

Effect and Mechanism of Microorganism for Oil Degradation Enhanced by Magnetic Field

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Abstract: One strain was isolated from activated sludge of oily wastewater biochemical treatment and named *Acinetobacter* sp B11 according to the determination of morphology, physiological, biochemical tests and 16S rDNA sequence analysis. In present research, magnetic field was used in oil biodegradation process to improve the degradation efficiency of microorganism for oil pollution and the effect and mechanism of oil-degrading bacteria (strain *Acinetobacter* sp B11) with magnetic field were conducted. The results showed that oil removal efficiency was 11.9% higher when magnetic field (25mT) was added in biodegradation process. Lower intensity of magnetic field could promote microbial growth and improve microbial enzyme activity and the results had showed that microbial logarithmic phase was shortened and the enzyme activity of bacteria increased by 27.4% at lower magnetic strength (25mT).

Keywords: Oil Degradation Bacteria, Magnetic Field, Microbial Activity, Microbial Growth.

1. Introduction

As an emerging technology, magnetic field had attracted more and more attentions in the field of water and wastewater treatment in recent years [1-3]. The magnetic field had been used in biodegradation process for pollutant removal and some studies showed that magnetic field could affect the growth of microbe. Nakamura et al [4] found that the cell number of *Bacillus subtilis* M113 in an inhomogeneous (5.2-6.1T) magnetic field was about twofold higher than that of the reference (7T) in the stationary phase. Liu et al [5] studied the effect of steady magnetic force on the growth of *Lentius edodes* and concluded that magnetic field improved the biological efficiency by speeding up the formation and division of fungus' original medium. Zheng et al [6] studied the effects of electromagnetic field on *Spirulina* growth and found that lower magnetic field intensity(0~40kA/m) had a positive effect on *Spirulina* growth and it was most significant when the magnetic field intensity was 24 kA/m. Rutkowska et al [7] observed that magnetic field at induction of 7mT generated by permanent magnets was a factor improving p-nitroaniline biodegradation by microorganisms inhabiting activated sludge.

On the other hand, magnetic field could affect the biodegradation ability of microbes. It made the activity of catalase, peroxidase and three kinds of phosphatase increase in different degrees and the magnetic field effect could continue for a long time. Koneracka et al [8] found that the bioactivity could be maintained more than 90% if the proteins and enzymes were immobilized on magnetic particle surface. Studies of Li et al [9] showed that the activity of catalase increased by 48.6% after the enzyme liquid treated by 100mT. Celik et al [10] found that moderate intensity of the magnetic field (below 5mT) increased the activity of superoxide dismutase and catalase more than 20% and the increases of magnetic field exposure times did not cause linear increases in enzyme activities. Sui et al [11] compared the different magnetic field intensity in improving the efficiency of urban wastewater biodegradation with the contrastive system and found that the

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activated sludge system enhanced by additional magnetic field (1500 Gs) increased the removal rate of COD, NH₃-N and PO₄³⁻P by 1.3%, 0.2% and 10.0%.

The process for magnetic field on biodegradation for pollutant removal was complicated. The aim of this work was to determine the effect of magnetic field (generated by permanent magnets) on the bacteria oil biodegradation ability and the mechanism of magnetic field on oil degradation bacteria was also investigated. Although the bacteria from a specific zone of Daqing oil wastewater treatment were tested in the work, it was a reference and preparation for similar research on oily waste-water by biological process.

2. Methods

2.1. Bacteria isolation and cultivation

One strain was isolated from Daqing oilfield wastewater biochemical treatment plant. The test of microbial physiology and biochemistry was conducted and the results showed that the colony of strain was round with the diameter of 1~2mm. The surface of colony was smooth, moist, neat-edged and the central uplift slightly with yellow teeth. The strain was in the pattern of short rod, blunt at the two sides, usually in the form of single or paired and no spores. The results of physiological and biochemical identification showed that strain was catalase positive, oxidase negative, gram negative without growth factors.

Genetic sequence was conducted by SANGON biotech and compared with sequence homology in NCBI (National Center for Biotechnology Information) and the ribosomal database (<http://rdp.cme.msu.edu/index.jsp>). The result of 16S rDNA was also carried on sequence homology comparison by Blast in GenBank. Comparison showed that the results were similar to the genetic sequences of *Acinetobacter*, and the similarity reached 99.6% (FJ494703).

With the determination of morphology, physiological and biochemical tests and 16S rDNA sequence analysis, the strain was identified as *Acinetobacter* and named *Acinetobacter sp* B11.

2.2. Preparation of oily wastewater

The preparation process of oily wastewater was following: a certain amount of crude oil and Tween-80 was mixed according to the proportion of 1:5. The water heated to 35°C and was gradually poured into above mix solution. The emulsified liquid was transferred to a separatory funnel for around 30min. The emulsified oil solution released from the bottom of the separatory funnel and the upper oil was also removed in order to obtain the stable oily wastewater.

