Effects of Degradation and Biostimulation on Phenolic Pollutants with Targeting Strains

Zhao Liang, Yanju Li*, Dongxu Zhao and Pan Shang

School of life science, Beijing Institute of Technology, Str. Zhongguancun, Hai Dian District, Beijing, P.R. China

*Corresponding author e-mail: liyanju721@163.com

Abstract. Aiming at the problems of high content and high environmental pollution of phenols in petrochemical wastewater, a strain capable of efficiently degrading phenol, catechol, m-cresol and 2,4-DCP was isolated from petrochemical wastewater and identified as *Stenotrophomonas maltophilia*, named DSM2. The highest degradation percentages of strain DSM2 to phenol, catechol, m-cresol and 2,4-DCP were 82.65%, 92.28%, 95.39% and 49.28%, respectively. When strain DSM2 was cultured with strain B29 which could produce biosurfactant, the results showed that strain PB29 could promote the degradation to catechol, m-cresol and 2,4-DCP, increased by 4.67%, 30.11% and 17.39%, respectively. And adding biosurfactant produced by strain PB29 enhanced the degradation of phenol and 2,4-DCP, increased by 6.11% and 11.90%.

1. Introduction

In recent years, a large amount of waste water containing phenolic compounds has been discharged into the environment. Studies have showed that phenol at the concentration of 1/1 (g/kg) of body weight is deathful to animals and human beings [1]. It has been proved that a certain concentration catechol leads to DNA damage, coma, and death [2]. *m*-Cresol and 2,4-dichlorophenol (2,4-DCP) are also difficult to degrade and could cause great harm to the creature [3] [4].

Although chemical and photochemical oxidationmethod have been adopted [5], microbial degradation is widely considered to be environmental friendly and cost effective, has become an attractive way to degrade phenols [6] [7].

Biosurfactants are a class of microbial metabolites with surface-active properties and they could enhance the degradation to organic pollutants [8]. They are structural diversity, low toxicity, biodegradability [9]. Ding Ying [10] explore the effects of biosurfactants on the biodegradation of phenol by *Candida tropicalis*, the result showed that the biosurfactant enhanced phenol degradation.

The aims of the study were to (i) screen a strain could degrade 4 kinds of phenols and the properties of the strain degraded phenol, catechol, *m*-cresol and 2,4-DCP were studied; (ii) the stimulation effect of biosurfactant-producing strain on biodegradation of phenols was studied through the co-culture of degrading strain and stimulating strain, which provided scientific basis for the efficient degradation of phenols.

2. Materials and Methods

2.1. Microorganis

Strain PB29, was isolated in this laboratory previously, the main components of biosurfactant produced by it was glycolipid.

2.2. Chemicals and materials

Petrochemical waste water samples were collected from Shandong Environmental Protection Engineering Co., Ltd. China, April 12, 2014. All chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd.

2.3. Methods

2.3.1. Screening and Degradation Spectrum of Strain. Mineral salt medium (MSM) was used as the liquid degradation medium and was composed of 1.5g/L of K_2HPO_4, 1.5g/L of KH_2PO_4, 0.5g/L of NaCl, 0.1g/L of $MgSO_4{\cdot}7H_2O$, 0.1g/L of $CaCl_2$, 0.001g/L of $FeSO_4{\cdot}6H_2O$ and 1000mL distilled water at pH 7.0. 10% (V/V) petrochemical wastewater was added to MSM with 200mg/L of phenol, catecho, m-cresol and 20mg/L of 2,4-DCP as carbon sources, respectively. All flasks were shaken at 160rpm and 30℃. Then the culture was diluted and spread onto sterilized MSM agar plates containing phenols and incubated at 30℃ in the dark. The strains could tolerate 4 kinds of phenols were used in the experiment below: 8% (V/V) of bacteria cell suspensions (OD_{600}=0.8) was inoculated in MSM containing 200mg/L of phenol, catecho, m-cresol and 20mg/L of 2,4-DCP, respectively. The controls were not inoculated with cell suspensions. After 24h/7d, the samples of phenol, catechol, m-cresol/2,4-DCP were taken for determination. The degradation percentages of 4 kinds of phenols were calculated.

