Isolation of Highly Efficient Phenol Degradation Strain and Characterization of Degradation of Phenol

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Abstract: The biodegradation of phenol was carried out from the sludge in the sediment of the chemical plant in the Heilongjiang Chemical Industry Group. The phenol was used as the only carbon source of the inorganic salt medium to obtained from the biodegradable strain of phenol to obtain more efficiency antimicrobial phenol and study its degradation characteristics. The phenol degradation rate of FB-2 was the highest, 87.9% and the phenol degradation is FB-4, which is 65.5%. pH7.2, the degradation rate of phenol was about 78.0%, and the degradation rate of phenol was 56.9%. When the phenol concentration was 100 mg/L, the degradation rate of phenol was reduced to 38.8% when the phenol concentration was 91.2% and the concentration of phenol increased to 2200 mg/L. The optimum conditions for inoculation were 20%, the pH was equal to 7.1, the temperature was 25°C, and the strain was screened with high efficiency phenol degradation, named FB-2.

1. Introduction

Petroleum, chemical, gas, coking and phenols and other production plants discharge of waste water which contains a lot of phenol [1]. Phenol wastewater can cause water pollution, resulting in fish deaths, causing damage to crops, and ultimately threatening human health. Many countries have classified phenol as one of the most important contaminants. At present, the domestic and foreign methods of dealing with phenol wastewater are physical, chemical, microbial and various combinations [2]. The microbial method mainly uses the microbial metabolic activity to remove the toxic substances in the waste water; the treatment method has no secondary pollution and is safe and economical. At present, the microbes that have been identified to degrade phenol are mainly *Pseudonomonas. Sp.*[3], *Bacillus sp.*[4], *Yeast trichosporon*[5], *Rhizobia*[6], *A. calcoaceticus*[7] and so on, Phenol-degrading strains are mostly present in the waste water, sludge and wastewater contaminated by phonetic pollutants[8]. In this paper, the strains were screened from the sludge contaminated with phenol wastewater to obtain phenol-resistant bacteria, and the phenol-degrading strains were screened on the inorganic salt culture with phenol as the sole carbon source to further determine the influencing factors of phenol degradation. The application value of phenol-specific wastewater was studied.

2. Materials and Method

2.1 Source of strain

The sludge was collecting from the sewage sludge at the Jiamusi Petrochemical Plant in Heilongjiang Province.

2.2 Medium
Basal medium: NaCl 5.0g/L, peptone 10.0g/L, agar 15-20.0g/L, yeast extract 5.0g/L, adjusted pH 7.0. Ingredients: CaCl2 0.1g/L, FeSO4.7H2O 0.01g/L, K2HPO4 0.5g/L, MnSO4.7H2O 0.05g/L, NaCl 0.2g/L, KH2PO4 0.5g/L, MgSO4.H2O 0.01g/L, NH4NO31.0g/L Phenol added according to the experimental requirements, adjust the pH to 7.0. Enrichment medium: glucose 10.0g/L, nutrient agar 33.0g/L, yeast leaching powder 10.0g/L, adjusted pH 7.5.

