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Effect of low-strength magnetic fields on the oil removal performance of oil-degrading microorganisms

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ABSTRACT

Using oil-degrading bacteria (*Acinetobacter sp*B11) as the reference strain, this article explored the effect of a low-strength magnetic field on strain B11 for oil removal. The magnetic field had a positive effect on the degradation of n-alkanes by microorganisms, especially short-chain hydrocarbons. Under the premise of not destroying the cell membrane, lower magnetic fields (15–25 mT) enhanced the permeability of the cell membrane and improved the activity of the oil-degrading bacteria. In the 25-mT magnetic field, the hydrophobicity and oil-spreading diameter of the B11 cells were increased by 6.7% and 21.9%, respectively, and the surface tension was decreased by 6% compared with that measured when bacteria were applied without magnetic field application. A test of the bio-surfactant levels showed that the magnetic field increased the surfactant content released by the microorganisms but had no significant impact on the surfactant properties.

Keywords: Magnetic field effects; Hydrophobicity; Surface tension; Surfactant

1. Introduction

As a bioaugmentation technology, magnetic fields have been used in biodegradation processes [1,2]. A wide range of studies have dealt with biotransformation, biodegradation, and bioremediation of oil pollution by magnetic fields [3–5]. Magnetic field application can affect microorganism growth and reproduction [6,7] and the biodegradation abilities of microbes [8,9]. In previous studies, a low-strength magnetic field (15 mT–35 mT) was applied during the biodegradation process. The results showed that the magnetic fields affected microbial characteristics and improved the efficiency of hydrocarbon biodegradation. Moreover, the biomass and oil degradation increased by 12.5% and 65.6%, respectively, compared to the values obtained under conditions without an external magnetic field [10]. However, the effect of magnetic fields on the growth of microorganisms is a very complex process, and the effects of magnetic fields on organic removal by microorganisms are not fully understood [11,12]. A highly efficient oil-removing bacterial strain (*Acinetobacter sp.* strain (B11)) was selected as the reference bacteria. In an attempt to further explore the effect of a low-strength magnetic field on the enhancement of microbial oil degradation, the efficiency of oil degradation, hydrophobicity of the oil-degrading bacteria, release of biosurfactant, surface tension of the liquid and cell membrane permeability were evaluated. The effects of magnetic fields on oil treatment by *Acinetobacter sp* B11 were investigated by measuring the growth, cell activity, cell surface morphology and cell permeability of the bacteria.

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2. Materials and methods

2.1. Experimental water samples

The oily wastewater preparation process was as follows. Crude oil and Tween-80 were mixed at a volume ratio of 1:5 and gradually poured into water at a temperature of 35°C, which was controlled by a water bath. The emulsified liquid was transferred into a separatory funnel and left standing for approximately 30 min. Then, the emulsified oil solution was released from the bottom of the separatory funnel and collected as the oily wastewater, and the upper oil was discarded. The oil concentration was approximately 200 mg/L.

2.2. Isolation and cultivation of the bacteria

Twenty-two strains were isolated from the Daqing Oilfield wastewater biochemical treatment plant. In view of the 7-d oil removal rate, the strain with the highest removal rate (53.6%) was selected as the reference bacteria, as described in our previous study [13]. The microbial physiology and biochemistry of the strain were tested. The strain colonies were round with a 1–2-mm diameter. The surfaces of the colonies were smooth and moist with well-defined edges, and a slight uplift with yellow teeth was observed in the center. The bacterial morphology was short rods that were blunt at the two ends and usually single or paired, and no spores were detected. The physiological and biochemical identification results showed that the strain was catalase-positive, oxidase-negative, and Gram-negative without growth factors.

Genetic sequencing was conducted (Sangon Biotech, Shanghai, China), and the sequence homology was compared using the National Center for Biotechnology Information and the ribosomal database (http://rdp.cme.msu.edu/index.jsp). Comparison of the 16S rDNA sequences using Blast in GenBank showed that the genetic sequences of the selected strain had 99.6% similarity to an *Acinetobacter* strain (FJ494703).

According to the morphological analysis, the physiological and biochemical tests and the 16S rDNA sequence analysis, the strain was identified as *Acinetobacter* and was named *Acinetobacter sp* B11.

