/10.1021/es0341844

Aravind, M.K., Kappen, J., Varalakshmi, P., John, S. A., Ashokkumar, B., 2020. Bioengineered Graphene Oxide Microcomposites Containing Metabolically Versatile Paracoccus sp. MKU1 for Enhanced Catechol Degradation. *ACS omega*, 5(27), 16752-16761. https://doi.org/10.1021/acsomega.0c01693

Cueva-Yesquén, L.G., Goulart, M.C., Attili de Angelis, D., Nopper Alves, M., Fantinatti-Garboggini, F., 2020. Multiple plant growth-promotion traits in endophytic bacteria retrieved in the vegetative stage from passionflower. *Frontiers in Plant Science,* 11, 2282. https://doi.org/10.3389/fpls.2020.621740

Das, B., Mandal, T. and Patra, S., 2016. Biodegradation of phenol by a novel diatom BD1IITG-kinetics and biochemical studies. *International Journal of Environmental Science and technology*, 13(2), 529-542. https://doi.org/10.1007/s13762-015-0857-3

Der Yang, R. and Humphrey, A.E., 1975. Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. *Biotechnology and Bioengineering*, 17(8), 1211-1235. https://doi.org/10.1002/bit.260170809

Dobereiner, J., Marriel, I. and Nery, M., 1976. Ecological distribution of Spirillum lipoferum Beijerinck. *Canadian Journal of Microbiology*, 22(10), 1464-1473. https://doi.org/10.1139/m76-217

El-Naas, M.H., Al-Zuhair, S. and Makhlouf, S., 2010. Batch degradation of phenol in a spouted bed bioreactor system. *Journal of Industrial and Engineering Chemistry*, 16(2), 267-272. https://doi.org/10.1016/j.jiec.2009.09.072

Ereqat, S.I., Abdelkader, A.A., Nasereddin, A.F., Al-Jawabreh, A.O., Zaid, T.M., Letnik, I., Abdeen, Z.A., 2018. Isolation and characterization of phenol degrading bacterium strain Bacillus thuringiensis J20 from olive waste in Palestine. *Journal of Environmental Science and Health*, Part A, 53(1), 39-45. https://doi.org/10.1080/10934529.2017.1368300

Imran, A., Saadalla, M.J.A., Khan, S.-U., Mirza, M.S., Malik, K.A., Hafeez, F.Y., 2014. Ochrobactrum sp. Pv2Z2 exhibits multiple traits of plant growth promotion, biodegradation and N-acyl-homoserine-lactone quorum sensing. *Annals of Microbiology*, 64(4), 1797-1806. https://doi.org/10.1007/s13213-014-0824-0

Ke, Z., Xiangling, W., Jian, C., Jia, C., 2019. Biodegradation of diethyl phthalate by Pseudomonas sp. BZD-33 isolated from active sludge. Paper presented at the IOP Conference Series: Earth and Environmental Science. https://doi.org/10.1088/1755-1315/295/2/012070

Khleifat, K. and Abboud, M., 2003. Correlation between bacterial haemoglobin gene (vgb) and aeration: their effect on the growth and amylase activity in transformed Enterobacter aerogenes. *Journal of Applied Microbiology*, 94(6), 1052-1058. https://doi.org/10.1046/j.1365-2672.2003.01939.x

Khleifat, K., Abboud, M., Al-Shamayleh, W., Jiries, A., Tarawneh, K., 2006. Effect of chlorination treatment on gram negative bacterial composition of recycled wastewater. *Pakistan Journal of Biological Sciences*, 9, 1660-1668. https://doi.org/10.3923/pjbs.2006.1660.1668

Khleifat, K.M., 2007a. Biodegradation of phenol by Actinobacillus sp.: Mathematical interpretation and effect of some growth conditions. *Bioremediation Journal*, 11(3), 103-112. https://doi.org/10.1080/10889860701429328

Khleifat, K.M., 2007b. Effect of substrate adaptation, carbon starvation and cell density on the biodegradation of phenol by Actinobacillus sp. *Fresenius Environmental Bulletin*, 16(7), 726-730.