2.3 Experimental Methods
2.3.1. Domestication and Separation of Degradation Phenol Strains. In the clean bench, 10.0mL containing 0.1g/L phenol into the basic culture medium, take 10.0g of sludge and 90.0mL of distilled water for 15min, put it aside for 5min; take the supernatant as the bacteria solution[8]. 1.0mL of the bacteria solution into the sterile water were made 100, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} gradient of the bacteria, and then from the bacteria in the test tube to take 1.0 mL with the coating method Inoculated on basal medium plates and cultured at pH 7 and 25℃ for 24h to 48h. Pick a single colony in the enrichment of the medium plate on the crossed and spread out. The plates were placed in a constant temperature incubator at 25℃ for 24 hours to 48 hours and stored in a refrigerator at 4℃. Microbiological identification was according to Gram stain.
2.3.2. Screening of Phenol. A single strain with OD of 600 was added to the inorganic salt medium at 20% (volume fraction) inoculated to screen the best strain with the highest degradation ability of phenol at the same time.
2.3.3. Effect of culture temperature on phenol-reducing ability of strain. Dipped in the appropriate amount of bacterial liquid culture and biochemical incubator temperature adjustment was Gradient setting at pH 7. Determination of phenol concentration was during 24h to calculate the phenol degradation rate.
2.3.4. Effect of pH Value on Phenol Tolerance. The culture conditions were 28℃, 220r/min, adjusted to pH 5.0, 6.0, 7.0, 8.0 and 9.0, and the pH was adjusted to 20%. The effect of the value on the phenol was reducing ability of the strain. After 24h the phenol concentration was determined and the phenol degradation rate was calculated.
2.3.5. Effects of Substrate Concentration on Phenol Tolerance. The culture conditions were 270r/min, 28℃, pH7 and the culture medium was inoculated at the concentration of 0.1, 0.5, 1.0, 1.5 and 2.2 g/L in 20% The effect of phenol concentration on the degradation rate of phenol was determined. After 24 h, the concentration of phenol was determined and the degradation rate of phenol was calculated [9].
2.3.6 Effect of Inoculation Amount on Phenol Tolerance. The results showed that the inoculation amount of the strain was 5%, 10%, 15%, 20%, 25% and 30% respectively. The culture conditions were as follows: pH value was 7, 270r/min and 28℃, substrate concentration was 1000mg.The amount of phenol was measured and the phenol degradation rate was calculated.

2.4 Analysis method
The effects of temperature, inoculation amount, pH value and substrate concentration on the degradation performance of the phenol degradation bacteria were analyzed. The concentration of phenol was measured by spectrophotometer with 4-aminoanantipyrine [10].

3. Results and discussion

3.1 Preliminary identification of degrading bacteria
FB-2, FB-3, FB-4, FB-5 and FB-6 were identified, and the growth rate of FB-2 strain was the fastest on the culture medium. The diameter of 89.1mm (plate diameter 90mm), FB-4 strain was growth rate of the slowest in the medium diameter of 56.4mm. Microscopic examination of Gram-negative bacteria, single cells were rod-shaped, single or several cells connected. (See Table 1).

Table 1. Preliminary results of phenol-resistant strains

number of the strain	Colony diameter / mm（a week）	pattern	Stained Grams
FB-1	72.7	Bacilli,	purple
FB-2	89.1	Bacilli,	purple
FB-3	68.3	Bacilli,	purple
FB-4	56.4	Bacilli,	purple
FB-5	78.2	Bacilli,	purple
FB-6	79.2	Bacilli,	purple

3.2 Effect of Temperature on Degradation Performance of Phenol Degradation Bacteria

The degradation rate of phenol degradation bacteria at different temperatures is shown in Fig. Compared with the temperature, the degradation rate of the phenol decreased with the increase of the temperature at 25℃-40℃. The degradation ability of the six strains was the best at 25℃. Among them, the degradation rate of phenol was the highest, reaching 87.9%, the lowest degradation rate of FB-4 was 65.5%. When the culture temperature was 30℃-40℃, the degradation ability of the strain decreased gradually, and the degradation rate of FB-2 was reduced to 57.6% at 40℃. At this time, the degradation rate of phenol was the lowest at 49.1. According to the data analysis, the optimum degradation rate of phenol was 25℃.

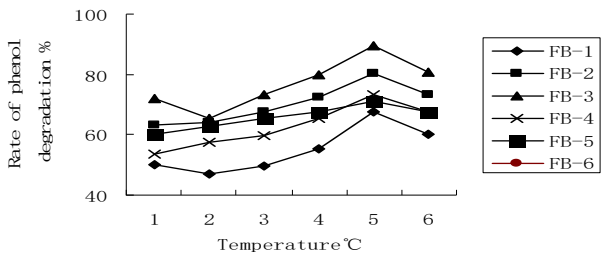

Fig.1 Effect of temperature on the degradation performance of phenol degradation bacteria.

