Biodegradation of Higher Concentration Phenol by Pseudomonas aeruginosa HS-D38

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Abstract: Phenol is an important and common environmental pollutant. In this study, Pseudomonas aeruginosa HS-D38 strain was used to degrade phenol, and the degradation characteristics were investigated. Results indicated that HS-D38 strain could degrade phenol and utilize it as the sole carbon, nitrogen and energy source. UV-visible spectra analysis showed that 300 mgL⁻¹ phenols could be completely degraded within 10 h by HS-D38 strain after an initial lag phase of 2 h, and the peak decreased gradually with the degradation of phenol by HS-D38. At the same time, an increase of OD₆₀₀ values in the culture was also observed. Phenol could be mineralized at higher concentration of 500 mgL⁻¹ by HS-D38 strain, whereas the degradation did not occur when the concentration was exceeded 700 mgL⁻¹ even after 72 h. A key intermediate was detected analyzing the degradation solution with HPLC and it was identified to p-hydroxybenzoic acid. The peaks disappeared after 15 h indicating intermediates were also degraded by HS-D38 strain and the metabolism proceeded through the p-hydroxybenzoic acid pathway.

Keywords: phenol; Pseudomonas aeruginosa; biodegradation; intermediate Metabolites

I. Introduction

Phenol and phenolic compounds are aromatic pollutants, which the distributions in various environmental sites are in the form of either natural or artificial mono-aromatic compounds. These compounds are common constituents of aqueous effluents as waste by-products from industrial processes, including oil refineries, petrochemical plants, ceramic plants, chemical, textiles, dye stuffs, steel industries, phenolic resin industries and pharmaceutical industries [1,2]. Phenol can be toxic to some aquatic species at concentrations in the low concentration range, and phenolic contaminants can damage sensitive cells, including permeabilisation of cellular membranes and cytoplasmic coagulation [3,4]. Toxic and highly soluble phenolic compounds have been introduced into many aquifers as a result of industrial activity and careless waste disposal practices, and thus cause profound health and environmental problems [5]. The wide use of phenols in some area and their potential toxicity has led the U.S. Environmental Protection Agency to include them on its list of priority pollutants [6].

The environmental clean-up of phenol by adsorption, solvent extraction, chemical oxidation, incineration and a biotic treatment procedure suffers from serious drawbacks such as economic issues and the production of hazardous byproducts [7]. Biodegradation is generally preferred, due to rapid, mild reaction conditions, cost effective, the complete mineralization, friendly environment and no secondary pollution, and it offers a promising strategy by which some wastes may be economically and safely detoxified. Biodegradation of phenolic compounds by microorganism has been extensively studied and a large number of phenol-degrading bacteria have been isolated [8-10]. Although phenol is biodegradable both aerobically and anaerobically, it can be growth inhibitory to microorganisms at elevated concentrations, even to those species that can use it as a substrate [11]. Degradation of phenol was also found incomplete for concentrations higher than 400 mgL⁻¹, and the residual phenol might inhibit the removal of N and P in wastewater treatment [8,12]. Nowadays...
many study aims to isolation phenol degrader at higher concentration.

In this study, *Pseudomonas aeruginosa* HS-D38 strain, which was originally isolated from cotton soils [13], was used to degradation phenol. The characteristics of biodegradation were investigated, including dynamic degradation, effects of different initial concentration and the metabolic pathway.

II. MATERIALS AND METHODS

A. Bacterial strain and culture medium

The wide-type bacterial strain HS-D38 was originally isolated from cotton soils contaminated by organophosphorus pesticides and identified as *Pseudomonas aeruginosa* in our laboratory (Zheng et al., 2006).

HS-D38 strain was incubated into LB medium at 35°C for 15 h, then the cells were harvested, centrifuged (5000 r/min, 10 min, 4°C), washed for 3 times and suspended with sterile MSM medium (OD600=0.5) as prepared inoculums.

Mineral salt medium (MSM) was used for cultured medium of phenol degradation and the composition was as follows (gL-1): KH₂PO₄ 0.5, K₂HPO₄ 1.5, NaCl 0.5, MgSO₄·7H₂O 0.5, and 1.5 ml Trace solution (Li et al., 2006). All media were adjusted pH to 7.0 and autoclaved for 30 min.

B. Metabolites extraction and preparation

On the basis of phenol mineralization time in MSM medium, the samples were collected at 8 h and 12 h for metabolite study. To recover enough metabolites, extraction were carried out from 1000 ml degradation samples by ethyl acetate, and dehydrated with sodium sulfate, and evaporated using a rotary evaporator, and then the sediment was redissolved into 1ml methanol and filtered with a 0.22-μm membrane as the samples of UV-vis spectral and HPLC analysis.

C. Analytical methods

Bacteria growth was evaluated spectrophotometrically at 600 nm using a 722S spectrophotometer (Shanghai, China). The dynamic curves of phenol degradation were determined by sampling at each hour and scanning with UV-vis spectrophotometer (S-3100, SCINCO Co. LTD., Korea).