Khleifat, K.M., Abboud, M., Omar, S., Al-Kurishy, J., 2006. Urinary tract infection in South Jordanian population. *Journal of Medical Sciences*, 6(1), 5-11. https://doi.org/10.3923/jms.2006.5.11

Khleifat, K.M., Abboud, M.M. and Al-Mustafa, A.H., 2006. Effect of Vitreoscilla hemoglobin gene (vgb) and metabolic inhibitors on cadmium uptake by the heterologous host Enterobacter aerogenes. *Process Biochemistry*, 41(4), 930-934. https://doi.org/10.1016/j.procbio.2005.10.012

Khleifat, K.M., Abboud, M.M., Al-Mustafa, A.H., Al-Sharafa, K.Y., 2006. Effects of carbon source and Vitreoscilla hemoglobin (VHb) on the production of -galactosidase in Enterobacter aerogenes. *Current Microbiology*, 53(4), 277-281. https://doi.org/10.1007/s00284-005-0466-3

Khleifat, K.M., Al-limoun, M.O., Alsharafa, K.Y., Qaralleh, H., Al Tarawneh, A.A., 2019. Tendency of using different aromatic compounds as substrates by 2, 4-DNT dioxygenase expressed by pJS39 carrying the gene dntA from Burkholderia sp. strain DNT. *Bioremediation Journal*, 23(1), 22-31. https://doi.org/10.1080/10889868.2018.1516619

Khleifat, K.M., Halasah, R.A., Tarawneh, K.A., Halasah, Z., Shawabkeh, R., Wedyan, M.A., 2010. Biodegradation of linear alkylbenzene sulfonate by Burkholderia sp.: Effect of some growth conditions. *International Journal of Agricultural and Biological Engineering*, 12, 17-25.

Khleifat, K.M., Sharaf, E.F. and Al-limoun, M.O., 2015. Biodegradation of 2-chlorobenzoic acid by enterobacter cloacae: Growth kinetics and effect of growth conditions. *Bioremediation Journal*, 19(3), 207-217. https://doi.org/10.1080/10889868.2015.1029113

Khleifat, K.M., Tarawneh, K.A., Wedyan, M.A., Al-Tarawneh, A.A., Al Sharafa, K., 2008. Growth kinetics and toxicity of Enterobacter cloacae grown on linear alkylbenzene sulfonate as sole carbon source. *Current Microbiology*, 57(4), 364-370. https://doi.org/10.1007/s00284-008-9203-z

Krijgsheld, K. and Van der Gen, A., 1986. Assessment of the impact of the emission of certain organochlorine compounds on the aquatic environment: Part I: Monochlorophenols and 2, 4-dichlorophenol. Chemosphere, 15(7), 825-860. https://doi.org/10.1016/0045-6535(86)90051-2

Levén, L. and Schnürer, A., 2005. Effects of temperature on biological degradation of phenols, benzoates and phthalates under methanogenic conditions. *International Biodeterioration & Biodegradation*, 55(2), 153-160. https://doi.org/10.1016/j.ibiod.2004.09.004

Liu, Y., Zhang, A. and Wang, X., 2009. Biodegradation of phenol by using free and immobilized cells of Acinetobacter sp. XA05 and Sphingomonas sp. FG03. *Biochemical Engineering Journal*, 44(2-3), 187-192. https://doi.org/10.1016/j.bej.2008.12.001

Loh, K.-C. and Wang, S.-J., 1997. Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. *Biodegradation*, 8(5), 329-338. https://doi.org/10.1023/A:1008267607634

Margesin, R. and Schinner, F., 1997. Bioremediation of diesel-oil-contaminated alpine soils at low temperatures. *Applied Microbiology and Biotechnology*, 47(4), 462-468. https://doi.org/10.1007/s002530050957

Marks, T.S., Smith, A.R. and Quirk, A.V., 1984. Degradation of 4-chlorobenzoic acid by *Arthrobacter* sp. *Applied and Environmental Microbiology*, 48(5), 1020-1025. https://doi.org/10.1128/aem.48.5.1020-1025.1984

