Study on the culture and degradation characteristics of dominant phenol-degrading bacterium for coal chemical wastewater

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Abstract. To culture dominant phenol-degrading bacterium for bioaugmentation treatment of coal chemical wastewater, 3 phenol-degrading bacterium such as PDB-1, PDB-2 and PDB-3 were enriched and screened from activated sludge in the coal gasification wastewater treatment plant. The culture conditions such as temperature, pH and additional carbon source were analyzed. At last, the degradation effects of raw water were investigated. Results indicated that PDB-2 was the best bacterial strain with the strongest phenol-degrading ability. The degradation rate was 96.6% in 24 h under the initial phenol concentration of 400mg/L. The degradation rates were respectively 89.5% and 96.1% in 24 h and 36h under the initial phenol concentration of 800mg/L. Under the initial phenol concentration of 400mg/L, the dominant phenol-degrading bacterium grew best with the temperature of 30 ℃ and pH of 7~7.5, and 100mg/L or 200mg/L addition of glucose could further increase the 24 h degradation rate. When the preprocessed coal gasification wastewater was used to acclimate PDB-2, the total phenol and COD concentration of raw water were 18.2 mg/L and 193 mg/L with the corresponding 48h degradation rate of 95.8% and 92.5%.

1. Overview
Wastewater of the coal chemical industry mainly refers to wastewater produced during coal gasification, liquefaction and coking. This type of wastewater is featured by complicated substances, high concentration of phenol and ammonia, many toxic and harmful substances, and its degradation is difficult [1]. Phenolic compounds may cause cancer, teratogenesis and mutation, and they are internationally recognized as priority pollutants for control. The high-phenol coal chemical industry wastewater generally flows into the biological treatment unit after extraction and dephenolization pretreatment. Due to the weak tolerance of general microorganisms to phenol [2], the removal efficiency of phenol in the existing biological treatment process is not ideal [3-4]. Phenolic compounds may also inhibit denitrification, make bacteria start self-protection mechanism, and seriously affect the process and efficiency of nitrification, denitrification or anaerobic ammonia oxidation [5-7]. It is difficult to achieve the ideal removal effect and the degradation is relatively slow merely through the degradation of phenolic substances by activated sludge. The high-efficiency bacteria which are added to biological treatment system with the bioaugmentation technology may promote the content and activity of functional microorganisms in the system and achieve ideal phenol removal effect [8-10]. However, up to now, the research on bioaugmentation technology of coal chemical wastewater treatment is still not deep, and it limits its application and promotion. Factors such as pollutant
concentration, inhibitor, pH, temperature, microbial biomass, microbial competition and adaptability may affect the adaptability and degradation rate of microorganisms to phenolic substances \[11\]. In addition to the microorganisms with good phenol degradation performance and strong tolerance, the effects of various factors on the degradation of phenolic substances shall also be investigated \[12\]. Its application prospect may not be clear until the effectiveness, application scope and economic applicability of the application of phenol degradation microorganism are fully understood \[13\]. In this study, selective subculture and directional acclimation are used to cultivate superior phenol degradation bacteria. The degradation features and main influencing factors are analyzed to improve the efficiency of phenol degradation by bioaugmentation of coal chemical wastewater.

2. Materials and approaches

2.1 Source of bacteria
The superior phenol degradation bacteria were separated and screened from the activated sludge in the sedimentation tank of a wastewater treatment station in a synthetic natural gas enterprise in Xinjiang.

2.2 Raw water quality
The test water was taken from the effluent from the air flotation tank of a wastewater treatment station of a synthetic natural gas enterprise in Xinjiang. The company adopted the gasification technology of crushed coal pressurized slag to produce natural gas. It had been processed by phenol ammonia recovery, oil separation & sedimentation, air flotation and other technologies in the front end. The following was the water quality. Total phenol was 432 mg/L, COD was 2,576 mg/L, NH4+-N was 136 mg/L, total alkalinity was 1,832 mg/L, and pH was 7.85.

2.3 Medium
Enriched medium: beef extract 5g/L, peptone 10g/L, NaCl 5g/L, pH 7.4-7.5;
   Inorganic salt medium: (NH4)2SO4 1g/L, KH2PO4 0.5g/L, KH2PO4 0.5g/L, MgSO4·7H2O 0.2 g/L, CaCl2 0.1g/L, FeSO4·7H2O 0.01 g/L, pH 7.5;
   Medium domestication: further add phenol or raw water to the inorganic salt medium;
   Medium isolation and preservation: 15 g/L agar and a certain amount of phenol are added to the enriched medium.