2.3. Biodegradation experiment

In order to prepare the bacterial suspension, the single colonies taken from the agar slant culture medium was inoculated to beef extract peptone medium and placed in a thermostatic shaker with 200r/min for 24h at 30°C. Then the bacterial suspension was diluted to OD₆₀₀ value for 0.8. The 5mL above bacteria suspension was collected into a 150mL conical flask with 50mL liquid oil medium, which was placed in the thermostatic shaker with 100r/min and 30°C for 7 days.

The oil concentration was determined by ultraviolet spectrophotometer with referring to the method of Determination of oil - UV Spectrophotometry of 93-1994 SL.

A circular ferrite permanent magnet purchased from Changzhou was as the magnetic field generating device. The diameter and thickness of magnet were 70mm and 10mm, respectively. The magnetic field intensity was measured by Teslometer. Experimental setup for the biodegradation process is shown in Fig. 1.



Fig. 1: Experimental setup for the biodegradation process

For a good linear relationship between the concentration of bacterial suspension and the absorbance, the absorbance at a wavelength of 600nm (OD600) was used to characterize the biomass.

With reference to “Identification Manual of Common Bacterial System” and "Berger Bacterial Identification Manual" (Eighth Edition), morphological observation and physiological and biochemical characteristics experiment were carried on for isolated petroleum degrading bacteria with high efficiency, the preliminary result was genus. At the same time, through the sequence analysis of 16S rDNA, the species of strains were further identified.

Dehydrogenase activity was conducted in present research and was determined according to the reduction method of chloride three TTC (2, 3, 5-Triphenyl Tetrazolium Chloride, TTC).

In order to better understand the effect of strain *Acinetobacter sp* B11 for oil removal, gas chromatography coupled with electron impact ionization tandem mass spectrometry was used to analyze the residual oil composition. Operating conditions of GC-MS: a capillary column of HP-5MS (60 m ×250µm×0.25µm);Carrier gas: He; The GC oven temperatures were: held at 45 °C for 3 min, ramped at 10 °C/min to 320 °C, with a final hold at 10 min. Constant voltage no shunt; The injector and detector temperatures were the same set at 280 °C. Scanning: 40-400amv; Electron energy: 70ev; Helium was used as the carrier gas at a flow rate of 1.2mL/min. Ion source temperature: 200 °C; transmission line temperature: 250 °C.

3. Results and Discussion

3.1. Effects of magnetic field on oil-degrading bacteria for oil removal

The effect of magnetic field on microorganism biodegradation performance had an important relationship with the magnetic field intensities [12]. The strain *Acinetobacter sp* B11 were inoculated in the culture medium. The liquid petroleum oil concentration was around 200mg/L, temperature was controlled at 30°C and the magnetic field of 0, 18, 25, 30, 35, 43 and 60mT were also added respectively. The performance of strain *Acinetobacter sp* B11 at different magnetic field intensity was studied and the result was shown in Fig. 2.

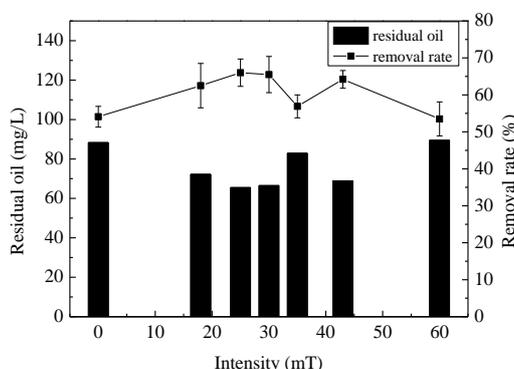


Fig. 2: Effect of different magnetic field intensity on oil degradation

In Fig. 2, the result showed magnetic field intensities had different effects on strain *Acinetobacter sp* B11 for oil degradation. When there was no external magnetic field, the concentration of the residual oil was

88.3mg/L and oil degradation rate was 54.1%. With the magnetic field intensity increased, oil removal rate also increased. When the magnetic field intensity reached 25mT, oil removal rate of strain *Acinetobacter sp* B11 reached a maximum value of 66% and the oil removal rate increased by 11.9% compared with the situation without magnetic field. As the magnetic field intensity was over 30mT, the performance of strain *Acinetobacter sp* B11 on oil degradation was not stable. The external magnetic field had few effects on the microbial oil degradation when magnetic field intensity reached 35mT and 60mT. This result was similar with Jia [13], who studied the magnetic field enhancement effect of the biodegradation ratio of COD and found that the biodegradation ratio went up first as the magnetic field intensity increased to 20mT, and then decreased sharply with further increased.

3.2. Effect of the magnetic field on the growth of oil-degrading bacteria

The impact on microbial growth with magnetic field was multifarious, and different magnetic field strength may have different impacts on microbes in different growth periods. The effect of magnetic field on microbial growth was conducted. The strain *Acinetobacter sp* B11 had been transferred from beef extract peptone medium to the oil liquid medium in the condition of 30°C and 100r/min shaking culture with the magnetic field from 0 to 60mT. The growth of oil-degrading bacteria in liquid petroleum medium was shown in Fig. 3.

