Study on Growth Characteristics of Oily Sludge Degrading Bacteria

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Abstract. Regarding oily sludge, microbial treatment of oily sludge will be a future development trend. It will be of great significance to apply microbial degradation of oily sludge to practical engineering. However, there are still many problems and deficiencies in biological treatment technology, such as Screening and cultivation of petroleum-degrading bacteria, long treatment cycle, etc. The purpose of this study is to cultivate a more active petroleum-degrading bacterium, which is used in the best state for the degradation of oily sludge.

1. Introduction

As people's environmental pollution problems become more and more serious, the problem of oily sludge treatment has also received widespread attention. Among the many methods for treating oily sludge, biological treatment is becoming more and more dominant, and its application is gradually becoming wider. In the future, biological treatment will become an inevitable trend in the treatment of oily sludge. However, there are still many problems and deficiencies in biological treatment technology, such as the screening and cultivation of petroleum-degrading bacteria, and the treatment cycle is long. The purpose of this study is to determine the optimum growth conditions for the isolated and purified petroleum-degrading bacteria, and to understand their physiological and biochemical characteristics, so as to cultivate the most active petroleum-degrading bacteria, in the best state, for oily sludge degradation.

2. Determination of optimum pH of oily sludge degrading bacteria

The optimum pH measurement results of oily sludge degrading bacteria are as follows:

| pH | OD600 1 | OD600 2 | OD600 3 | average value |
|----|---------|---------|---------|--------------|
| 3  | 0.005   | 0.028   | 0.004   | 0.013        |
| 4  | 0.039   | 0.031   | 0.043   | 0.038        |
| 5  | 0.065   | 0.073   | 0.097   | 0.079        |
| 6  | 0.349   | 0.398   | 0.372   | 0.373        |
| 7  | 0.468   | 0.489   | 0.378   | 0.445        |
| 8  | 0.477   | 0.542   | 0.459   | 0.493        |
| 9  | 0.341   | 0.320   | 0.397   | 0.353        |
As shown in Fig. 1, when the pH value is 3-5, the growth of the degrading bacteria is very poor, the growth rate is slow, and the growth is obviously inhibited, indicating that the degrading bacteria cannot adapt to the acidic environment; when the pH is 5-6, the degradation is performed. There was a mutation in the growth of the bacteria, and the number of bacteria increased rapidly. At pH 6-9, the growth of the degrading bacteria was not much different and was maintained at a high level. When the pH was 8, the growth was best. The growth of degrading bacteria increased first and then decreased with the increase of pH, of which 8 was the best. The degrading bacteria are more suitable for growth under neutral alkaline conditions and are significantly inhibited under acidic conditions.

Figure 1. Effect of pH on the growth of degrading bacteria

3. Isolation, purification and colony morphology of degrading bacteria
Figure 2 shows the two kinds of slopes after 24 hours of inoculation. The growth of the slope in the normal enrichment medium is better than that in the crude oil. Figure 3 shows the colony after 24 hours of coating. The colonies of the degrading bacteria are very small, round, neat edges, milky white, translucent, smooth and convex colony surface.

Figure 2. Culture and preservation of degrading bacteria (a is a liquid bacterial solution, b is a plain enrichment medium inclined surface, and c is an enriched medium inclined surface to which oily sludge is added)
4. Determination of optimum nitrogen to phosphorus ratio of oily sludge degrading bacteria

As shown in Fig. 4, a nitrogen source was additionally added to the medium of 30 ml, and the growth of the degrading bacteria first increased with the increase of the nitrogen source and then decreased. When the nitrogen source is not added and the ratio of nitrogen to phosphorus is 1:1, the growth is relatively good. When the ratio of nitrogen to phosphorus is 1:1, the growth is better. Although the addition of a suitable nitrogen source to the medium will accelerate the growth of the microorganisms relatively, the addition of too much nitrogen source will inhibit the growth of the microorganisms. In 30 ml of medium, the nitrogen source should not be added too much, without adding a nitrogen source or by controlling the nitrogen to phosphorus ratio to 1:1, the degrading bacteria can grow better.