2.3. Magnetic treatment of Acinetobacter spB11

Single colonies taken from the agar slant culture medium were inoculated into beef extract peptone medium and cultivated with a thermostatic shaker at 200 rpm for 24 h at 30°C to obtain the bacterial suspension. The bacterial suspension was freshly prepared before each experimental batch. Then, the bacterial suspension was diluted to obtain a working solution with an OD_{600} value of 0.8, and 5 mL of the working solution was placed into a 150-mL conical flask with 50 mL of liquid oil medium. The flask was placed in a thermostatic shaker at 100 rpm and 30°C, and the degradation process continued for 7 d. Subsequently, 50-mL samples of the *Acinetobacter sp*B11 cell suspension were exposed to different magnetic fields at 15, 25, 35, 45 or 60 mT. Each experiment was conducted in triplicate, and *Acinetobacter sp*B11 not exposed to a magnetic field served as the control. The experimental setup of the biodegradation process using a static magnetic field (SMF) is shown in Fig. 1. The magnetic field was generated by a commercially available circular ferrite permanent magnet (Changzhou, China). The diameter and thickness of the magnet were 70 mm and 10 mm, respectively. The static magnetic field intensity was measured using a Tesla meter (HT20, Shanghai Hengtong Electromagnetic Technology Co. Ltd.).

2.4. Experimental methods

The oil concentration was determined according to the UV spectrophotometric method for the determination of oil (93-1994 SL).

The bacterial cell surface hydrophobicity was assessed using the microbial adhesion to hydrocarbons (MATH) method (Rosenberg, 2006). Cell surface hydrophobicity is given by the following equation: $CSH = (M–N)/M \times 100\%$ (2-1), where *CSH* indicates the cell surface hydrophobicity, *M* is the OD_{600} value of the bacterial suspension before it is mixed with n-dodecane and *N* is the OD_{600} of the aqueous phase after the bacterial suspension is mixed with n-dodecane.

The oil-spreading diameter was measured using the following procedure. First, 0.1mL of crude oil preheated

Fig. 1. The experimental setup for the biodegradation process.

to 45°C and dyed with oil red O was dripped into a Petri dish with 30 mL of distilled water. When the oil film was evenly distributed on the surface of the water, 0.1 mL of the B11 suspension was dripped onto the oil film. The oil film was squeezed to the edge; when it stabilized, the oil-spreading diameter was measured 3 times to determine the average.

Superoxide dismutase (SOD) activity in kidney homogenates was measured by a competitive inhibition assay using xanthine-xanthine oxidase system to reduce NBT. The surfactant activity was measured by the critical micelle concentration (CMC). The surfactant was measured by thin layer chromatography. The OD_{260} and OD_{280} values were used to reflect the nucleic acid and protein concentrations in the solution.

3. Results

3.1. Effects of magnetic fields on the oil degradation efficiency

The activated B11 suspension was inoculated into liquid oil medium at a concentration of 200 mg/L. Degradation in the 25-mT magnetic field was tested and compared to degradation in the absence of a magnetic field. An oil medium sample without bacteria was used as the blank control. After 7 d of degradation on the shaker at 30°C and 100 rpm, the residual oil was extracted with dichloromethane for GC-MS analysis. The GC-MS analysis results are shown in Fig. 2.

As shown in Fig. 2, the oil in the blank control included saturated hydrocarbons with an even number of carbon atoms (C_{14} to C_{22}) and C_{22} to C_{26} saturated hydrocarbons. Remarkably, the biodegradation of n-alkanes occurred independently of the presence of a magnetic field. The effective degradation of C_{14} to C_{26} alkanes implies that the B11 strain grows well when C_{14} to C_{26} alkanes serve as the only carbon source. The B11 strain had a better degradative effect on long-chain alkanes (C_{18}^{+}) than on C_{14} to C_{16} short-chain alkanes, which was in conformity with the observation that most oil-degrading bacteria would first degrade n-alkanes with high carbon numbers into n-alkanes with low carbon numbers when crude oil served as the carbon source.

Compared with degradation without an external magnetic field, a better degradative effect on oil was observed with a 25-mT external magnetic field. The magnetic field had a clear positive effect on the degradation of C_{14} to C_{26} alkanes, especially on the degradation of short chain alkanes. As shown in Fig. 2, the degradation rate of n-alkanes with low carbon numbers was relatively low without an external magnetic field. For example, the degradation rate of C_{14} alkanes was only 38.8%. The degradation rate gradually increased as the alkane carbon numbers grew. The degradation rate of C_{26} alkanes reached 78.6%, which was the highest among all alkanes. However, with an external 25-mT magnetic field, the degradation rate of C_{14} alkanes was the highest (88.9%) and was 50.1% higher than the rate without an external magnetic field.