2.3.2. Strain Identification. To identify DSM2, 16S rDNA gene sequence analysis was performed [11].

2.3.3. Degradation of Phenols In Different Concentrations. The concentrations (mg/L) were phenol: 200, 400, 600, 800, 1000, 1200; catechol: 200, 400, 600, 800, 1000, 1200; m-cresol: 100, 200, 300, 400, 500, 600; 2,4-DCP: 10, 20, 40, 60, 80, 100. The inoculation amount was 8% (V/V). The controls were not inoculated with cell suspensions. Every 2h/1d, the samples of phenol, m-cresol, catechol/2,4-DCP were taken for determination. The degradation percentage of different concentrations were calculated.

2.3.4. The Biostimulation Of Phenolic Degradation By Pb29. In order to explore the effects of biostimulation on the degradation of strain DSM2 to 4 kinds of phenols, the biosurfactant-producing strain PB29 was cultured with DSM2 and the biosurfactant produced by strain PB29 was used in the experiment below: 4% of DSM2 + 4% of PB29 cell suspensions, 8% of DSM suspensions +100mg/L biosurfactant, 8% of DSM2 cell suspensions, 8% of PB29 cell suspensions were inoculated in MSM containing phenol (600mg/L), m-cresol (1000mg/L), catechol (400mg/L) and 2,4-DCP (60mg/L), respectively. The controls were not inoculated with cell suspensions. The degradation percentages of phenols were calculated.

2.3.5. Determination Method. The residual amount of phenol and m-cresol and catechol were determined by 4-anino-antipyrine [12] and concentration of the 2,4-DCP was determined using a UV-spectrophotometer [13].

2.3.6. Calculation and Statistical Analysis. The degradation percentagse were calculated from equation:

$$Y= [C_{ck} - C_t]/C_{ck} \times 100\%$$

Y*ck* (%): degradation percentage of phenols; C*ck* (mg/L): control concentration; C*t* (mg/L): treatment residual concentration of phenols; All treatments were prepared separately in triplicate.

2.4. Results and discussion

2.4.1. Screening and Degradation Spectrum of Strain

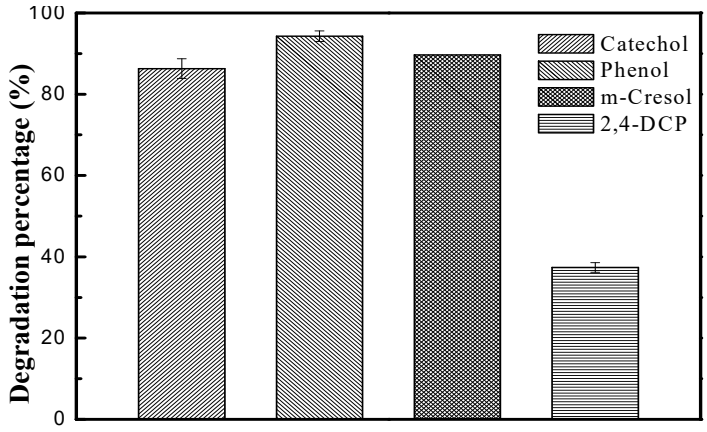

Figure 1. The degradation of catechol, phenol, m-cresol, and 2,4-DCP by strain DSM2.

The results showed that strain DSM2 could tolerate to phenol, catechol, *m*-cresol and 2,4-DCP. The degradation percentages of DSM2 to them were 94.30%, 86.32%, 89.68% and 37.38%, at 24h, 24h, 24h and 7d, respectively (Figure 1).

