Biodegradation of Fluoranthene Contaminated Soil by Different Combinations of Bacteria

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Abstract. In this study, different combinations of three fluoranthene degrading bacteria YN420, YN1, E420 complex microorganisms were constructed through orthogonal experiment, and applied to the biodegradation of fluoranthene contaminated soil to determine the optimal combination of complex microorganism. The results showed that the degradation rate of fluoranthene in a soil slurry of different combinations of complex bacteria was higher than that of three strains inoculated alone. The optimal combination ratio of complex microorganism was YN420: YN1:E420=2:1:1, and different ratios of complex microorganism could be enhanced to the bioremediation of fluoranthene in contaminated soil.

1. Introduction
Polycyclic aromatic hydrocarbons (PAHs) are a kind of persistent organic pollutant in agricultural soils [1]. Bioremediation is the way to treat PAHs contaminated soil. However, only a single strain has limited effect on the degradation of PAHs, and it is more difficult to degrade high molecular weight PAHs because of its strong chemical stability. Increasing the number of degrading bacteria is one of the methods to improve the degradation rate of PAHs by microorganisms [2]. Shweta Mishra used two strains of bacteria Pseudomonas aeruginosa PSA5, Rhodococcus sp. NJ2 and their complex microorganism to carry out the degradation experiment of fluoranthene to promote the degradation of fluoranthene [3].

In this experiment, three fluoranthene degrading bacteria were used for the biodegradation of fluoranthene contaminated soil by different combinations.

2. Materials and methods

2.1. Strain and medium
Three kinds of fluoranthene degrading bacteria were used for the experiment, Bacillus paranthracis YN420, Bacillus paramycoides YN1, Bacillus paranthracis E420. Mineral salts medium (MSM): 0.01 g NaCl, 0.5 g KH2PO4, 0.5 g K2HPO4, 0.2 g MgCl•6H2O, 0.339 mg MnSO4, 0.428 mg ZnSO4, 0.026 g CaCl•6H2O, 0.347 mg H8MoN2O4, 0.2 g Yeast extract, in 1 L distilled water.

2.2. Preparation of inoculation solution and antagonistic experiment of strains
The fluoranthene degrading bacteria were cultured in the MSM with 1g of glucose as the growth substrate. Each of the bacterial cultivation was transferred to a 50 ml centrifuge tube and centrifuged
with 4000 r/min, 10 min, and the supernatant were discarded. Add 10 ml PBS buffer sol. to the centrifuge tube, mixing, centrifuge again, discard the supernatant, and repeat three times. The concentrated bacterial sol. was diluted with PBS buffer sol., and finally the bacterial sol. was adjusted to OD\textsubscript{600nm}=1.0 for seeding.

Before the combinatorial experiment, the antagonistic experiments of three strains were carried out to observe whether the strains affected each other[4].

2.3. Degradation characteristics of fluoranthene by different bacteria
The black soil was used for this experiment. Its properties were pH 6.83, SOM 6.78%, Clay 10.8%. Weigh 2 g of soil through a 1 mm sieve, put it into 20 ml glass sample bottle, add fluoranthene standard solution as required for the experiment to contaminate the soil, add 10 ml of MSM, and cultivated at room temperature for 48 h and used in the experiment. Three different strains of fluoranthene degrading bacteria were inoculated into 2 g of sterilized soil slurry with contamination concentration of 20 mg/kg to evaluate the degradation effect. The seeding size was 200 μl, incubation 10 d. Residue of fluoranthene was extracted by ultrasonic, and analyzed by HPLC[5].

2.4. Degradation characteristics of fluoranthene by complex bacteria with different proportions
It designed the three-factor two-level orthogonal test as shown in Table 1. After mixing the prepared bacterial sol. in the proportions was listed in the orthogonal table, inoculated into 2 g, 20 mg/kg sterilized soil slurry. The total inoculation amount was 200 μl, culture 10 d. And residue fluoranthene was analyzed. ANOVA and range analysis were carried out on the experimental results to get the best proportion of combined bacteria.

| Level | Factors |
|-------|---------|
|       | YN420   | YN1    | E420   |
| 1     | 1       | 1       | 1       |
| 2     | 2       | 2       | 2       |

3. Results and Discussion

3.1. Strain antagonistic experiment
Through observation, there was no bacteriostatic zone between each strain in this experiment, indicating that there was no antagonism between the strains, and three strains could be used in combination. The experiment results were shown in figure 1.

![Figure 1. Antagonistic effect.](image)

3.2. Degradation characteristics of fluoranthene in contaminated soil by different bacteria
The fluoranthene residues of each strain were shown in figure 2. The degradation rate of fluoranthene of the three strains relative to the control was shown in figure 3. The lowercase letters in the figure
indicate significant differences between the groups ($p < 0.05$). Compared with the control, the three strains all could degrade fluoranthene. Among them, the degradation rate of fluoranthene by strain YN420 and YN1 were higher than the strain E420, which were 10.17%, 9.19%, 2.9%, respectively.

3.3. Effects of different combinations of complex microorganism on the degradation of fluoranthene

According to the Table 1, orthogonal experiments were carried out in a soil slurry. The range analysis of the experimental results was shown in Table 2, and the results of the analysis of variance experimental result was shown in Table 3. The degradation rate of the fluoranthene in different proportions was higher than that of three strains alone. Treatment of different proportions of fluoranthene degrading complex bacteria in a soil slurry improved biodegradation. The $F$ value of the variance analysis results showed that the influence of three factors on the degradation rate of fluoranthene was ranked as $C > A > B$. E420 was the main factor in the combination of composite bacteria, which affects the degradation of fluoranthene. There was a significant difference between factor A (YN420) and factor C (E420) on the degradation rate of fluoranthene ($p < 0.05$), while no significant difference on factor B (YN1) ($p > 0.05$). In the next step, the optimal combination proportion could be determined according to the range analysis of experimental result[6]. From $K_1$ and $K_2$, it could be concluded that the optimal combination ratio was $C_1A_2B_1$, the optimal combination ratio of complex microorganism was YN420: YN1: E420=2: 1: 1.
Table 2. Range analysis of experimental results.

| No. | A (YN420) | B (YN1) | C (E420) | Results (Degradation rate) |
|-----|-----------|---------|----------|----------------------------|
| 1   | 1         | 1       | 1        | 16.31                      |
| 2   | 1         | 2       | 2        | 11.80                      |
| 3   | 2         | 1       | 2        | 13.29                      |
| 4   | 2         | 2       | 1        | 17.69                      |
| K₁  | 14.055    | 14.8    | 17       |                            |
| K₂  | 15.49     | 14.745  | 12.545   |                            |
| R   | 1.435     | 0.055   | 4.455    |                            |

Table 3. Variance analysis of experimental results.

| Sources | SS    | df | V   | F    | P   |
|---------|-------|----|-----|------|-----|
| A (YN420) | 6.221 | 1  | 6.221 | 129.218 | .000 |
| B (YN1)   | .009  | 1  | .009 | .182  | .681 |
| C (E420)  | 59.443| 1  | 59.443| 1234.772| .000 |
| e         | .385  | 8  | .048 |       |     |
| Total     | 2685.660| 12 |      |      |     |

4. Conclusion
In this experiment, the degradation rate of fluoranthene in the soil slurry of complex bacteria with different ratios was improved compared with the three strains alone inoculation. The treatment of fluoranthene degrading composite bacteria with different ratios enhanced the bioremediation of fluoranthene in contaminated soil. The best combination ratio of complex bacteria was YN420: YN1: E420=2: 1: 1.

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