3.2. Effect of magnetic fields on microbial activity

After activation, the B11 strain were transferred to nutrient liquid medium and placed at 30°C on a 100 rpm shaking bed for culture. The magnetic fields applied were 0, 15, 25, 35, 45, and 60 mT; three parallel samples were cultured at each magnetic field intensity overnight. The microbial activity analysis results are shown in Fig. 3.

As shown in Fig. 3, the SOD activity in the absence of the magnetic field treatment was stable at 29 U/L. The lower magnetic fields (15, 25, and 35 mT) had positive effects on the activity of the B11 strain. The maximum SOD value was 32.71 U/L at 8 h when the magnetic field intensity was 25 mT, which was 12.3% higher than the activity measured without the magnetic field treatment. Conversely, the cell activity was inhibited after long-term treatment with higher magnetization (45 mT and 60 mT). The SOD activity increased at 2 h and then decreased gradually with the 45-mT and 60-mT magnetic fields, showing that high-strength magnetic fields might inhibit or damage bacterial activity.

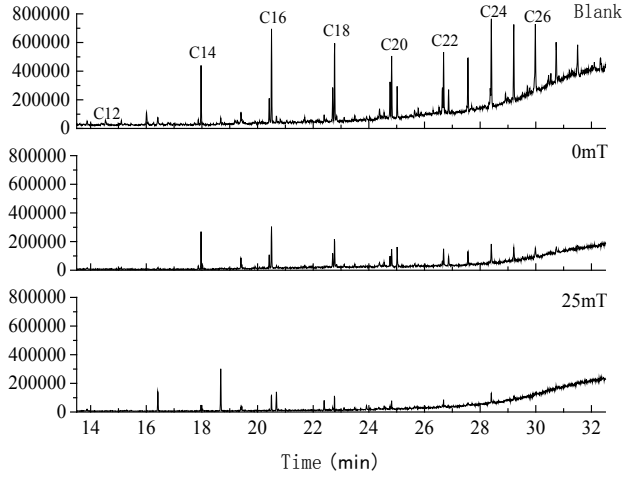

Fig. 2. The effect of magnetic field intensities on microbial oil degradation.

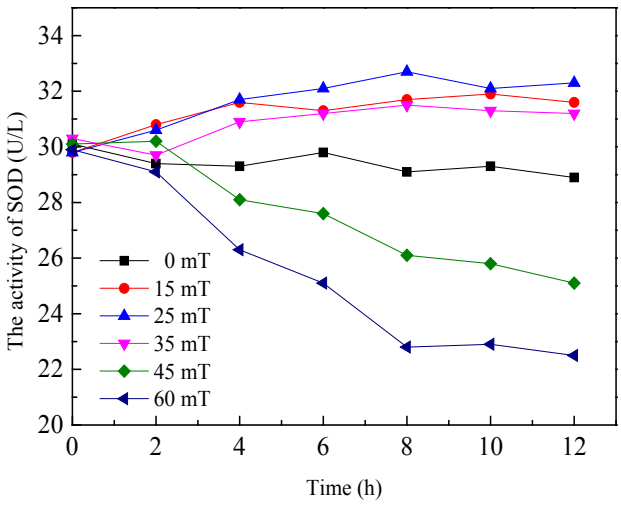

Fig. 3. Effect of the magnetic field on microbial activity.

3.3. Effect of magnetic fields on cell membrane permeability

In the present study, the activated B11 strain was transferred to nutrient liquid medium and placed in the 100 rpm shaking bed at 30°C for culture with external magnetic fields of 0, 25, 35, 45 and 60 mT. Three parallel samples were cultured with each magnetic field strength overnight. The OD_{260} and OD_{280} values reflected the nucleic acid and protein concentrations in the solutions.