2.4.2. Strain Identification

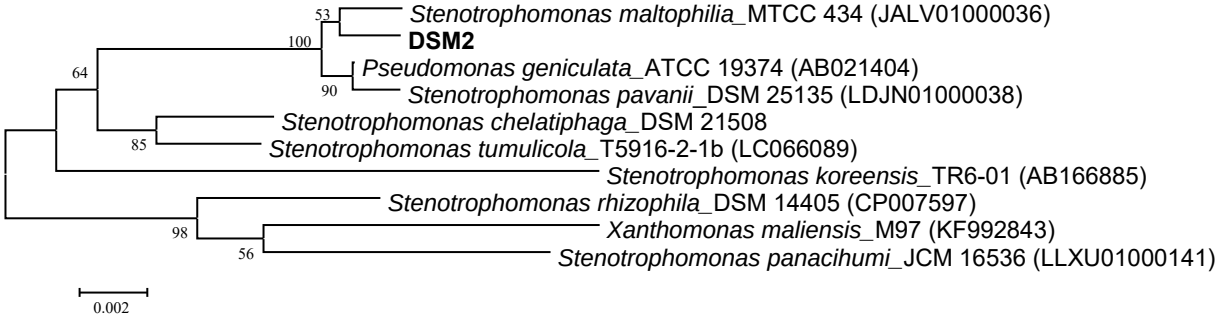

Figure 2. Phylogenetic tree of strain DSM2 based on the 16S rRNA gene homology.

After 16S rDNA sequencing analysis and the data were compared with those of Genbank. Then the related softwares were used to analyze the phylogenetic tree. The results showed that the similarity between DSM2 and *Stenotrophomonas maltophilia* was 99.23% (>95%), so strain DSM2 was identified as *Stenotrophomonas maltophilia.* (Figure 2).

2.4.3. Degradation of Phenols in Different Concentrations. Recently, many phenolic-degrading strains have been isolated from petrochemical wastewater [14]. N. M. Arif [15] reported that Rhodococcus sp. strain AQ5NOL2 could degrade 900mg/L phenol, at 100h reached highest degradation rate, and the strain also could degrade 100 mg/L of 2,4-DCP.

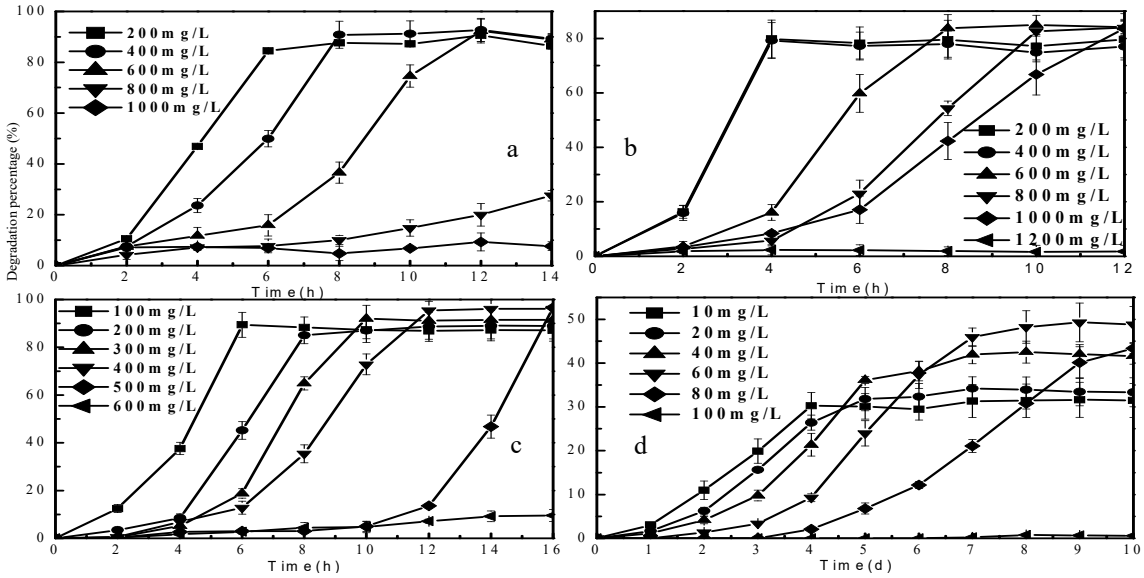

Figure 3. Degradation percentage of DSM2 to different concentrations of phenol (a), catechol (b), m-cresol (c) and 2,4-DCP (d).

The results showed that strain DSM2 could degrade 200-800mg/L of phenol, and the optimum degradation percentage was 92.28% at 12h (Figure 3a). Besides, highest degradation concentration of the strain to catechol, *m*-cresol and 2,4-DCP were 1000mg/L, 500mg/L and 80mg/L, the highest degradation rates were 83.76%, 95.58% and 49.28% at 10h, 12h and 9d (Figure 3b,3c,3d).