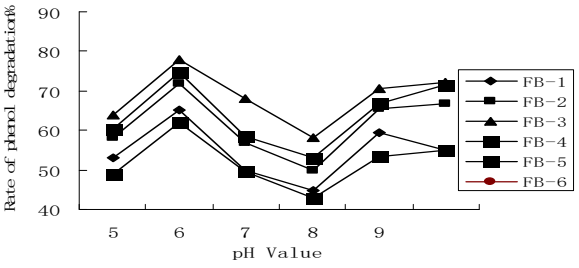

Fig.2 Effect of pH on degradation performance of phenol degradation bacteria

3.3 Effect of pH on Degradation Performance of Phenol Degradation Bacteria

The degradation rate of phenol degradation bacteria at different pH values is shown in Fig. 2, and the degradation rate of phenol is only 71.4% at only pH 8.0 when cultured for 48h, and the degradation of phenol at pH 7.0. The degradation rate of FB-2 was the best, and the degradation rate of phenol was 78.0%. The degradation rate of FB-4 was the worst and the degradation rate of phenol was 56.9%. In the pH value of 5.0 and 9.0 due to acidic and alkaline is strong that lead to protein degeneration and thus inactivated. It can be seen that the neutral or weak alkaline environment is more conducive to the degradation of phenol by phenol degradation bacteria. According to the experimental data, it was found that the optimum degradation of phenol degradation was pH 7.0 or partial alkalinity.

3.4 Effect of Substrate Concentration on Degradation Performance of Phenol Degradation Bacteria

The degradation rate of phenol degradation bacteria at different substrate concentrations was shown in Fig.3. The degradation effect of FB-2 was the best among the six strains when the concentration of phenol was 100-2200mg/L. The degradation rate of phenol in FB-2 was 91.2% when the concentration of phenol was 100mg/L. The degradation rate of FB-4 was 67.5%. When the concentration of phenol was 2200mg/L, the degradation rate of FB-2 decreased to 38.8%. At this time, the degradation rate of FB-4 was only 14.9%. According to the experimental data, it was found that the degradation rate of phenol decreased with the increase of phenol concentration.

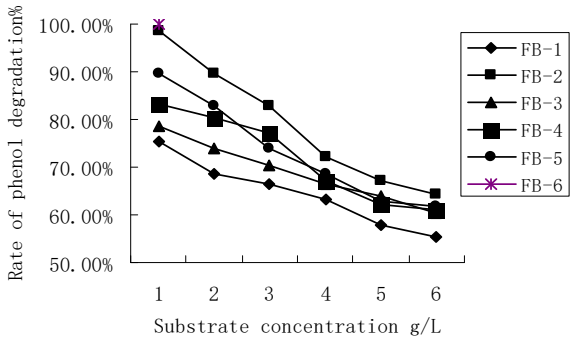

Fig.3 Effect of substrate concentration on the degradation performance of phenol.

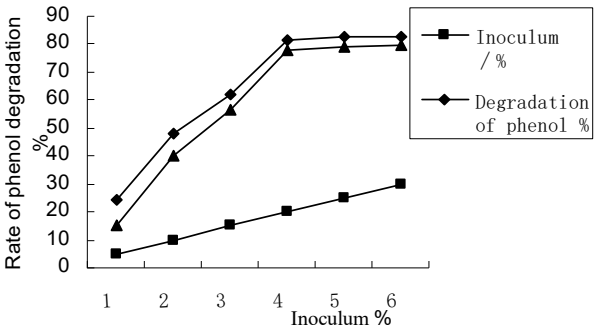

Fig.4 Effect of inoculation amount on degradation of phenol.