2.4 Test method
Enriched culture: 5g source bacteria centrifuged at 4500r/min were added into a conical flask containing 100ml of liquid medium, cultivated and shaken at 30°C and 120r/min. 5ml of culture liquid was transferred from the previous culture medium for further culture every 1 cycle.

Separation, purification and screening: 0.1mL of culture medium with high phenol degradation rate in the parallel sample was absorbed and then diluted. The diluted culture medium was coated on the separation plate and cultured for 2 days in a biochemical incubator at 30°C. Three strains of typical single colonies (named PDB-1, PDB-2 and PDB-3) were selected from the screening plate of phenol degradation bacteria, and they were respectively inoculated into 100ml domesticated medium with phenol as the single carbon source, and the constant temperature oscillation culture and the screening of superior phenol degradation bacteria were continued;

Culture condition analysis: in the conical flask containing 100ml of liquid medium (400mg/L phenol added in the inorganic salt medium), 5mL of superior phenol degradation strain was added and cultured for 24h under the condition of 120r/min. The effects of temperature, pH and carbon source on the phenol degradation were analyzed;

Analysis of raw water degradation effect: in the conical flask containing 100 ml of raw water, 10 ml of superior phenol degradation bacteria solution was added and cultured in the condition of 30°C and 120r/min. The removal effect of superior phenol degradation bacteria on total phenol and COD in raw water was analyzed;
The water quality indexes such as total phenol, phenol, COD and pH were determined by bromination capacity method, 4-Aminoantipyrine spectrophotometry, rapid digestion spectrophotometry and glass electrode method.

3. Results and discussion

3.1 Screening of superior phenol degradation bacteria
PDB-1, PDB-2, PDB-3 and other superior bacteria were inoculated into the liquid medium with phenol concentration of 400mg/L and 800mg/L respectively. The change of phenol degradation rate along with degradation time can be seen in Figure 1. Under the condition of different initial phenol concentration, each strain could realize the rapid degradation of phenol. According to the degradation capacity, the sequence was PDB-2, PDB-1 and PDB-3, and PDB-2 had the fastest degradation rate. When the initial concentration of phenol was 400mg/L, the 24-hour degradation rates of phenol by PDB-2, PDB-1 and PDB-3 were 96.6%, 91.1% and 84.2% respectively; the degradation rate of PDB-2 phenol increased rapidly in the range of 0~20 hours, and it became stable after 20 hours. When the initial concentration of phenol was 800mg/L, the 24-hour phenol degradation rates of PDB-2, PDB-1 and PDB-3 were 89.5%, 78.4% and 69.3% respectively, and the 36-hour phenol degradation rates were 96.1%, 89.1% and 84.8% respectively; the degradation rate of PDB-2 phenol increased rapidly in the range of 0~24 hours, and became stable after 24 hours.

3.2 Morphological features of superior phenol degradation bacteria
The physiological and biochemical tests and scanning electron microscope analysis were carried out on PDB-2. The isolated strains were gram negative, aerobic test was positive, oxidizable glucose produced acid, contact enzyme was positive, oxidase was positive, acetylmethylalcohol (V-P) test was negative, nitrate reduction test was positive. The scanning electron microscope image can be seen in Figure 2. The bacteria were rod-shaped and their size was about (0.5~0.6) μm * (1.5~2.4) μm. They did not have spore, but had flagella. According to the analysis of morphological and biochemical features, the isolated strain was Pseudomonas.
3.3 Effect of culture conditions on degradation effect

3.3.1 Effect of temperature on degradation effect
When pH was 7.5, the effect of temperature change on the degradation effect can be seen in Figure 3. Each strain was mesophilic, and the degradation rate changed along with the temperature. In the range of 10~30℃, the degradation rate of phenol increased along with the rise of temperature, and it reached the maximum value at 30℃. In the range of 30~40℃, the rise of temperature had a negative effect on the effect of phenol degradation, and the degradation rate decreased. The degradation rate of PDB-2 was higher than that of PDB-1 and PDB-3 in the test range. The suitable temperature range was 25~35℃, and the degradation rate of PDB-2 was 88.6%~96.6%. The degradation effect of PDB-1 was better than that of PDB-3 in the range of 15~30℃, but PDB-3 was more resistant to low temperature at 10℃. The water temperature of coal chemical wastewater in the biochemical stage was generally 20-30℃, and it might be low at 15℃ in winter. The volatile phenol represented by phenol could be effectively removed by adjusting the temperature or degradation time.

