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Screening of Efficient Phenol-Degrading Bacteria and Analysis of Their Degradation Characteristics

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Abstract: A strain with high phenol degradation capacity was isolated from the activated sludge of an urban wastewater treatment system in Panshan County, Liaoning Province, by enrichment culture, gradient domestication and plate delineation, and named strain LA1. After morphological observation of colonies, physiological and biochemical identification and 16S rDNA gene sequencing, strain LA1 was initially identified as *Stenotrophomonas* sp. Examination of environmental factors showed that pH 7, a growth temperature of 35 °C and shaking at 150 r/min were the optimal degradation conditions for strain LA1. Under these culture conditions, strain LA1 could completely degrade 1000 mg/L phenol in 18 h. LA1 is the most powerful strain with phenol-reducing ability among the reported genera, which provides some theoretical basis for the treatment of phenol-containing wastewater and technical support for the sustainable development of the wastewater treatment industry.

Keywords: phenol; biodegradation; *Stenotrophomonas* sp.

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1. Introduction

Phenol (C_6H_5OH), also known as hydroxybenzene, phenol, or petrolatum, has a special odor and strong corrosive properties. Phenol has a wide range of uses in industry: it can be used in the production and manufacture of paints, coke, rubber, plastics, phenolic resins, etc., and is a common raw material for the chemical industry [1]; it can be used in the production and manufacture of salicylic acid, aspirin and sulfa drugs and other pharmaceuticals, and is an important raw material for medicine; it can also be used in the production and manufacture of stones, spices, dyes and paints in daily life [2]. With the development of the domestic economy and the improvement of phenol production technology and the production process, the production volume of phenol has been growing rapidly. According to statistics, the total production of phenol in China grew from 1,414,800 tons in 2014 to 4.21 million tons in 2022, while the total annual consumption of phenol showed a gradual growth trend with the increase in total production. In recent years, China's demand for bisphenol A, phenolic resins and other phenol series downstream products has also shown an upward trend, and the consumption of phenol will also steadily increase.

Phenol will be discharged into the environment with the waste gas, waste water and waste residue from industrial production, which will then cause pollution of the atmosphere, water environment and soil environment and endanger the health and safety of human beings and organisms [3]. Studies have shown that long-term inhalation of phenol or drinking water contaminated with phenol can cause dizziness and vomiting and neurological disorders, and, in serious cases, physical poisoning; when the concentration of phenol in water is 0.1~0.2 mg/L, it can cause slow poisoning of aquatic organisms and affect the reproduction of fish [4]; irrigating agricultural land with wastewater containing phenol greater than 100 mg/L can cause soil modification, crop yield decline and even death. Therefore, many countries have strict regulations on the concentrations of phenol

and its derivatives in drinking water and the environment, trying to minimize its contamination level. The EPA lists phenol as a toxic pollutant and a priority pollutant on its list, and it also has strict controls on the discharge of phenol-containing wastewater [5]. The discharge standards for phenolic compounds are $\leq$0.01 mg/L in surface water and $\leq$0.001 mg/L in drinking water. According to national requirements and the trend in social development, efficient measures must be taken to strictly control the discharge of phenol-containing wastewater and reduce the concentration of phenol in the environment. How to find an efficient and fast way to deal with it is a pressing issue at present and a focus of sustainable development concern. With improvement in people′s awareness of the hazards of phenol-containing wastewater and the development of wastewater treatment technology, treatment methods for phenol-containing wastewater have been gradually developed, and, with continuous innovation in treatment technology, the interpenetrating combination of physical–chemical–biological treatment methods has made the treatment of phenol-containing wastewater achieve better results. There are three main types of treatment methods for industrial phenol-containing wastewater: physical (extraction [6], adsorption [7] and salting), chemical (precipitation, oxidation [8] and photocatalysis) and biological (biofilm [9], enzymatic treatment and activated sludge). A high concentration of phenol in wastewater is commonly treated by physical and chemical methods, but there are defects, such as a large floor space requirement, high investment costs, high energy consumption, easiness to produce secondary pollution, and more stringent requirements for the wastewater treatment process. Low-concentration phenol-containing wastewater is now commonly treated by biological methods, which use microorganisms′ own metabolism to metabolize phenolic substances in wastewater into stable CO_2, SO_2, NH_3 and other small molecules, and use enzymes in the body to decompose phenolic substances to synthesize the organic matter they need, so that phenolic wastewater can be treated. Compared with physical and chemical methods, the biological method has the advantages of a simple pre-treatment process, low investment in equipment, high treatment capacity and no secondary pollution; this is the most widely used treatment technology for phenol-containing wastewater in China at present and has received widespread attention. Therefore, studying the mechanisms of bioremediation for phenolic wastewater is crucial for the sustainable development of the environmental protection industry.

Among phenol-degrading microorganisms, bacteria occupy a major position due to their multiple biochemical adaptations and susceptibility to induce mutations. The phenol-lowering bacterial classes of microorganisms that have been isolated and identified include *Alcaligenes* [10], *Micrococcus, Bacillus, Pseudomonas* [11], *Rhodococcus, acinetobacter, Sphingomonas, Rhizobium, Ochrobactrum* [12], etc. Hao et al. studied the kinetic model of phenol degradation by *Acinetobacter* sp. at different substrate concentrations and showed that it took 6 h for complete degradation at a phenol concentration of 200 mg/L [13]. Mohite isolated *Citrobacter freundii* and *Proteus mirabilis* in petroleum-contaminated soil, strains capable of degrading about 90% of 100 mg /L phenol within 80 h [14]. Among these reported phenol-degrading bacteria, some strains have low tolerance to phenol concentrations and some strains require too long a period for phenol degradation. If these strains are used for the biological degradation of phenol, further studies are needed to improve their properties to enhance the degradation of phenol. Therefore, it is still necessary to isolate strains with high degradation ability, high environmental adaptability and high application potential from special polluted environments and to explore novel biodegradation methods, which are of great importance in the field of environmental biotechnology as well as in the field of sustainable development.

In this study, phenol was used as the only carbon source, and an efficient phenol-degrading strain was obtained from the activated sludge of a municipal wastewater treatment plant, which was identified as *Stenotrophomonas* sp. by a combination of morphological observation, physiological and biochemical experiments and molecular identification. Meanwhile, the effects of pH, temperature, shaking bed speed, strain dosage and salinity on the degradation of phenol by strain LA1 were studied to determine the optimal growth

conditions, improve the treatment efficiency of phenol pollution, provide a theoretical basis and technical support for microbial remediation of phenol-containing wastewater and improve the ecological environment's quality, with a view to contributing to the sustainable development of the environmental protection industry.

2. Materials and Methods

2.1. Sample Source and Experimental Apparatus

The samples were taken from the activated sludge of the urban wastewater treatment system in Panshan County, Liaoning Province. Information about the experimental apparatus is shown in Table 1.

Table 1. Experimental apparatus.