3.5 Effect of Inoculation Amount on Degradation Performance of Phenol Degradation Bacteria

The effect of inoculation amount on phenol degradation rate is shown in Fig 4. When the inoculation amount was 5% -20%, the degradation ability of the strain increased with the increase of inoculation amount. When the inoculation amount was equal to 20%, the degradation rate of phenol was reached at the same time. Among them, the degradation efficiency of FB-2 was the best, the degradation rate was 91.5%; the degradation efficiency of FB-3 was the worst, the degradation rate was 67.7%. When the inoculation amount was 20%-30%, the degradation rate of phenol decreased gradually. The above results show that excessive increase in the amount of bacteria will not only reduce the degradation rate, and cause waste of resources, and the appropriate increase in the amount of bacteria, is conducive to the rapid removal of phenol in water, that should be added in accordance with the scientific proportion of phenol degradation bacteria.

4. Conclusion

The effects of different strains on pH, temperature, concentration of phenol and inoculation were studied. The effects of different pH value, temperature, concentration of phenol and inoculation were studied. The results showed that the removal efficiency of phenol was 100 mg/L and below, and the removal efficiency of phenol was the highest at 25℃, pH was neutral or weakly alkaline, and the inoculation amount was 20% When the concentration of phenol reached 2200mg/L, there was still some degradation effect. Consider the various environmental factors, applied to the actual should take full account of the actual situation, to further improve the efficiency of phenol wastewater treatment, give full play to the role of high efficiency Phenol in solving practical problems. Therefore, in the screening of phenol degradation strains, not only need to have high yield of the strain, but also need to be able to achieve the maximum degradation rate in a short time the strain. That is, for the screening of highly efficient phenol-resistant strains should be further explored in order to shorten the time required to achieve the maximum degradation rate and improve the vitality of phenol.

Acknowledgments

Graduate Science and Technology Innovation Project, Jiamusi University, №: YZ2016_011.

References

[1] Fakhru'l-Razi A, Pendashteh A, Abdullah L C, et al. Review of technologies for oil and gas produced water treatment[J]. Journal of hazardous materials, 2009, 170(2): 530-551.

[2] Oller I, Malato S, Sánchez-Pérez J A. Combination of advanced oxidation processes and biological treatments for wastewater decontamination—a review [J]. Science of the total environment, 2011, 409(20): 4141-4166.

[3] Pandey A, Singh P, Iyengar L. Bacterial decolorization and degradation of azo dyes[J]. International Biodeterioration & Biodegradation, 2007, 59(2): 73-84.

[4] Saratale R G, Saratale G D, Chang J S, et al. Bacterial decolorization and degradation of azo

dyes: a review[J]. Journal of the Taiwan Institute of Chemical Engineers, 2011, 42(1): 138-157.
[5] Khan R, Bhawana P, Fulekar M H. Microbial decolorization and degradation of synthetic dyes: a review[J]. Reviews in Environmental Science and Biotechnology, 2013: 1-23.
[6] El-Naas M H, Al-Muhtaseb S A, Makhlouf S. Biodegradation of phenol by Pseudomonas putida immobilized in polyvinyl alcohol (PVA) gel[J]. Journal of Hazardous Materials, 2009, 164(2): 720-725.
[7] Cordova-Rosa S M, Dams R I, Cordova-Rosa E V, et al. Remediation of phenol-contaminated soil by a bacterial consortium and Acinetobacter calcoaceticus isolated from an industrial wastewater treatment plant[J]. Journal of hazardous materials, 2009, 164(1): 61-66.
[8] Basha K M, Rajendran A, Thangavelu V. Recent advances in the biodegradation of phenol: a review[J]. Asian J Exp Biol Sci, 2010, 1(2): 219-234..
[9] Yan J, Jianping W, Hongmei L, et al. The biodegradation of phenol at high initial concentration by the yeast Candida tropicalis[J]. Biochemical Engineering Journal, 2005, 24(3): 243-247.
[10] Munaf E, Zein R, Kurniadi R, et al. The use of rice husk for removal of phenol from waste water as studied using 4-aminoantipyrine spectrophotometric method[J]. Environmental Technology, 1997, 18(3): 355-358.