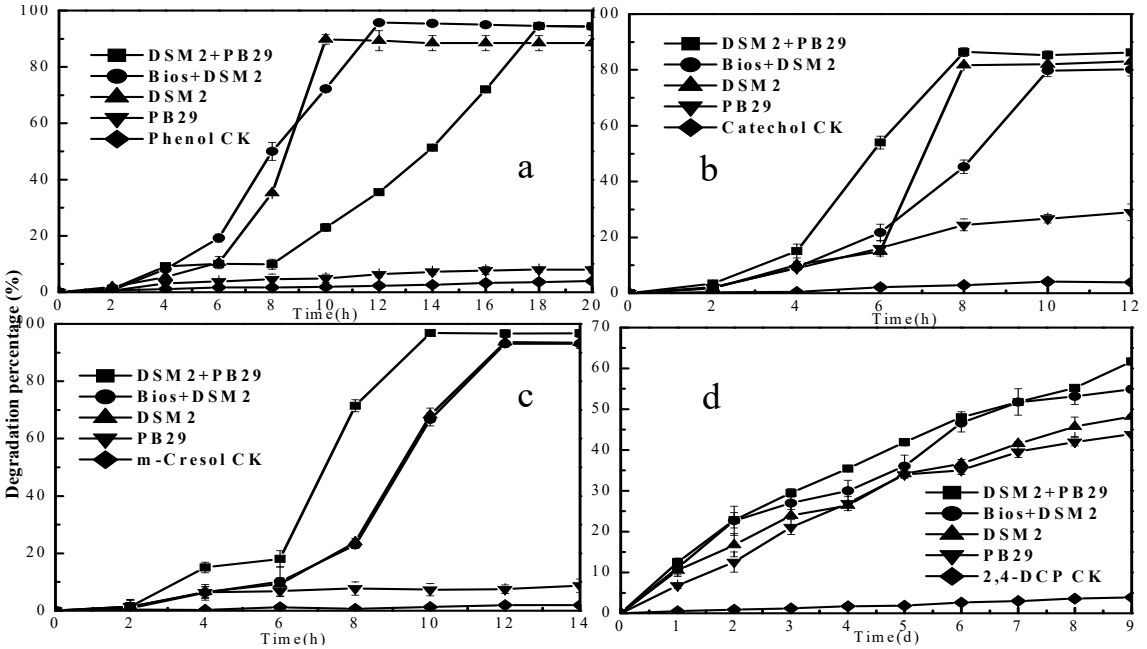

Figure 4. Degradation percentage of DSM2 to phenol (a), catechol (b), m-cresol (c), and 2,4-DCP (d) at different conditions. Biosurfactant was abbreviated as Bios in this figure above.

2.4.4. The Biostimulation Of Phenolic Degradation By Pb29. At 12h, the degradation percentages of mixed strains DSM2+PB29 and strain DSM2 were 35.54% and 88.44%, but these were 94.55% and 88.55% at 18h, increased by 6.00% (Figure 4a). Compared to single strain DSM2, mixed strains DSM2+PB29 enhanced the degradations of catechol, m-cresol and 2,4-DCP at 8h, 10h and 9d, increased by 4.67%,30.11% and 17.39% (Figure 4b,4c,4d). Compared to the degradation percentages of strain DSM2 to phenol, catechol, m-cresol and 2,4-DCP, were 88.44%, 81.70%, 66.71% and 47.09% at 12h, 8h, 10h and 9d, biosurfactant+ DSM2 groups were 94.55%, 45.28%, 68.18%, 56.99%. So strain PB29 enhanced the degradation of DSM2 to catechol, m-cresol and 2,4-DCP. Biosurfactant promoted the degradation of phenol and 2,4-DCP. Probably because biosurfactant increased the solubility of phenol and 2,4-DCP in water and could be metabolized with the sole carbon source.

3. Conclusion

The newly isolated strain DSM2 (*Stenotrophomonas maltophilia*) could degrade efficiently phenol, catechol, *m*-cresol, and 2,4-DCP. The optimum degradation percentages of DSM2 to 600mg/L phenol, 600mg/L catechol, 400mg/L *m*-cresol and 60mg/L 2,4-DCP were 92.28%, 83.76%, 95.58% and 49.28%. The co-culture of biodegradation-producing strain PB29 and DSM2 enhanced the biodegradation of catechol, *m*-cresol, and 2,4-DCP, increased by 4.67%, 30.11% and 17.39%, at 8h, 10h and 9d, respectively. However, the biosurfactant produced by strain PB29 just promote the biodegradation of phenol and 2,4-DCP, increased by 6.11% and 11.90%, at 12h and 9d.