Mirza, B.S. and Rodrigues, J.L., 2012. Development of a direct isolation procedure for free-living diazotrophs under controlled hypoxic conditions. *Applied and Environmental Microbiology*, 78(16), 5542-5549. https://doi.org/10.1128/AEM.00714-12

Mohammad Abdulraheem Al-kafaween , H.A.N.A.-J., Abu Bakar Mohd Hilmi , Nour Amin Elsahoryi , Norzawani Jaffar, Mohd Khairi Zahri., 2020. Antibacterial properties of selected Malaysian Tualang honey against Pseudomonas aeruginosa and Streptococcus pyogenes. *Iranian Journal of Microbiology*, 12(6), 565-576. https://doi.org/10.18502/ijm.v12i6.5031

Mohite, B.V., 2015. Efficient Biotransformation of Phenol and Its Derivatives using *Streptococcus epidermis* BY Catechol 2, 3-Dioxygenase Metabolism. *Environmental Engineering & Management Journal (EEMJ)*, 14(4). https://doi.org/10.30638/eemj.2015.094

Nadeem, S.M., Zahir, Z.A., Naveed, M., Asghar, H.N., Arshad, M., 2010. Rhizobacteria capable of producing ACCdeaminase may mitigate salt stress in wheat. *Soil Science Society of America Journal*, 74(2), 533-542. https://doi.org/10.2136/sssaj2008.0240

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, 170(1), 265-270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Nithyapriya, S., Lalitha, S., Sayyed, R., Reddy, M., Dailin, D.J., El Enshasy, H.A., Herlambang, S., 2021. Production, Purification, and Characterization of Bacillibactin Siderophore of Bacillus subtilis and Its Application for Improvement in Plant Growth and Oil Content in Sesame. *Sustainability*, 13(10), 5394. https://doi.org/10.3390/su13105394

Onysko, K.A., Budman, H.M. and Robinson, C.W., 2000. Effect of temperature on the inhibition kinetics of phenol biodegradation by Pseudomonas putida Q5. *Biotechnology and Bioengineering*, 70(3), 291-299. https://doi.org/10.1002/1097-0290(20001105)70:3<291::AID-BIT6>3.0.CO;2-Y

Orhan, F., 2016. Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (Triticum aestivum). *Brazilian Journal of Microbiology*, 47, 621-627. https://doi.org/10.1016/j.bjm.2016.04.001

Patten, C.L. and Glick, B.R., 2002. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, 68(8), 3795-3801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002

Pérez-Miranda, S., Cabirol, N., George-Téllez, R., Zamudio-Rivera, L., Fernández, F., 2007. O-CAS, a fast and universal method for siderophore detection. *Journal of Microbiological Methods*, 70(1), 127-131. https://doi.org/10.1016/j.mimet.2007.03.023

Rai, A., Gowrishetty, K.K., Singh, S., Chakrabarty, J., Bhattacharya, P., Dutta, S., 2021. Simultaneous Bioremediation of Cyanide, Phenol, and Ammoniacal-N from Synthetic Coke-Oven Wastewater Using Bacillus sp. NITD 19. *Journal of Environmental Engineering*, 147(1), 04020143. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001835

Rughöft, S., Vogel, A.L., Joye, S.B., Gutierrez, T., Kleindienst, S., 2020. Starvation-dependent inhibition of the hydrocarbon degrader Marinobacter sp. TT1 by a chemical dispersant. *Journal of Marine Science and Engineering*, 8(11), 925. https://doi.org/10.3390/jmse8110925

Samadi, A., Sharifi, H., Nejad, Z.G., Hasan-Zadeh, A., Yaghmaei, S., 2020. Biodegradation of 4-Chlorobenzoic Acid by Lysinibacillus macrolides DSM54T and Determination of Optimal Conditions. *International Journal of Environmental Research*, 1-10. https://doi.org/10.1007/s41742-020-00247-4

Schwartz, A.R., Ortiz, I., Maymon, M., Herbold, C.W., Fujishige, N.A., Vijanderan, J.A., Sanders, E.R., 2013. Bacillus simplexa little known PGPB with anti-fungal activityalters pea legume root architecture and nodule morphology when coinoculated with Rhizobium leguminosarum bv. viciae. *Agronomy*, 3(4), 595-620. https://doi.org/10.3390/agronomy3040595