3.3.2 Effect of pH on degradation effect
Under the condition of 30℃, the influence of pH change on degradation effect can be seen in Figure 4. The trend of degradation rate of each strain along with pH change was relatively consistent. The degradation rate of PDB-2 strain in the test range was higher than that of PDB-1 and PDB-3. When the pH was 6~7, the degradation rate of phenol increased along with the rise of pH; under the condition of pH 7.5~9, the increase of pH had a negative effect on the activity of phenol degradation bacteria, which resulted in the decrease of degradation rate. The degradation rates of PDB-2, PDB-1 and PDB-3
were 96.6%~97.0%, 90.2%~91.1%, 83.2%~84.2% respectively. The pH value of coal chemical wastewater in the biochemical stage was generally 7-8, which was suitable for the superior phenol degradation bacteria to give full play to its degradation of phenolic compounds.

3.3.3 Effect of external carbon source on degradation effect
Taking glucose as the external carbon source, the effect of PDB-2 strain on the phenol degradation in the presence of other organic carbon sources was analyzed, as shown in Figure 5. In the presence of glucose as a high-quality carbon source, PDB-2 could still degrade phenol effectively. At the initial stage, the addition of glucose had a certain negative effect on phenol degradation. The higher the concentration of glucose was, the more obvious the negative effect would be. Along with the degradation time, the degradation rate of 100mg/L and 200mg/L glucose concentration was accelerated, which promoted the effect of phenol degradation. Compared with not addition of carbon source, the glucose concentration of 300mg/L inhibited the phenol degradation, and the phenol degradation rate and the final phenol degradation rate decreased. The results show that glucose can promote the growth of superior phenol degradation bacteria, and co-metabolism will be caused for the degradation of phenol, which is beneficial to the growth of phenol degradation bacteria and the deep removal of phenol. In addition, the sufficient degradation time can ensure the improvement of phenol degradation rate.

![Fig.5 Influence of additional carbon source on degradation effects](image1)

![Fig.6 Total phenol and COD degradation effects of raw water](image2)

3.4 Degradation effect of raw water
The degradation effect of PDB-2 strain on total phenol and COD in coal gasification wastewater can be seen in Figure 6. After 48 hours of acclimation, PDB-2 strain could effectively degrade total phenol and COD. The concentrations of total phenol and COD were reduced to 18.2mg/l and 193mg/L respectively. The degradation rates of total phenol and COD were 95.8% and 92.5% respectively. Comparing Fig. 6 with Fig. 1 (a), it can be found that when the initial total phenol concentration was basically identical, it took longer to degrade the phenolic compounds in the raw water, and the degradation rate of the total phenol in the initial stage of domestication culture was low, compared with the liquid culture medium with phenol as the single carbon source. It is analyzed that coal chemical industry wastewater represented by synthetic natural gas wastewater had complex components, and after pretreatment (phenol ammonia recovery + oil removal), it still contained high concentration of ammonia nitrogen and a small amount of biochemical inhibitors, and it would inhibit the reproduction and activity of phenol degradation bacteria to a certain extent, so it required a longer time to achieve the effective degradation of total phenol. In addition, the refractory organics in the raw water, except phenolic compounds, were difficult to be effectively degraded by a single strain, so the raw water still contained a certain concentration of COD in 48 hours after degradation. In general, it is feasible and stable to apply the superior phenol degradation bacteria to the bioaugmentation treatment
of coal gasification wastewater.

4. Conclusion
(1) PDB-1, PDB-2 and PDB-3 are enriched and screened from the activated sludge of a synthetic natural gas wastewater treatment plant. PDB-2 has the strongest ability to degrade phenol. The degradation rate in 24 hours reaches 96.6% at the initial phenol concentration of 400mg/L, and 89.5% and 96.1% at the initial phenol concentration of 800mg/L in 24 hours and 36 hours respectively.

(2) When the initial concentration of phenol is 400mg/L, the most suitable conditions for the growth of superior phenol degradation bacteria are the temperature at 30°C and pH between 7–7.5, and 100mg/L or 200mg/L glucose can further improve the 24-hour degradation rate.

(3) The PDB-2 strain is acclimated and cultured for 48 hours with the synthetic natural gas wastewater after pretreatment (phenol ammonia recovery and oil removal). The concentrations of total phenol and COD in the raw water are reduced to 18.2mg/l and 193 mg/L respectively. The degradation rates of total phenol and COD in 48 hours are 95.8% and 92.5% respectively.

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