The optimum nitrogen to phosphorus ratio of oily sludge degrading bacteria is as follows:

Table 2. OD value of oil-sludge sludge degrading bacteria under different nitrogen-phosphorus ratios

| Nitrogen to phosphorus | OD600 1 | OD600 2 | Average value |
|-----------------------|----------|----------|---------------|
| 0                     | 0.886    | 0.896    | 0.891         |
| 1:1                   | 0.885    | 0.931    | 0.908         |
| 2:1                   | 0.830    | 0.849    | 0.8395        |
| 3:1                   | 0.815    | 0.779    | 0.797         |
| 5:1                   | 0.660    | 0.728    | 0.694         |
| 8:1                   | 0.442    | 0.557    | 0.4995        |
| 10:1                  | 0.446    | 0.343    | 0.3945        |
| 15:1                  | 0.219    | 0.233    | 0.226         |
| 20:1                  | 0.125    | 0.158    | 0.1415        |
| 30:1                  | 0.028    | 0.033    | 0.0305        |

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![Graph showing effect of nitrogen to phosphorus ratio on growth of degrading bacteria]

**Figure 4.** Effect of nitrogen to phosphorus ratio on growth of degrading bacteria

5. **Gram staining**

The results of Gram staining are as follows:

![Gram staining images]

**Figure 5.** Gram staining of oily sludge degrading bacteria

Figure a, b, c, and d are screenshots of different regions of the same Gram stain. Figures e and f are screenshots of different areas of the same staining. It can be seen from the figure that Gram staining results of oily sludge degrading bacteria it is red and is a Gram-negative bacteria.

6. **Physiological and biochemical characteristics of oily sludge degrading bacteria**

The results of the determination of physiological and biochemical characteristics of oily sludge degrading bacteria are as follows:
Table 3. Determination table of physiological and biochemical characteristics of oily sludge degrading bacteria

| Identification feature | hydrogen peroxide | Methyl red | Acetymethylmethanol | Oxidase | Starch hydrolysis |
|------------------------|-------------------|------------|---------------------|---------|------------------|
| result(+/-)            | +                 | -          | +                   | +       | -                |

*Note: + is positive, - is negative

Figure 6. Test results of some physiological and biochemical characteristics (a is methyl red test, b is yinduo test)

7. Determination of the optimum amount of bacteria for oily sludge degrading bacteria

The results of determining the optimum bacterial amount of oily sludge degrading bacteria are as follows:

Table 4. OD value of different sludge in oily sludge degrading bacteria

| number | 0.5ml | 1ml | 2ml | 3ml | 5ml | 8ml | 10ml |
|--------|-------|-----|-----|-----|-----|-----|------|
| 1      | 1.013 | 1.066 | 1.075 | 1.054 | 1.149 | 1.134 | 1.321 |
| 2      | 1.006 | 1.254 | 1.023 | 1.161 | 1.161 | 1.143 | 1.327 |
| 3      | 1.166 | 1.076 | 1.026 | 1.055 | 1.146 | 1.298 | 1.324 |
| average | 1.062 | 1.132 | 1.041 | 1.09 | 1.152 | 1.192 | 1.324 |
| 1      | 1.287 | 1.029 | 0.944 | 1.166 | 1.115 | 1.150 | 1.215 |
| 2      | 0.940 | 0.968 | 0.964 | 1.044 | 1.155 | 1.103 | 1.194 |
| 3      | 1.074 | 1.158 | 0.943 | 1.074 | 1.138 | 1.207 | 1.121 |
| average | 1.095 | 1.052 | 0.050 | 1.095 | 1.136 | 1.153 | 1.177 |

Since the growth of the degrading bacteria is determined by ultraviolet spectrophotometry, that is, the degree of turbidity of the culture solution is used to understand the growth of the degrading bacteria, 30 ml of the medium is relatively small, and the turbidity of the culture solution itself is 10 ml. It is much larger than when I received 1 ml. Even after 16 hours of cultivation, it is impossible to judge the optimum amount of bacteria. However, as shown in Fig. 7, when the inoculation amount is 0.5 ml, 1 ml, and 2 ml, after 16 hours, the level can be as long as 10 ml, so the subsequent experiment is still to take 1 ml.

Improved method: After initial inoculation, the initial OD value of each inoculum was measured at a wavelength of 600 nm. After 16 hours of culture, the OD value was measured and the growth rate in the 16 hours was calculated. The optimum amount of bacteria is determined according to the growth rate.
8. Summary
Oily sludge is one of the environmental pollution problems that have been widely concerned by the society. Microbial treatment of oily sludge will be a future development trend. It will be of great significance to apply microbial degradation of oily sludge to practical engineering. It is to cultivate the oil-degrading bacteria with strong activity and use it in the best state for the degradation of oily sludge.

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