Instrument Name	Models	Manufacturers
Electronic balance	AR224CN	Shanghai OHAUS Instruments Co., Shanghai, China
UV–visible spectrophotometer	UV-5800	Shanghai Yuananalysis Instrument Co., Shanghai, China
Constant temperature water bath	HH-2	Changzhou Guohua Electric Co., Jintan, China
Vertical pressure steam sterilizer	BXM-30R	Shanghai Boxun Medical Instrument Co., Shanghai, China
Tabletop high-speed centrifuge	H1850	Hunan Xiang Yi Laboratory Instrument Development Co., Xiangtan, China
Constant temperature culture oscillator	ZWY-1102C	Shanghai Zhicheng Analytical Instrument Manufacturing Co., Shanghai, China
Electric thermostat incubator	HPX-9272MBE	T Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory, Shanghai, China
Vacuum drying oven	DZG-6050	Shanghai Senxin Experimental Instruments Co., Shanghai, China

2.2. Culture Medium and Reagents

All the chemicals used in the study were of analytical grade unless otherwise specified and obtained from Tianjin Damao Chemical Reagent Factory and Beijing Aoboxing Biotechnology Co. The composition of the inorganic salt medium for the bacterial culture was K_2HPO_4, 0.5 g, KH_2PO_4, 0.5 g, NaCl, 0.2 g, $FeCl_3$, 0.02 g, $MgSO_4{\cdot}7H_2O$, 0.2 g, $MnSO_4{\cdot}H_2O$, 0.01 g, $Na_2MoO_4{\cdot}2H_2O$, 0.01g, $(NH4)_2SO_4$, 1.0 g, deionized water, 1000 mL. The composition of the enrichment medium was beef paste, 3 g, peptone, 10g, NaCl, 5 g and deionized water, 1000 mL [15]. The pH of the above medium should be adjusted to 7.0~7.2 and sterilized at 121 °C for 20 min. If the corresponding solid medium is needed, add 1.5~2.0% agar.

2.3. Experimental Methods

An appropriate volume of mud–water mixture was inoculated into the enrichment medium with an initial phenol concentration of 100 mg/L and placed in a shaker at 30 °C and 150 r/min; when the culture became turbid, 5 mL of the supernatant was inoculated into the medium with a phenol concentration of 400 mg/L; thus, repeatedly transferred, the phenol concentration was set in a gradient of 100 mg/L, 400 mg/L, 700 mg/L, 1000 mg/L, 1200 mg/L, and 1500 mg/L. The last rounds of the enrichment solution were diluted with 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} gradients, and 200 uL of each dilution gradient was evenly spread on an inorganic salt solid medium with a phenol concentration of 1000 mg/L and incubated in a constant temperature incubator at 30 °C. After visible colonies grew on the plate, the single colonies of different forms were picked and purified several times to obtain the pure strain.

2.4. Strain Identification

Morphological observations: these were mainly for colony morphology, including the morphology, luster, size, etc. of the strain growing on the plate.

Physiological and biochemical identification: the purified strains were added to 2 mL of saline, shaken and mixed, and, then, the turbidity of the suspensions was measured to

reach 0.5 Mackay′s concentration using a Mackay′s turbidimeter and analyzed with the VITEK 2 COMPACT automatic microbiological analysis system according to the operating instructions of the GN Gram-negative bacteria identification card.

Molecular identification: genomic DNA was used as the template to amplify 16S r DNA, and the universal primers for the PCR reaction were 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR reaction system: 10 × PCR buffer, 3 μL, dNTP (2.5 mmol/L), 1 μL, template DNA, 1 μL, Taqase, 0.2 μL, and ddH_2Oto, 25 μL [16]. The purification and sequencing of PCR products were performed at Biotech Bioengineering (Shanghai) Co. The 16S r DNA sequencing results were compared with the homology of 16S r DNA sequences in the Genbank database using BLAST software.

2.5. Determination of Phenol Content

The 4-aminoantipyrine method was used to measure the phenol content [17]. When the solution's pH is 10 ± 0.2, under the condition of potassium hexacyanoferrate, 4-aminoantipyrine can chemically react with phenol and produce orange-red indoleantipyrine, whose solution has the maximum absorbance value at 510 nm.

$$\text{Phenol degradation rate } (\%) = \left(1 - \frac{\text{phenol concentration after reaction}}{\text{Initial concentration of phenol}}\right) \times 100\%$$

2.6. Growth Curve of the Strain

The strain was inoculated with a phenol concentration of 500 mg/L medium at 5% inoculum and incubated in a shaker at 30 °C and 150 r/min, and the absorbance, OD_{600}, was measured every three hours to plot the growth curve of the strain.

The growth of bacteria goes through four stages: delayed, logarithmic, stable, and declining. During the logarithmic growth period, the bacterial growth rate was the fastest and the utilization of carbon sources was the highest. Therefore, the growth curves of the strains were measured, and the strains within the logarithmic growth period were prepared as seed solutions for phenol degradation experiments.

2.7. Degradation Characteristics of the Strains

The effects of different pHs, temperatures, shaker speeds, strain dosages, salinities and additional carbon and nitrogen sources on growth and phenol degradation were investigated in an inorganic salt medium containing 1000 mg/L phenol.

The effects of different pHs (4~10) on the growth of the bacteria and the degradation of phenol were investigated at a temperature of 30 °C, a shaker speed of 150 r/min and an inoculum of 5% to determine the optimal pH.

At the optimum pH, a shaker speed of 150 r/min and an inoculum of 5 %, the effects of different temperatures (20~45 °C) on the growth of the bacteria and the degradation of phenol were investigated to determine the optimum T.

The effects of different shaking speeds (0~200 r/min) on the growth of bacteria and phenol degradation were investigated under the optimal T, pH and inoculum conditions to determine the optimal shaking speed.

The effects of different inoculum levels (1~20%) on the growth of the bacteria and the degradation of phenol were investigated at the optimal T and pH and a shaker speed of 150 r/min to determine their optimal inoculum levels.

The effects of different salinities (0~6%) and additional carbon (glucose, amylum and maltose) and nitrogen (NH_4Cl, NH_4NO_3 and CH_4N_2O) on the growth and phenol degradation of the strains were investigated under optimal degradation conditions.

3. Results and Discussion

3.1. Screening and Identification of Strains

The samples were continuously enriched, screened and purified to obtain a strain, named the LA1 strain, which can efficiently degrade phenol with a tolerance of 3500 mg/L. The bacterium had a smooth, round, neatly edged, creamy white surface on LB plates. Its Gram stain was negative (e.g., Figure 1).

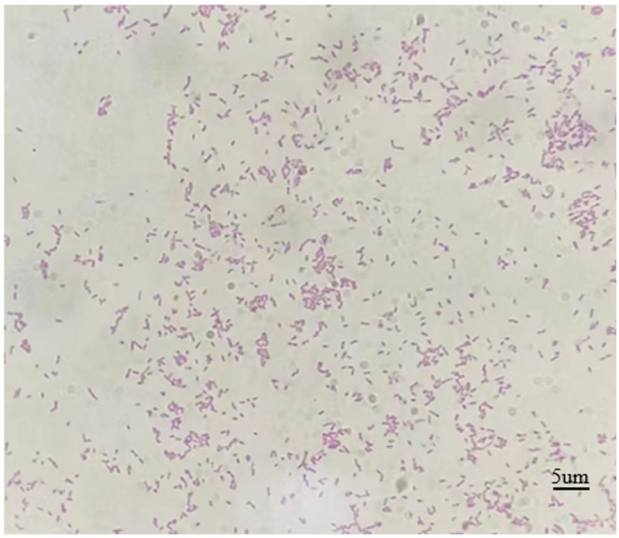

Figure 1. Gram staining results of strain LA1 (1000×).