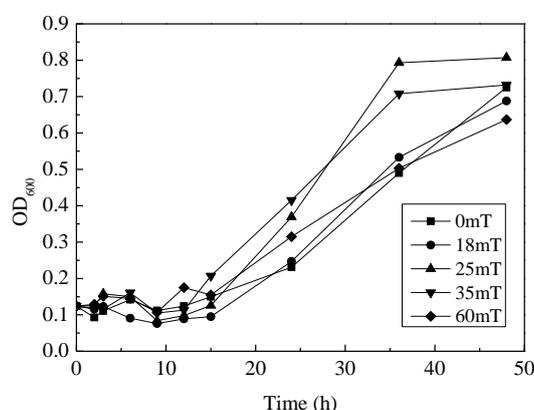


Fig. 3: Effect of magnetic field on the growth of oil-degrading bacteria

In Fig. 3, with different magnetic field strength, there was a large difference for oil-degrading bacteria of *Acinetobacter sp* B11 to adopt oily wastewater. At the beginning of 15 hours, no matter whether the external magnetic field was added or not, the lag phase of strains was no significant change. In the logarithmic phase, the slope of the curve became greater for the external magnetic field, which indicated the microbial growth rate increasing. Especially with the magnetic field of 25mT, the microbial growth was the fastest, the period of logarithm was the shortest, and biomass of strains firstly reached the maximum biomass.

In this context, results from Fig. 3 suggested that low-level magnetic field tended to benefit the bacterial growth compared without magnetic field. Probably due to the proper strength of the magnetic field could change the generation time of microorganism. This result was same with Cheng [14], who found that generation time of bradyrhizobium with magnetization treatment was shorter than the control strain. Zheng et al [6] also found that the magnetic field had an effect on microbial growth mainly in the logarithmic phase, and when the magnetic field intensity was 25mT, the promoting effect on strain growth was the most obvious.

3.3. Effect of the magnetic field on the microbial activity of oil-degrading bacteria

Acinetobacter sp B11 strain was inoculated in the culture medium with liquid petroleum oil at the magnetic field of 0, 18, 25, 30, 35, 43 and 60mT. Under the condition of 30°C and 100r/min in the shaking culture, the dehydrogenase activity of bacteria liquid were determined and the results were shown in Fig. 4.

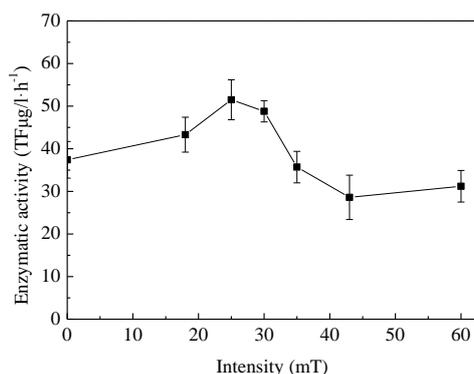


Fig. 4: Effect of the magnetic field on the microbial activity

In Fig. 4, the magnetic field had a promoting effect on the microbial enzyme activity when the magnetic field intensity was between 18-30mT. When the magnetic field intensity was 25mT, the promoting effect was the strongest, the microbial enzyme activity was 51.5 TFµg/L·h⁻¹ and the activity was increased by 27.4% compared with no magnetic field. When the magnetic field intensity was more than 35mT, the magnetic field had an inhibitory effect on the enzyme activity, which tended to be stable and did not drop.

The active center of the enzyme contains iron, manganese, zinc, copper and other paramagnetic metal ions has an enzyme molecule with certain magnetic moment of the side chains. Orientation distribution will change with the Lorentz force generated by the magnetic field, which results in the effect on the conformation and activity of enzyme [15]. The conformational change of enzyme molecular can be better fit with the substrate, producing the formation of the enzyme substrate complex, in general this process is very fast, but when the substrate diffusion limitation exists, the magnetic field can promote the rapid formation of complexes [16]. From the changes of enzyme activity, the magnetic field can promote the adsorption and utilization of microorganisms on organic matter and improve the degradation effect of organic matter. However, mechanisms that completely explain how magnetic fields may initiate changes in biological systems have not yet been elucidated [17].

4. Conclusion

The performance and mechanism of magnetic field on oil-degrading bacteria (*Acinetobacter sp* B11) for oil removal was conducted. The result showed that lower magnetic field intensity not only had a positive effect on the oil degradation efficiency, especially for short chains alkane degradation, but also had the most significant effect on the activity of enzymes. When the magnetic field intensity was 25mT, the oil removal rate of microorganisms increased by 11.9% and the enzyme activity of bacteria increased by 27.4% compared with no magnetic field.

5. Acknowledgments

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6. References

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