Acknowledgements

This Study was supported by the Open Project Program of State Key Laboratory of Petroleum Pollution Control (Grant No. PPC2016016), CNPC Research Institute of Safety and Environmental Technology. *YANJU LI is corresponding author.

References

[1] ATSDR, Agency for Toxic Substances and Disease Registry. 2015. Toxicological profile for phenol. Accessed 20 December 2016. Sheppard S C., (1998), Environmental Contaminant Reference Databook, Volume III [J]. Van Nostrand Reinhold, (4):985-986.

[2] S. Suresh, Vimal Chandra Srivastava, Indra Mani Mishra. 2011. Study of Catechol and Resorcinol Adsorption Mechanism through Granular Activated Carbon Characterization, pH and Kinetic Study [J]. Separation Science & Technology, 46 (11):1750-1766.

[3] Zepeda A, Ben-Youssef C, Rincón S, et al. 2013.Complete and simultaneous removal of ammonium and m-cresol in a nitrifying sequencing batch reactor [J]. Biodegradation,24 (3):377-85.

[4] Zhang C, Zhou M, Ren G, et al. 2015. Heterogeneous electro-Fenton using modified iron-carbon as catalyst for 2,4-dichlorophenol degradation: influence factors, mechanism and degradation pathway [J]. Water Research, 70:414.

[5] Zhang F, Zhang W, Zhao L F, et al. 2016. Degradation of phenol with Horseradish Peroxidase immobilized on ZnO nanocrystals under combined irradiation of microwaves and ultrasound [J]. Desalination & Water Treatment, :1-11.

[6] Acikgoz E, Ozcan B. 2016. Phenol biodegradation by halophilic archaea [J]. International Biodeterioration & Biodegradation, 107:140-146.

[7] Patel B P, Kumar A. 2015. Optimization study for maximizing 2,4-dichlorophenol degradation by Kocuria rhizophila strain using response surface methodology and kinetic study [J]. Desalination & Water Treatment, 57 (39):1-12.

[8] Liu J F, Mbadinga S M, Yang S Z, et al. 2015. Chemical structure, property and potential applications of biosurfactants produced by Bacillus subtilis in petroleum recovery and spill mitigation. [J]. International Journal of Molecular Sciences, 16 (3):4814.

[9] Santos D K F, Rufino R D, Luna J M, et al. 2016. Biosurfactants: Multifunctional Biomolecules of the 21st Century: [J]. International Journal of Molecular Sciences, 17 (3):401.

[10] Chang Y C, Reddy M V, Umemoto H, et al. 2015. Bio-Augmentation of Cupriavidus sp. CY-1 into 2,4-D Contaminated Soil: Microbial Community Analysis by Culture Dependent and Independent Techniques [J]. Plos One, 10 (12):1-18.
[11] Ding Y, Yuan X Z, Zeng G M, et al. 2010. Effects of surfactants on the biodegradation of phenol by Candida tropicalis [J]. Environmental Science, 2010, 31 (4):1047-1052.
[12] Ailijiang N, Chang J, Wu Q, et al. 2016. Phenol Degradation by Suspended Biomass in Aerobic/Anaerobic Electrochemical Reactor [J]. Water Air & Soil Pollution, 227 (7):233.
[13] Elkarmi A Z, Abuelteen K H, Atta A A, et al. 2009. Biodegradation of 2,4-dichlorophenol originating from pharmaceutical industries. [J]. African Journal of Biotechnology, 8 (11):2558-2564.
[14] Basak B, Bhunia B, Mukherjee S, et al. 2013. Optimization of physicochemical parameters for phenol biodegradation by PHB5 using Taguchi Methodology[J]. Desalination & Water Treatment, 51 (34-36):6846-6862.
[15] Arif N M, Ahmad S A, Syed M A, et al. 2013. Isolation and characterization of a phenol‐degrading Rhodococcus sp. strain AQ5NOL 2 KCTC 11961BP[J]. Journal of Basic Microbiology, 53 (1):9-19.