As shown in Fig. 4, the amount of nucleic acid and protein leakage gradually increased with the magnetic field stimulation time, with an especially rapid increase from 4–8 h, and achieved stability after 10 h. With the increase in the magnetic field strength, the amount of nucleic acid and protein leakage also gradually increased. When the magnetic field strength was 15 mT and 25 mT, the amount of nucleic acid and protein leakage was not obvious. When the magnetic field strength was 60 mT, the amount of nucleic acid and protein leakage reached the maximum,whereas the leakage of nucleic acids and proteins in cells without magnetic field treatment was only 9.3% and 44%, respectively, of the leakage detected in the stimulation group with 60 mT.

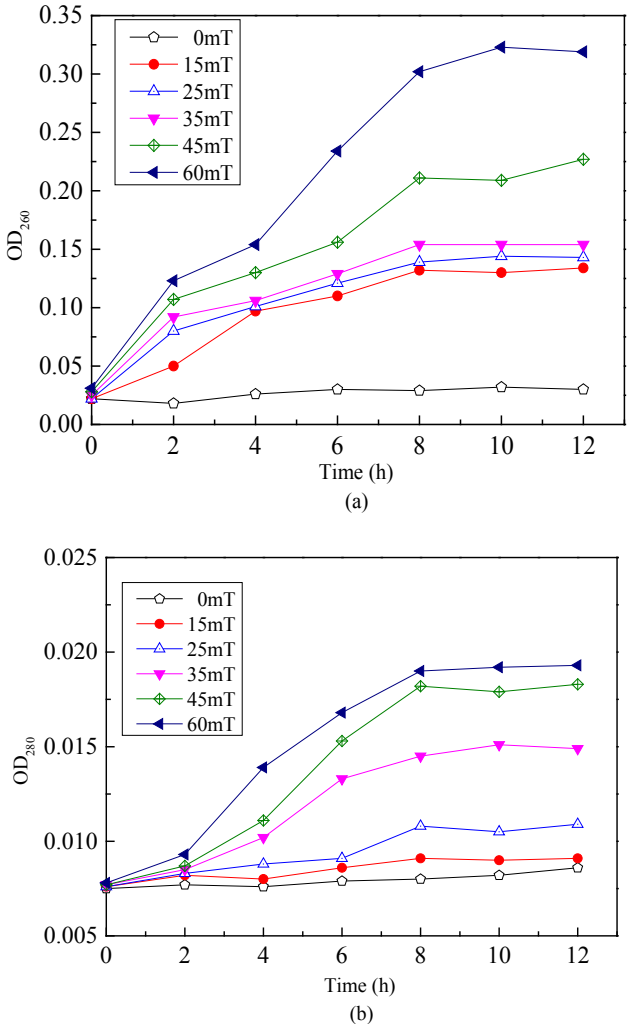

Fig. 4. Effect of magnetic fields on the extravasation of nucleic acids (a) and intracellular proteins (b).

3.4. Effect of magnetic fields on microbial hydrophobicity

Bacterial cell surface hydrophobicity is an important factor underlying the nonspecific adhesion of bacteria to various microorganisms and surfaces. In this experiment, activated B11 strain bacteria were inoculated into nutrient medium and placed on the shaker at 30°C and 100 rpm for one night in the presence of an external magnetic field of 0 mT, 15 mT, 25 mT, 35 mT, 45 mT or 60 mT. Three parallel samples were collected from each condition. The CSH of the B11 strain exposed to the different magnetic field intensities was tested using the MATH method; the results are shown in Fig. 5.

The average CSH of the B11 strain in the absence of a magnetic field was 24.1%. As the magnetic field intensity increased, the CSH of the B11 strain rose gradually to a peak value and then dropped. For example, the CSH in the presence of the 25-mT magnetic field peaked at 32.7%, which was 6.7% higher than the CSH in the untreated sample in the absence of a magnetic field. As the magnetic field intensity continued to increase, the increased margin of the CSH was reduced. The increased margin of the CSH was only 3.3% under a magnetic field intensity of 60 mT, which was the smallest increase in the different intensities of the magnetic fields.

3.5. Effect of magnetic fields on the oil-spreading diameter

Normally, the oil-spreading diameter has a linear relationship with the surfactant concentration [14]. Therefore, the surfactant concentration in a solution can be preliminarily measured using the oil-spreading method. In our experiment, the activated B11 strain was inoculated into nutrient medium and placed on a shaker at 30°C and 100 rpm for one night in the presence of an external magnetic field of 0 mT, 15 mT, 25 mT, 35 mT, 45 mT or 60 mT. Three parallel samples were prepared for each condition.