The specific physiological and biochemical data are shown in Table 2.

Table 2. Strain LA1: physiological and biochemical indexes.

Physiological and Biochemical Indicators	Test Results	Physiological and Biochemical Indicators	Test Results
dGLU	+	BGLU	+
dMAL	+	dMAN	+
dTAG	−	LIP	−
PLE	−	TyrA	−
SAC	+	URE	−
dTRE	+	BXY	−
CIT	−	dSOR	+

Note: "+" indicates positive; "−" indicates negative.

According to BLAST (basic local alignment search tool) analysis, it is now accepted that, when the homology of the 16S r DNA of two bacteria is greater than 95%, they can be grouped into the same genus [18]. The sequence of strain LA1 was amplified, and the sequence was submitted to the GenBank database, and the registration number was OQ414956. The sequencing results were compared in the BLAST database of NCBI, and the sequence homology of strain LA1 was 99% with *Stenotrophomonas* sp. It was identified as *Stenotrophomonas* sp. The 16S rDNA sequence of the bacterium is shown in Figure 2 and the phylogenetic tree is shown in Figure 3.

CATGGCTCAGAGTGAACGCTGGCGGTAGGCCTAACACATGCAAGTCGAACGGCAGCACAGGAGAGCT
TGCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGAATGCATCGGAATCTACTCTTTCGTGGGGGATAA
CGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCCGGGGACCTTCGGGCCTGGCG
CGAATGAATGAGCCGATGCCCGATTAGCTAGTTGGCGGGGTAAGAGCCCACCAAGGCGACGATCGGTA
GCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGC
AGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCTTTC
GGGTTGTAAAGCCCTTTTGTTGGGAAAGAAAAGCAGCTGGTTAATACCCGGTTGTTCTGACGGTACCC
AAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCG
GAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTCGTGAAAGCCCTGGGCTCAACCT
GGGAATTGCGATGGAAACTGGGCGACTAGAGTGTGGCAGAGGATAGTGGAATTCCTGGTGTAGCAGT
GAAATGCGTAGAGATCAGGAGGAACATCCGTGGCGAAGGCGACTGTCTGGGCCAACACTGACACTGA
GGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAAC
TGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTAC
GGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAAT
TCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGCGAACTTTCCAGAGATGGATTGGTGCC
TTCGGGAACGCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC
CCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCC
GGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACA
CGTACTACAATGGTGGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATC
TCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCAT
TGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGCACCA
GAAGCAGGTAGCTTAACCTTCGG

Figure 2. The 16S rRNA sequence of the strain.

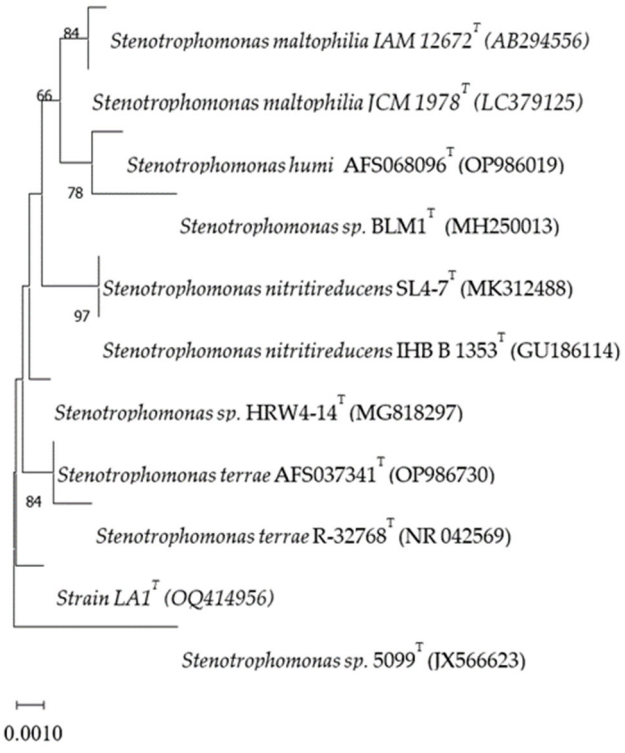

Figure 3. The phylogenetic tree of strain LA1.

3.2. Strain Growth and Degradation Curve

The growth curve of the microorganisms was determined to provide a reference for the culture and growth characteristics of the strain. The experimental results for the growth

curve of phenol-degrading bacteria are shown in Figure 4. The growth of strain LA1 was slow in the first 3h, indicating that the strain was in the delayed phase. In the following 12 h, the strain's proliferation ability was strong, indicating that the strain was in the logarithmic phase, the demand for a carbon source was large, the degradation was rapid, and the phenol concentration decreased quickly. At a time 15 h later, the growth of the bacterium becomes stable; the carbon source phenol content in the medium is relatively small; there are no new carbon and nitrogen sources to supplement it; coupled with the accumulation of metabolites, the growth of the bacterium is inhibited; the number of bacteria no longer increases; and degradation becomes relatively slow. During the whole process, the change in OD_{600} of the bacterial solution was consistent with the change in phenol concentration in the medium, indicating that the strain used phenol for metabolism and reproduction. Yan Jiang et al. found that the phenol-lowering effect mainly occurred in the late logarithmic growth phase of the strain, so this experiment prepared the strain in the logarithmic growth phase as a seed solution for the subsequent phenol degradation experiments [19].

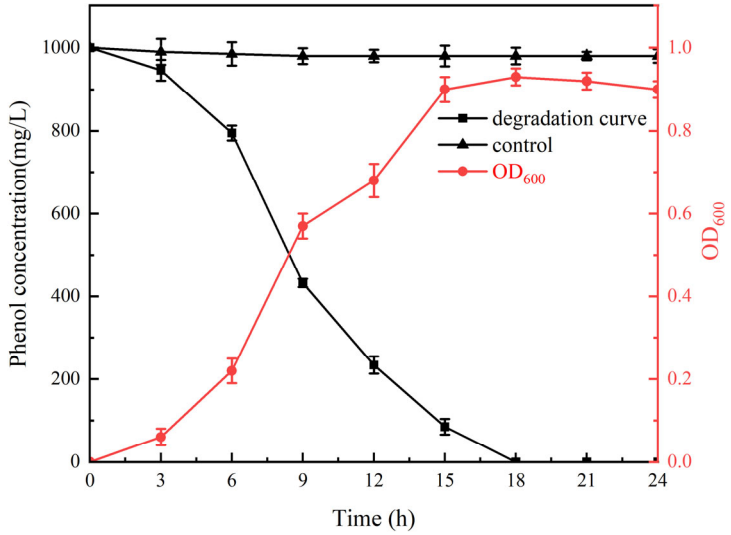

Figure 4. The growth and phenol degradation curve of LA1.