The degradation rates were quantified by high performance liquid chromatography (HPLC) equipped with 2487 UV detector (Waters 2695, Waters Corp., USA). A reverse-phase column C₁₈ (Waters XTerra 150 cm × 4.6 cm i.d., 3 μ particles) was used for chromatographic separations which maintained at 25 °C throughout the analysis. The mobile phase consisted of methanol and water (30/70, v/v), and a flow rate of 1.0 mlmin⁻¹ was used. The injection volume was 20 μl and the detector wavelength was fixed at 325 nm.

III. RESULTS AND DISCUSSION

A. Dynamic Biodegradation of phenol

In a mineral salt medium (MSM), HS-D38 strain could degrade phenol and used it as the sole carbon, nitrogen and energy source. 300 mgL⁻¹ phenols were total degraded within 10 h at 35°C, 150 rpm and 1% microbial inoculums. Samples of the degradation solutions were taken in each hour for measurement of biomass and phenol concentrations.

As shown in Fig 1A, an absorption peak of phenol at 263.5 nm was observed, and the strength of peak on UV–visible spectra decreased gradually with the degradation processing, suggesting that phenol was effectively degraded in MSM medium by HS-D38 strain. After an initial lag phase of 2 h, the peak decreased more rapidly in the next 8 h, meaning the phenol degradation became active. By 10 h, the peak eventually disappeared and phenol concentration was undetectable, indicating that 300 mgL⁻¹ phenols were completely degraded and HS-D38 strain could effectively degrade higher concentration phenol. At the same time, an increase of OD₆₀₀ values in the culture was also measured, corresponding to the phenol decrease. The biomass increased from the initial 0.098 to 0.41 at 10 h, and the concentration of phenol from the initial 300 mgL⁻¹ decreased gradually and finally no phenol was detected. In the contrast, no depletion of phenol concentration or increase in the values of OD₆₀₀ was noticed (Fig.1B).

B. Effects of different initial phenol concentration

In order to determine the degradation ability of *P. aeruginosa* HS-D38, experiment was carried out at different initial concentrations of phenol. HS-D38 strain could grow and
 degrade phenol in MSM medium with the phenol concentration was under 500 mgL$^{-1}$, and the strains grew well at the concentration range of 100 mgL$^{-1}$ to 500 mgL$^{-1}$, while at 600 mgL$^{-1}$ phenol, HS-D38 strain grew slowly but the enzyme involved in phenol degradation released by HS-D38 still reacted the substrate of phenol, phenol was degraded completely after 24 h (Fig. 2A), When the concentration was exceeded 700 mgL$^{-1}$, phenol could not be degradation even after 72 h, it concluded that high concentration of phenol was toxic to cells and restrained HS-D38 growth[8]. The optimal concentration of phenol was at 500 mgL$^{-1}$ concentration (Fig. 2B).

### C. Metabolites analysis by HPLC

Intermediate metabolites were analyzed using HPLC technique. Two peaks, which the retention times were at 2.38 min and 6.14 min, were detected in the filtrate of cultures sampled at time 8 h (Fig. 3A). Comparing with the authentic phenol eluted at time 2.318 min (Fig. 3B) and $p$-hydroxybenzoic acid at 6.141 min (Fig 3C). The compound peak of retention time 6.14 min was a key intermediate in degrade phenol in MSM medium with the phenol concentration was under 500 mgL$^{-1}$, and the strains grew well at the concentration range of 100 mgL$^{-1}$ to 500 mgL$^{-1}$, while at 600 mgL$^{-1}$ phenol, HS-D38 strain grew slowly but the enzyme involved in phenol degradation released by HS-D38 still reacted the substrate of phenol, phenol was degraded completely after 24 h (Fig. 2A), When the concentration was exceeded 700 mgL$^{-1}$, phenol could not be degradation even after 72 h, it concluded that high concentration of phenol was toxic to cells and restrained HS-D38 growth[8]. The optimal concentration of phenol was at 500 mgL$^{-1}$ concentration (Fig. 2B).

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biodegradation pathway of phenol, and it was identified to the
\( p \)-hydroxybenzoic acid which was corresponds exactly to the
authentic compound. Furthermore, with the process of phenol
degradation, the peaks were disappeared analyzing the sample
at 15 h, which meant phenol was completely degradation.
These results indicated that the phenol was mineralized by
HS-D38 strain and the metabolization proceeded via the
\( p \)-hydroxybenzoic acid pathway (Fig.4).

Figure 4. Proposed metabolic pathway of phenol degradation by HS-D38.

IV. CONCLUSIONS

\textit{P. aeruginosa} HS-D38 strain could mineralized phenol
and used it as the sole carbon, nitrogen and energy source. 300
mg\text{L}^{-1} \text{phenols} were completely degraded in 10 h in MSM
culture. Higher concentration phenol (above 500 mg\text{L}^{-1}) could
be degraded within 12. The key intermediate of phenol
biodegradation which the retention time was at 5.139 min was
determined and identified to the \( p \)-hydroxybenzoic acid,
suggested that HS-D38 degraded phenol through the
\( p \)-hydroxybenzoic acid pathway. It was significant for offering
a strategy of bioremediation the phenolic compound pollution
by \textit{P. aeruginosa} HS-D38.

ACKNOWLEDGMENTS

This work was supported by the Specialized Research Fund
for the Doctoral Program of Higher Education (20060511002)
and by the Construction Fund for “211” Project of the Ministry
of Education of China and by the Huanggang Science and
Technology Found of Hubei Province of China (07HG183).

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