As shown in Fig. 6, the oil-spreading diameter of the B11 solution increased to different extents as the magnetic field intensity increased from 15 mT to 45 mT. For example, the oil-spreading diameter peaked at 3.56 cm in the presence of

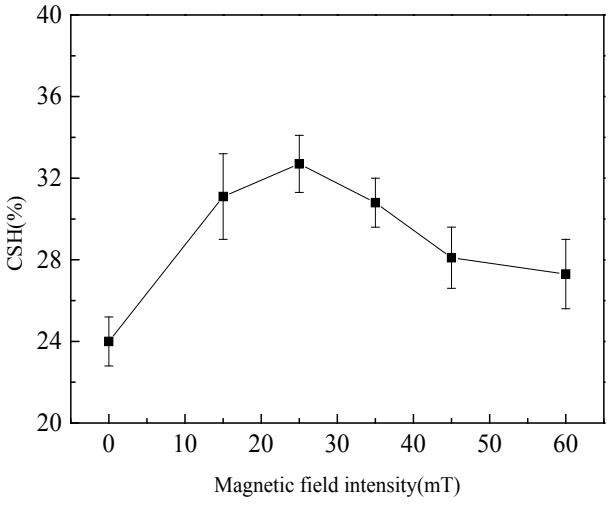

Fig. 5. The effect of magnetic fields on microbial hydrophobicity.

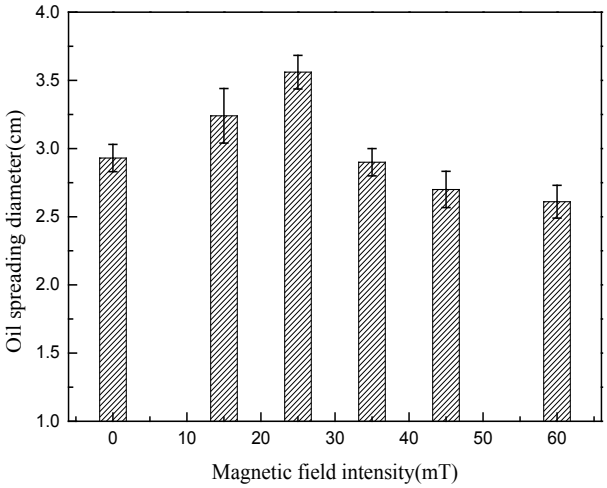

Fig. 6. Effect of the magnetic fields on the oil-spreading diameter.

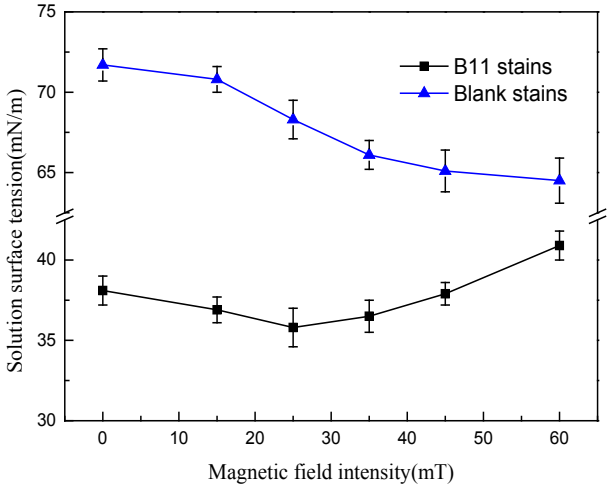

Fig. 7. Effect of the magnetic field on the microbial solution surface tension.

the 25-mT magnetic field, which was 21.9% greater than the diameter of the control. When the magnetic field intensity increased to 60 mT, the oil-spreading diameter was 1.1% less than that of the control.

3.6. Effect of magnetic fields on the microbial solution surface tension

The activated B11 strain was inoculated into nutrient medium and placed on the shaker at 30°C and 100 rpm for one night in the presence of an external magnetic field of 0 mT, 15 mT, 25 mT, 35 mT, 45 mT or 60 mT. Nutrient medium with no bacteria was used as the blank control. Three parallel samples were prepared for each magnetic field intensity. The surface tensions of the B11 solution and the nutrient medium under the different magnetic field intensities are shown in Fig. 7.