3.3. Optimal pH Conditions for Strain Growth

pH is one of the key factors affecting the growth of microorganisms and the degradation of organic matter in wastewater, and its main role is to influence the uptake of nutrients by microorganisms as well as metabolic activity, metabolic pathways, etc. The ability of bacteria to degrade phenol at different pH varies due to the variability of the strains' own properties. Some bacteria can tolerate acids, some can tolerate bases, and some can only degrade under neutral conditions [20].

The results are shown in Figure 5: strain LA1 can reproduce normally in a pH-5-to-9 environment and has a certain degradation ability, which is consistent with the trend reported in the screening of *Citrobacter* by Tao Deng, et al. [21]. When the pH was 7, the growth rate of the microorganism was the fastest, and degradation efficiency could reach more than 99%; when the pH was lower than 7, the degradation ability of the strain towards phenol gradually decreased; when the pH was 4 in the medium, the strain could hardly grow; when the pH was more than 7, the degradation rate of the strain towards phenol was significantly improved compared with the acidic condition (45.8% at pH 5, 67.1% at pH 6, 99.1% at pH 7, 89.9% at pH 8, 53.1% at pH 9). This is because organic acids such as adipic acid and pyruvic acid are produced during the degradation of phenol, leading to a decrease in the pH of the medium, so neutral and alkaline environments are more conducive to the degradation of phenol by the strain compared to acidic environments [22]. Therefore, a pH of 7 was selected as the optimal pH condition for this experiment.

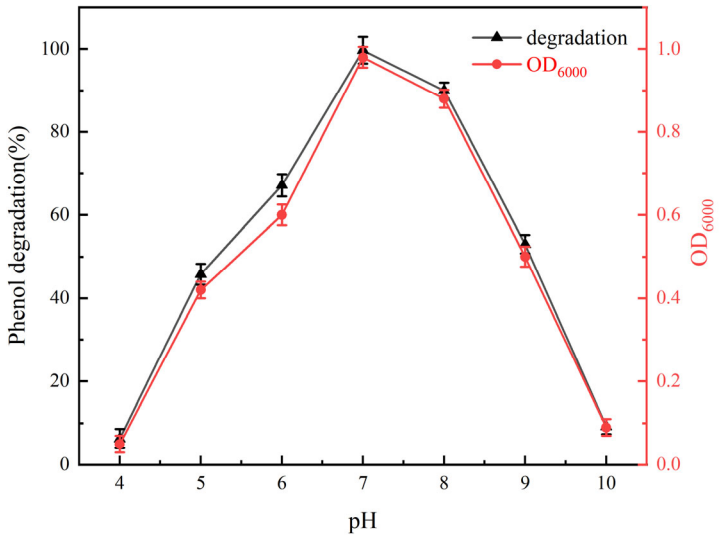

Figure 5. Effect of pH on the growth of strain LA1 and the degradation of phenol.

3.4. Optimal Temperature Conditions for Strain Growth

Temperature is an important factor affecting the growth and reproduction of microorganisms, and its influence on enzyme activity in bacteria is large. A high temperature will reduce or even inactivate enzyme activity, and a low temperature will also weaken the metabolic activity of the cells [23]. Due to the different characteristics of the strains, some bacteria can still degrade phenol at high temperatures, and some can only degrade phenol at room temperature or at low temperatures [24].

The results are shown in Figure 6: strain LA1 showed the best growth rate and phenol reduction ability at 35 °C, with 98.9% degradation of phenol within 24 h. At 30 °C, the strain also maintained strong activity, with 95.5% degradation of phenol. Strain LA1 can grow at 20 °C to 45 °C and is a typical mesophilic bacterium. The lowest biomass and degradation rates for the strains were reached at 15 °C and 45 °C (45.2% at 15 °C and 31.3% at 45 °C), which may be due to the fact that low temperature decreases the enzymatic activity of microorganisms involved in phenol degradation, thus leading to a decrease in the metabolic rate of these microorganisms, while high temperature tends to make microbial enzymes inactive and inhibits cell growth. This phenomenon is similar to many previous studies in which the ability of microorganisms to remove pollutants is reduced when the temperature is too low or too high [25].

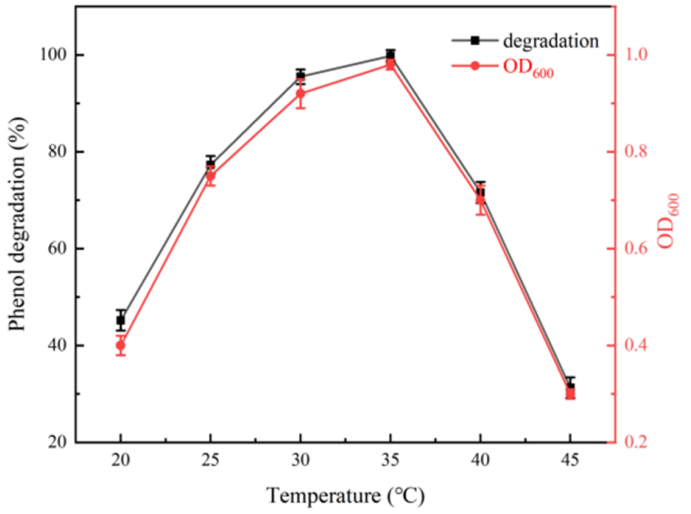

Figure 6. Effect of temperature on the growth of strain LA1 and the degradation of phenol.

3.5. Optimal Shaker Speed Conditions for Strain Growth

The speed of rotation of phenol-degrading microorganisms is closely related to dissolved oxygen; the faster the rotational speed, the higher the dissolved oxygen content in the water. The rotation of the shaker is to provide sufficient oxygen to the microorganisms. The effect of dissolved oxygen on the growth of the strain and the degradation of phenol was investigated by changing the speed of the shaker.

The results are shown in Figure 7: when the speed is 0, strain LA1 is less active due to the limitation of dissolved oxygen in the medium. With an increase in shaker speed, the oxygen supply gradually increased, and the degradation rate of phenol by strain LA1 also increased (66.7% at 50 r/min and 87.9% at 100 r/min), indicating that strain LA1 is aerobic, which is similar to the experimental results of Shahab Shahryari et al. [26]. When the rotational speed reached 150 r/min, the phenol degradation was basically complete, and further increase in rotational speed had little effect on its degradation rate, indicating that the oxygen supply was sufficient at this speed. Therefore, 150 r/min was determined as the optimal shaking speed for the growth of the strain and the degradation of phenol by combining the phenol removal rate and the degradation cost.