Schwyn, B. and Neilands, J., 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160(1), 47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Shawabkeh, R., Khleifat, K.M., Al-Majali, I., Tarawneh, K., 2007. Rate of biodegradation of phenol by Klebsiella oxytoca in minimal medium and nutrient broth conditions. *Bioremediation Journal*, 11(1), 13-19. https://doi.org/10.1080/10889860601185830

Sewidan, N., Khalaf, R.A., Mohammad, H., Hammad, W.E., 2020. In-Vitro Studies on Selected Jordanian Plants as Dipeptidyl Peptidase-IV Inhibitors for Management of Diabetes Mellitus. *Iranian Journal of Pharmaceutical Research: IJPR*, 19(4), 95.

Suhaila, Y.N., Hasdianty, A., Maegala, N., Aqlima, A., Hazwan, A.H., Rosfarizan, M., Ariff, A., 2019. Biotransformation using resting cells of Rhodococcus UKMP-5M for phenol degradation. *Biocatalysis and Agricultural Biotechnology*, 21, 101309. https://doi.org/10.1016/j.bcab.2019.101309

Tarawneh, K.A., AlTawarah, N.M., AbdelGhani, A.H., AlMajali, A.M., Khleifat, K.M., 2009. Characterization of verotoxigenic Escherichia coli (VTEC) isolates from faeces of small ruminants and environmental samples in Southern Jordan. *Journal of Basic Microbiology*, 49(3), 310-317. https://doi.org/10.1002/jobm.200800060

Vílchez, J.I., Navas, A., González-López, J., Arcos, S.C., Manzanera, M., 2016. Biosafety test for plant growth-promoting bacteria: Proposed environmental and human safety index (EHSI) protocol. *Frontiers in*

Microbiology, 6, 1514.
https://doi.org/10.3389/fmicb.2015.01514

Vilchez, S. and Manzanera, M., 2011. Biotechnological uses of desiccation-tolerant microorganisms for the rhizoremediation of soils subjected to seasonal drought. *Applied Microbiology and Biotechnology*, 91(5), 1297-1304.
https://doi.org/10.1007/s00253-011-3461-6

Wen, Y., Li, C., Song, X., Yang, Y., 2020. Biodegradation of Phenol by Rhodococcus sp. Strain SKC: Characterization and Kinetics Study. *Molecules*, 25(16), 3665.
https://doi.org/10.3390/molecules25163665

Ying, W., Ye, T., Bin, H., Zhao, H.-b., Bi, J.-n., Cai, B.-l., 2007. Biodegradation of phenol by free and immobilized Acinetobacter sp. strain PD12. *Journal of Environmental Sciences*, 19(2), 222-225.
https://doi.org/10.1016/S1001-0742(07)60036-9

Younis, S.A., ElGendy, N.S. and Nassar, H.N., 2020. Biokinetic aspects for biocatalytic remediation of xenobiotics polluted seawater. *Journal of Applied Microbiology*, 129(2), 319-334.
https://doi.org/10.1111/jam.14626

Zou, S., Zhang, B., Yan, N., Zhang, C., Xu, H., Zhang, Y., Rittmann, B.E., 2018. Competition for molecular oxygen and electron donor between phenol and quinoline during their simultaneous biodegradation. *Process Biochemistry*, 70, 136-143.
https://doi.org/10.1016/j.procbio.2018.04.015

Table S1. Effect of phenol concentration on the biodegradation rate by *B. simplex*

Concentration (ppm)	Biodegradation rate (ppm/h)
200	1.53
400	5.56
700	6.60
800	6.48
1000	5.56
1200	5.16

Table S2. Effect of growth conditions on the biodegradation rate of phenol by *B. simplex*

Condition	Value	Biodegradation rate (ppm/h)
Incubate temperature (c)	25	10
	28	20.8
	33	15
	37	14.2
pH	5.5	15
	7	24.16
	8	12.5
	9	10