With or without an external magnetic field, the surface tension of the B11 solution dropped more sharply from baseline than that of the nutrient medium. In the presence of the 25-mT magnetic field, the surface tension of the B11 solution dropped 47.6% from baseline, whereas the surface tension of the nutrient medium dropped only 4.7%. The result suggested that the surfactant released during the growth of the B11 strain played a dominant role in the decrease in surface tension. In addition, when the magnetic field was 15 mT, 25 mT and 35 mT, the surface tensions of the B11 solution were 3.2%, 6%, and 4.2% lower than those obtained without a magnetic field, respectively. In the25-mT magnetic field, the B11 solution surface tension dropped to 35.8 mN/m, which was the lowest surface tension value obtained. When the magnetic field intensity rose to 60 mT, the B11 solution surface tension increased to 40.9 mN/m, which was 7.1% higher than the tension obtained without a magnetic field.

3.7. Effect of magnetic fields on the release of bio-surfactants by microorganisms

The activated B11 strain was inoculated into nutrient medium and placed in a shaker at 30°C and 100 r/min for one night with or without the25-mT magnetic field. Three parallel samples were prepared for each magnetic field intensity.

After one night of cultivation, the surfactants were extracted, and the surfactant contents in each 100-mL bacterial solution were calculated. The results showed that the yield of surfactants was 0.114 g/100 mL in the 25-mT magnetic field, which represented a 17.57% increase compared to the bacterial solution without an external magnetic field. Xu [15] found that water magnetized with 175 mT significantly improved the lipoprotein concentration in the blood serum, which indicated that an appropriate magnetic field intensity facilitated the synthesis of macromolecular substances by microorganisms, such as proteins and lipids.

4. Discussion

Magnetic fields have been used in biodegradation processes, and some studies have shown that magnetic fields can improve pollutant removal performances. Yavuz and Celebi [16] found that the substrate removal rate was increased by increasing the magnetic field strength, reached a maximum at 17.8 mT and then decreased with a further increase in the magnetic field. This finding indicated that the magnetic field had a positive effect on the acclimation of bacteria and revealed that the activated sludge acclimatization and organic pollutant biodegradation processes were stimulated under magnetic fields, resulting in higher efficiency wastewater treatment [17]. Fig. 2 shows that the enhancing effect of the magnetic field on the biodegradation of petroleum hydrocarbons decreases as the carbon chain length increases. Although the gap between the degradation rates with and without external magnetic fields tended to close, the degradation rates of alkanes with different lengths of carbon chains remained above 80% with an external magnetic field (except for the degradation rate of C_{16} alkanes, which was 79.8%), which were superior to the degradation rates obtained without an external magnetic field. These results suggest that magnetic fields can enhance the degradation of n-alkanes by microorganisms, especially

short-chain alkanes. One possible explanation may be that magnetic fields affect both the degradation efficiency of microorganisms and the properties of short-chain alkanes.

The effects of magnetic fields on microbial activity shown in Fig. 3 were similar to the results of Li's research, which found that the proliferation activity of vascular endothelial cells was obviously promoted by the application of a 0.05-mT magnetic field, was not sensitive to the 0.1-mT magnetic field, and was inhibited by the 1-mT and 5-mT magnetic fields [18]. A magnetic field can affect some active centers, coenzymes or metal enzymes with auxiliary groups containing trace metal atoms or ions [19,20]; these metal atoms and ions in the dynamic magnetic field generated by the Lorenz force change the arrangement and thus affect the enzyme activity. The enzymatic reaction is usually the pairing reaction between the electrons produced and reactant substances; the magnetic field can produce a magnetic force on these unpaired electrons, making them spin and thus affecting the enzymatic reaction. If the magnetic field intensity is too high, the bio-activity may decrease or even become inactivated. Therefore, choosing the appropriate magnetic field intensity can enhance the oxidation resistance ability of the microorganism, which is beneficial for the growth and reproduction of the microorganism and protective against the harsh environment [21,22].