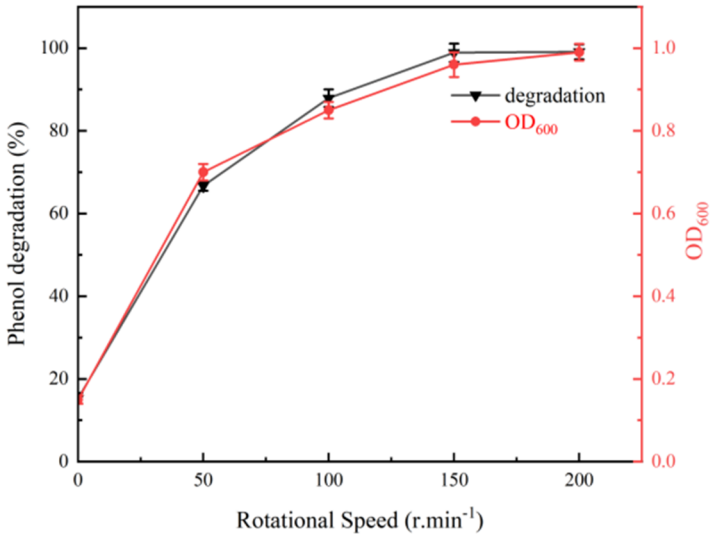

Figure 7. Effect of rotation rate on the growth of LA1 and the degradation of phenol.

3.6. Conditions of Optimal Inoculum Size for Strain Growth

Inoculum level directly affects the delay period of bacterial growth; too little inoculum will result in strains more susceptible to phenol inhibition, while too much inoculum will not only increase the input cost but also cause competition between strains for carbon sources and affect the degradation effect.

The results are shown in Figure 8: the degradation of 1000 mg/L phenol by strain LA1 was only 39.8% in 24 h when the inoculum was 1% of the bacterial solution. With increasing inoculum, the delay period of bacterial growth was significantly shortened and the concentration of phenol in the medium decreased. The degradation rate of phenol was 85.7% in 24 h when inoculated with 5% bacterial solution and increased to 96.3% in 24 h when inoculated with 10% bacterial solution. This indicates that an appropriate increase in inoculum is an effective way to enhance the phenol degradation effect of the strain. The degradation of phenol by the strain could reach more than 95% at 10% or higher inoculation percentages, and the degradation effect of further increasing the strain dosing on phenol pairs was unknown, which is consistent with results reported in the literature [27]. In order to ensure high phenol degradation efficiency, and for cost reasons, a 10% inoculum was chosen for the follow-up experiments.

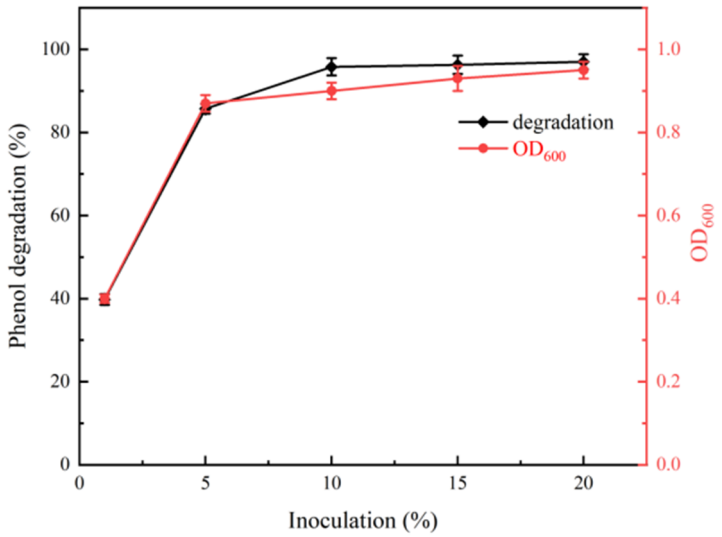

Figure 8. Effect of inoculation on the growth of LA1 and the degradation of phenol.

3.7. Examination of the Salt Tolerance of the Strain

Actual wastewater usually contains a certain degree of salinity, and the high salt content of industrial wastewater can greatly affect the effectiveness of biological treatment. Inorganic salts play an important role in promoting enzymatic reactions, maintaining membrane homeostasis and regulating osmotic pressure during the growth of microorganisms [28]. However, a too high salt concentration will have an inhibitory effect on microbial activity. In previous studies, a few strains were salt-tolerant and were still able to degrade phenol at a 3~5% salt concentration.

The results are shown in Figure 9: when the salinity in the medium was less than 3%, the strain was able to degrade phenol at an initial concentration of 1000 mg/L by more than 96.8% within 24 h. When the salinity in the medium was greater than 3%, the growth and phenol degradation of the strain were significantly inhibited with increasing NaCl concentration (43.3% at 4% salinity, 4.6% at 5% salinity and 2.5% at 6% salinity). It is known that the strain has basically no ability to degrade phenol when the salinity is greater than 5%, which is due to the phenomenon of salinization when the salinity is too high, which can reduce the activity of microbial proteins and biological enzymes or even inactivate them, affecting the growth and metabolism of microorganisms and their performance in degrading phenol [29].

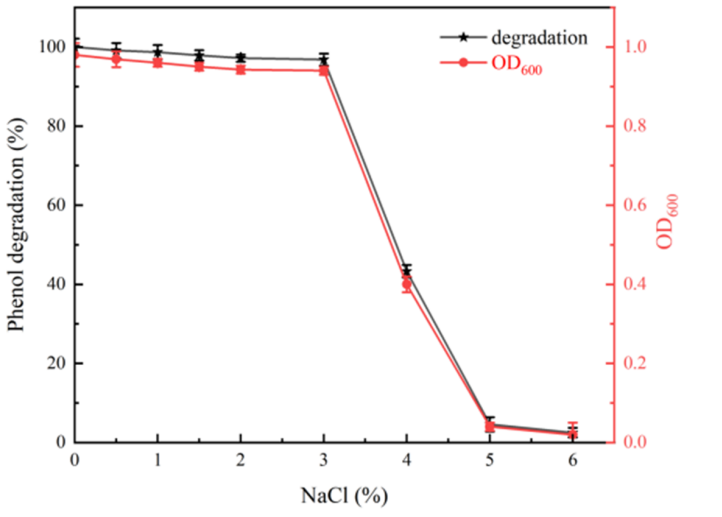

Figure 9. Effect of NaCl concentration on the growth of LA1 and the degradation of phenol.

3.8. Effect of Added Carbon Source on the Degradation of Phenol by the Strain

It has been reported in the literature that the addition of carbon sources other than phenol (especially glucose) has a facilitative effect on the biodegradation of phenol; for example, Liming Wang et al. found that the addition of low concentrations of glucose can greatly promote the biodegradation of phenol [30]. The reason for this is that the strain can first use these carbon sources to increase the strain′s own biomass and thus accelerate the use of phenol. In order to determine the effect of different auxiliary carbon sources on phenol degradation, 100 mg/L maltose, glucose and sucrose were selected and added to the inorganic salt medium, and the inorganic salt medium with phenol as the only carbon source was used as a blank control, and the degradation of 1000 mg/L phenol and the growth of the strain were measured under optimal conditions.

The results are shown in Figure 10: strain LA1 grew more slowly in all four media from 0 to 6 h, which may be a short adaptation process of the bacteria to the new environment. The biological growth of strain LA1 in the control medium and in the media with additional amylum and glucose was rapid from 6 to 24 h, and there was no significant difference between the three, indicating that the addition of amylum and glucose had basically no promoting effect on phenol degradation; however, when maltose was present, the phenol degradation rate was significantly inhibited, and the degradation rate of phenol by the strain in 24 h was 79.6%. The reason may be that, with an increase in external carbon sources in the environment, the bacteria will preferentially use the external carbon sources, which inhibits the production of enzymes to break down phenol, so enzymes capable of degrading phenol are synthesized only after the maltose is depleted, and the degradation rate decreases over a certain period of time; another reason is that the bacteria will produce acid while using external carbon sources such as maltose, and the acidic environment inhibits the degradation of phenol.

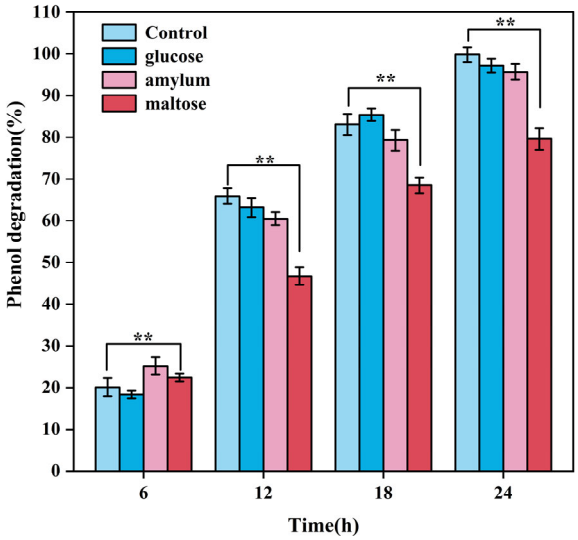

Figure 10. The influence of additional carbon sources on phenol degradation (** indicates a significant difference).

3.9. Effect of Applied Nitrogen Source on the Degradation of Phenol by the Strain

For most of the phenol-reducing bacteria, the addition of easily degradable nitrogen sources in the process of phenol reduction significantly promoted the degradation of phenol and reduced the degradation time. This is due to the preferential use of the easily degradable nitrogen source by the bacteria, which accelerates the growth and reproduction rate of the bacteria and thus increases the degradation rate. At the same time, a small number of phenol-reducing bacteria showed no improvement in degradation efficiency with the added nitrogen source [31]. In order to determine the effects of different auxiliary carbon sources on the degradation of phenol by strain LA1, 100 mg/L NH_4Cl, NH_4NO_3

and CH_4N_2O were selected and added to the inorganic salt medium, the inorganic salt medium with phenol as the single carbon source was used as a blank control, and the degradation of 1000 mg/L phenol and the growth of the strain were measured under optimal conditions to study the effects on the phenol reduction ability of strain LA1.

The results are shown in Figure 11: phenol reduction ability differed greatly with the addition of different nitrogen sources; with NH_4Cl and NH_4NO_3 as the nitrogen sources, phenol was basically degraded completely within 18 h, and the phenol reduction rate was faster than in the comparison group; the degradation of phenol was obviously inhibited when CH_4N_2O was used as the nitrogen source, and the degradation rate of the strain was 70.2% within 24 h for the initial concentration of 1000 mg/L phenol, which may be due to the presence of certain nitrogen sources being unsuitable for some intermediate metabolites produced by biodegradation, thus prolonging the degradation period.

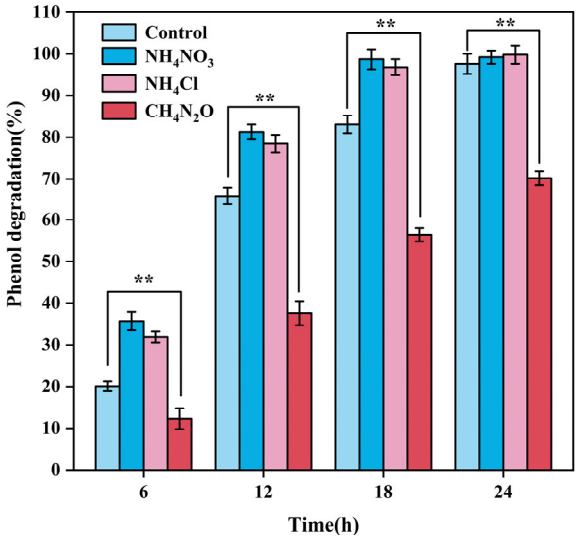

Figure 11. The influence of nitrogen source on phenol degradation (** indicates a significant difference).

3.10. Effect of Initial Concentration on the Degradation of Phenol

Among various phenol treatment methods, microbial degradation is popular because of its high removal rate, complete degradation, and absence of secondary pollution to the environment. After obtaining the optimal degradation conditions for strain LA1, the effect of initial phenol concentration on degradation was investigated under this condition [32]. As can be seen in Figure 12, there is no delay period for phenol degradation when the phenol concentration is lower than 600 mg/L, and phenol can be completely degraded quickly; when the phenol concentrations are 1000 mg/L and 1300 mg/L, delay periods of 3 h and 6 h can be observed, and the corresponding concentrations of phenol can be completely degraded within 18 h and 21 h; when the phenol concentration was 1700 mg/L, the delay period became longer and the degradation rate of phenol became slower. This phenomenon, that the lengthening of the hysteresis period and the degradation time of phenol gradually increased with an increase in phenol concentration, was due to the presence of certain toxic effects of phenol on the cells [33]. Studies have shown that phenol can induce changes in cell membranes and protect cells from cell membrane damage [34], and a similar situation was found by researchers such as Athar Hussain, who also noted that high concentrations of phenol toxicity inhibited the growth of free cells [35], thereby reducing the efficiency of pollutant removal. Geng and Lim et al. also found that high concentrations of phenol treatment influenced not only the inhibition of bacterial growth but also the upregulation of oxidative stress proteins. This also included upregulation of the expression of oxidative stress proteins, heat shock proteins and ABC-type sugar transport proteins. Therefore, the complete removal of phenol depends on the initial concentration of phenol due to the toxic effects caused by phenol [36].

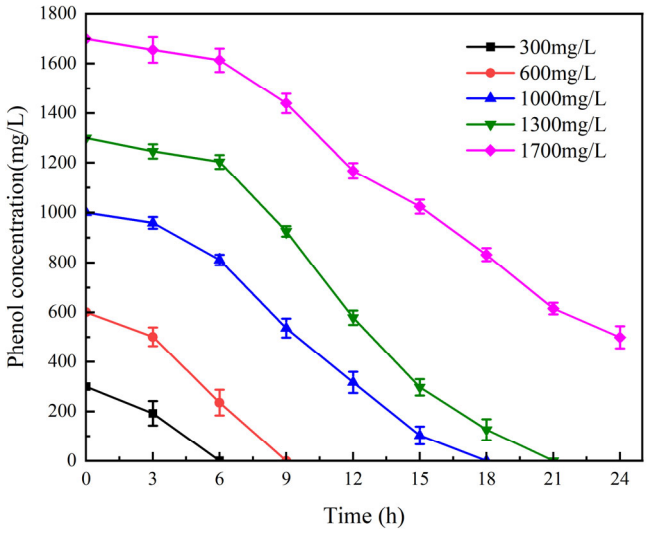

Figure 12. Effects of different initial concentrations on the degradation of phenol.

4. Conclusions

Using phenol as the sole carbon source, a phenol-degrading strain, LA1, was obtained from the activated sludge of an urban wastewater treatment system in Panshan County, Liaoning Province, and was identified as *Stenotrophomonas sp.* genus by 16SrDNA sequencing.

This degradation performance study on strain LA1 showed that the optimal conditions for degradation were pH 7, a growth temperature of 35 °C, a shaking speed of 150 r/min and an inoculum quantity of 10%.

The addition of a carbon source did not promote the growth or phenol degradation ability of the bacteria; especially in the presence of maltose, the phenol degradation rate was significantly inhibited. Phenol reduction ability varied greatly with the addition of different nitrogen sources, and the phenol reduction rate was faster with NH_4Cl and NH_4NO_3 as nitrogen sources than in the control group, while CH_4N_2O as a nitrogen source had a significant inhibitory effect. The novelty of this study is that the screened strain has a strong phenol degradation ability and can tolerate a phenol concentration of 3500 mg/L. Moreover, the degradation efficiency is high and the cycle time is short, and a phenol concentration of 1000 mg/L can be completely degraded in 18 h under optimal degradation conditions.

5. Problems and Prospects

Highly toxic and widespread sources of phenol-containing wastewater seriously threaten human health and safety and are issues that cannot be ignored in environmental protection. Therefore, screening microorganisms with high degradation ability towards phenols is of profound significance for bioremediation of phenol-containing wastewater, and this is crucial for the sustainable development of the environmental protection industry. After obtaining efficient phenol-reducing bacteria from the polluted environment, studying their degradation characteristics and applying them to phenol-containing wastewater treatment systems is one of the most cost-effective ways to treat phenol-containing wastewater today [37]. However, most phenol-reducing strains can only degrade if the phenol concentration is low, and the best degradation concentrations for phenol-reducing bacteria found so far are only in the range 300~1000 mg/L, while the actual concentrations in phenol-containing wastewater discharged from plants can be thousands or even tens of thousands of mg/L. Moreover, wastewater is a very complex mixed system, and it is difficult to meet national discharge standards by treating phenol-containing wastewater with a single strain of bacteria. Therefore, research on the biological aspects of high-concentration phenol-containing wastewater needs to be further explored.

With the rapid development of science and technology, modern technological tools are gradually being applied to the cultivation of efficient strains, such as the use of cell fusion technology to treat strains, biochemical technology to cultivate strains, etc. Moreover, the use of cell fusion, genetic recombination and other techniques to select efficient degrading bacteria has become a trend in this development. Research on and application of the technology of high-efficiency strains has been flourishing, and it can be expected that phenol-reducing strains will play a major role in practical applications to industrial wastewater treatment. A beautiful environment is necessary for the healthy survival of human beings, and it is imperative to screen for efficient, green and applicable phenol-reducing bacteria.

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References

1. Ahmed, S.; Rasul, M.G.; Martens, W.N.; Brown, R.; Hashib, M.A. Heterogeneous photocatalytic degradation of phenols in wastewater: A review on current status and developments. *Desalination* **2010**, *261*, 3–18. [CrossRef]
2. Arutchelvan, V.; Kanakasabai, V.; Nagarajan, S.; Muralikrishnan, V. Isolation and identification of novel high strength phenol degrading bacterial strains from phenol-formaldehyde resin manufacturing industrial wastewater. *J. Hazard. Mater.* **2005**, *127*, 238–243. [CrossRef] [PubMed]
3. Busca, G.; Berardinelli, S.; Resini, C.; Arrighi, L. Technologies for the removal of phenol from fluid streams: A short review of recent developments. *J. Hazard. Mater.* **2008**, *160*, 265–288. [CrossRef] [PubMed]
4. Edalatmanesh, M.; Mehrvar, M.; Dhib, R. Optimization of phenol degradation in a combined photochemical–biological wastewater treatment system. *Chem. Eng. Res. Des.* **2008**, *86*, 1243–1252. [CrossRef]
5. Ramakrishnan, A.; Surampalli, R.Y. Comparative performance of UASB and anaerobic hybrid reactors for the treatment of complex phenolic wastewater. *Bioresour. Technol.* **2012**, *123*, 352–359. [CrossRef]
6. Jiang, H.; Fang, Y.; Fu, Y.; Guo, Q. Studies on the extraction of phenol in wastewater. *J. Hazard. Mater.* **2003**, *101*, 179–190. [CrossRef]
7. Lin, S.; Juang, R. Adsorption of phenol and its derivatives from water using synthetic resins and low-cost natural adsorbents: A review. *J. Environ. Manag.* **2009**, *90*, 1336–1349. [CrossRef]
8. Esplugas, S.; Gimenez, J.; Contreras, S.; Pascual, E.; Rodriguez, M. Comparison of different advanced oxidation processes for phenol degradation. *Water Res.* **2002**, *36*, 1034–1042. [CrossRef]
9. Barriosmartinez, A.; Barbot, E.; Marrot, B.; Moulin, P.; Roche, N. Degradation of synthetic phenol-containing wastewaters by MBR. *J. Membr. Sci.* **2006**, *281*, 288–296. [CrossRef]
10. Essam, T.; Amin, M.A.; Tayeb, O.E.; Mattiasson, B.; Guieysse, B. Kinetics and metabolic versatility of highly tolerant phenol degrading Alcaligenes strain TW1. *J. Hazard. Mater.* **2010**, *173*, 783–788. [CrossRef]
11. Wang, Y.; Song, J.; Zhao, W.; He, X.; Chen, J.; Xiao, M. In situ degradation of phenol and promotion of plant growth in contaminated environments by a single Pseudomonas aeruginosa strain. *J. Hazard. Mater.* **2011**, *192*, 354–360. [CrossRef] [PubMed]
12. Afzal, M.; Iqbal, S.; Rauf, S.; Khalid, Z.M. Characteristics of phenol biodegradation in saline solutions by monocultures of Pseudomonas aeruginosa and Pseudomonas pseudomallei. *J. Hazard. Mater.* **2007**, *149*, 60–66. [CrossRef] [PubMed]
13. Hao, O.J.; Kim, M.H.; Seagren, E.A.; Kim, H. Kinetics of phenol and chlorophenol utilization by Acinetobacter species. *Chemosphere* **2002**, *46*, 797–807. [CrossRef]
14. Mohite, B.V.; Pawar, S.P.; Morankar, A. Isolation, Selection and Biodegradation Profile of Phenol Degrading Bacteria from Oil Contaminated Soil. *Bull. Environ. Contam. Toxicol.* **2011**, *87*, 143–146. [CrossRef] [PubMed]
15. Gąszczak, A.; Szczyrba, E.; Szczotka, A.; Greń, I. Effect of Nickel as Stress Factor on Phenol Biodegradation by Stenotrophomonas maltophilia KB2. *Materials* **2021**, *14*, 6058. [CrossRef] [PubMed]

16. Miyajima, M.; Matsuda, M.; Haga, S.; Kagawa, S.; Millar, B.C.; Moore, J.E. Cloning and sequencing of 16S rDNA and 16S-23S rDNA internal spacer region (ISR) from urease-positive thermophilic Campylobacter (UPTC). *Lett. Appl. Microbiol.* **2002**, *34*, 287–289. [CrossRef] [PubMed]
17. Munaf, E.; Zein, R.; Kurniadi, R.; Kurniadi, I. The Use of Rice Husk for Removal of Phenol from Waste Water as Studied using 4-Aminoantipyrine Spectrophotometric Method. *Environ. Technol.* **1997**, *18*, 355–358. [CrossRef]
18. Paisio, C.E.; Talano, M.A.; Gonzalez, P.S.; Pajuelo-Dominguez, E.; Agostini, E. Characterization of a phenol-degrading bacterium isolated from an industrial effluent and its potential application for bioremediation. *Environ. Technol.* **2013**, *34*, 485–493. [CrossRef]
19. Jiang, Y.; Wen, J.; Bai, J.; Jia, X.; Hu, Z. Biodegradation of phenol at high initial concentration by Alcaligenes faecalis. *J. Hazard. Mater.* **2007**, *147*, 672–676. [CrossRef]
20. Ibrahim, S.; Zahri, K.N.M.; Convey, P.; Khalil, K.A.; Gomez-Fuentes, C.; Zulkarnain, A.; Alias, S.A.; González-Rocha, G.; Ahmad, S.A. Optimisation of biodegradation conditions for waste canola oil by cold-adapted *Rhodococcus* sp. AQ5-07 from Antarctica. *Electron. J. Biotechn.* **2020**, *48*, 1–12. [CrossRef]
21. Deng, T.; Wang, H.; Yang, K. Phenol biodegradation by isolated Citrobacter strain under hypersaline conditions. *Water Sci. Technol.* **2018**, *77*, 504–510. [CrossRef] [PubMed]
22. Liu, Y.; Wang, W.; Shah, S.B.; Zanaroli, G.; Xu, P.; Tang, H. Phenol biodegradation by Acinetobacter radioresistens APH1 and its application in soil bioremediation. *Appl. Microbiol. Biot.* **2020**, *104*, 427–437. [CrossRef]
23. Sowparnika, S.; Rajani, V.; Aswathy, R. Effect of temperature on degradation potential of selected bacterial strains from effluent treatment plant of coir industry. *Int. J. Adv. Res.* **2016**, *4*, 248–254.
24. Gong, Y.; Ding, P.; Xu, M.; Zhang, C.; Xing, K.; Qin, S. Biodegradation of phenol by a halotolerant versatile yeast Candida tropicalis SDP-1 in wastewater and soil under high salinity conditions. *J. Environ. Manag.* **2021**, *289*, 112–525. [CrossRef] [PubMed]
25. Sachan, P.; Madan, S.; Hussain, A. Isolation and screening of phenol-degrading bacteria from pulp and paper mill effluent. *Appl. Water Sci.* **2019**, *9*, 100. [CrossRef]
26. Shahryari, S.; Zahiri, H.S.; Haghbeen, K.; Adrian, L.; Noghabi, K.A. High phenol degradation capacity of a newly characterized Acinetobacter sp. SA01: Bacterial cell viability and membrane impairment in respect to the phenol toxicity. *Ecotox. Environ. Saf.* **2018**, *164*, 455–466. [CrossRef]
27. Maza-Márquez, P.; Martínez-Toledo, M.V.; González-López, J.; Rodelas, B.; Juárez-Jiménez, B.; Fenice, M. Biodegradation of olive washing wastewater pollutants by highly efficient phenol-degrading strains selected from adapted bacterial community. *Int. Biodeterior. Biodegrad.* **2013**, *82*, 192–198. [CrossRef]
28. Wang, Y.; Wang, X.; Li, H.; Lin, K.; Wang, P.; Yang, J.; Liu, Y.; Sun, Z.; Fan, L.; Wu, Z. Treatment of high salinity phenol-laden wastewater using a sequencing batch reactor containing halophilic bacterial community. *Int. Biodeterior. Biodegrad.* **2014**, *93*, 138–144. [CrossRef]
29. Nowak, A.; Wasilkowski, D.; Mrozik, A. Implications of Bacterial Adaptation to Phenol Degradation under Suboptimal Culture Conditions Involving Stenotrophomonas maltophilia KB2 and Pseudomonas moorei KB4. *Water* **2022**, *14*, 2845. [CrossRef]
30. Wang, L.; Li, Y.; Yu, P.; Xie, Z.; Luo, Y.; Lin, Y. Biodegradation of phenol at high concentration by a novel fungal strain Paecilomyces variotii JH6. *J. Hazard. Mater.* **2010**, *183*, 366–371. [CrossRef]
31. Youssef, M.; El-Shatoury, E.H.; Ali, S.S.; El-Taweel, G.E. Enhancement of phenol degradation by free and immobilized mixed culture of Providencia stuartii PL4 and Pseudomonas aeruginosa PDM isolated from activated sludge. *Bioremediat. J.* **2019**, *23*, 53–71. [CrossRef]
32. Pal, B.S.; Sarkar, P.; Pal, P. Isolation and characterization of phenol utilizing bacteria from industrial effluent-contaminated soil and kinetic evaluation of their biodegradation potential. *J. Environ. Sci. Health A* **2014**, *49*, 67–77.
33. Kumari, S.; Chetty, D.; Ramdhani, N.; Bux, F. Phenol degrading ability of Rhodococcus pyrinidivorans and Pseudomonas aeruginosa isolated from activated sludge plants in South Africa. *J. Environ. Sci. Health A* **2013**, *48*, 947–953. [CrossRef] [PubMed]
34. Murínová, S.; Dercová, K. Response Mechanisms of Bacterial Degraders to Environmental Contaminants on the Level of Cell Walls and Cytoplasmic Membrane. *Int. J. Microbiol.* **2014**, *2014*, 873081. [CrossRef] [PubMed]
35. Hussain, A.; Dubey, S.K.; Kumar, V. Kinetic study for aerobic treatment of phenolic wastewater. *Water Resour. Ind.* **2015**, *11*, 81–90. [CrossRef]
36. GENG, A.; LIM, C.J. Proteome Analysis of the Adaptation of a Phenol-Degrading Bacterium Acinetobacter sp. EDP3 to the Variation of Phenol Loadings. *Chin. J. Chem. Eng.* **2007**, *15*, 781–787. [CrossRef]
37. Gu, Q.; Wu, Q.; Zhang, J.; Guo, W.; Wu, H.; Sun, M. Community Analysis and Recovery of Phenol-degrading Bacteria from Drinking Water Biofilters. *Front. Microbiol.* **2016**, *7*, 495–506. [CrossRef]

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