Fig. 4 shows that the magnetic field can enhance the permeability of the cell membrane, with a greater magnetic field strength having a greater influence on cell membrane permeability. When the magnetic field intensity reaches a certain level, the lipid bilayer of the cell membranes is destroyed, resulting in the release of macromolecules that cannot cross the membrane under normal circumstances. Cell membrane permselectivity is damaged to different extents during the process without maintaining the cell internal and external balances, resulting in leakage of material including cellular nucleic acids and proteins, exosmosis, and finally a decrease in cell activity [23,24]. At a magnetic field strength of 60 mT, the cell membrane of *Acinetobacter sp* strain B11 might be destroyed, which would cause the internal solution to flow out and sharply increase the nucleic acid and protein concentrations. Zhang et al. also found that the cell membrane permeability increased to a certain extent, which made the intracellular soluble outflow and seriously affected the cell activity [25].

The effects of magnetic fields on microbial hydrophobicity (Fig. 5) suggest that magnetic fields with different intensities may increase the CSH of the B11 strain to different extents. To degrade hydrocarbons, microorganisms must have effective physical contact with substrates, but most hydrocarbons are water-insoluble and display strong hydrophobicity. Therefore, the CSH of microorganisms needs to be adjusted to improve effective contacts with water-insoluble substrates. There are three major microbial pathways for hydrocarbon uptake: direct utilization of hydrocarbons dissolved in water, contact of cellular surfaces with hydrocarbon surfaces and transport of macromolecular hydrocarbons into the interior of microorganisms. Previous studies have shown that during the biodegradation of hydrocarbons in oil pollutants, the CSH has a significant impact on the adhesion of bacteria to pollutants, which affects the uptake of hydrocarbons [26]. Al-Tahhan et al. argued that the increase in CSH improved the effective contact of bacteria with hydrophobic organic substrates and thus enhanced hydrocarbon uptake [27]. Lan et al.'s study on activated sludge in glucose wastewater in a low-intensity magnetic field also found that the CSH of microorganisms could be affected by magnetic fields [28]. In our experiment, the average CSH of the B11 strain was 24.1%, indicating that the B11 strain had difficulty directly using hydrocarbons dissolved in water. In addition, B11 has no flagella, and its capacity to move in water is low. Therefore, the catch and transport of macromolecular hydrocarbons to the interior of B11 is unlikely, and the most likely pathway for hydrocarbon uptake by B11 is direct contact of its cellular surface with hydrocarbon surfaces. The magnetic field may increase the metabolism of the B11 strain and stimulate the release of surfactants into the surroundings. As a result, the surfactants will increase the hydrophobicity and adhesion of B11 and improve the degradation of hydrocarbons in the form of small droplets [29].

The effect of a magnetic field on the oil-spreading diameterin Fig. 6 shows that an increase in the oil-spreading diameter indicated a decrease in the surface tension of the B11 solution. This effect might be a result of the increased surfactant release induced by the magnetic field or changes in the liquid properties in response to the magnetic field. The water solubility, contact angle and surface tension may be affected by an electromagnetic field or a static magnetic field. Nie et al. [30] found that the surface tension of mine water was reduced from 65 mN/m to 56 mN/m in a magnetic field of 100 mT. Deng et al. [31] argued that the reason for the decrease in the water surface tension in a magnetic field might be changes in molecular bonding and polarity. Therefore, changes in the solution surface tension and surfactant release by bacteria should be examined.

The microbial solution surface tension first increases and then decreases with an increase in the magnetic field intensity (Fig. 7). Zhang et al. [14] found that the relationship between the medium surface tension and the surfactant concentration was normally linear. One explanation for the decrease in the bacterial solution surface tension in a magnetic field is that the reproduction of microorganisms is accelerated and more surfactants are released due to this increased metabolism. Wan et al. [32] showed that oil was classified as either polar or nonpolar and determined that modifications to the magnetic field made the surface tension of the nonpolar oil rise by 0.3%–4% with the increase in the magnetic intensity; additionally, the surface tension of the nonpolar oil presented a cyclical change, the water–oil interfacial tension was reduced, and the nonpolar oil dispersivity in water was improved. However, magnetic field modifications make the surface tension of polar oil decrease, similar to that of water. After mixing nonpolar oil with polar oil, nonpolar oil takes the leading role when the amount of polar oil is minor, and magnetic field modification makes the surface tension of the nonpolar–polar oil mixtures rise. However, with increases in the polar oil component in nonpolar–polar oil mixtures, polar oil takes the leading role gradually as the magnetic field modification makes the surface tension of the nonpolar–polar oil mixtures decrease. Further studies are needed to examine whether this decrease is a result of increased amounts of surfactant or changes in surfactant properties.

The critical micelle concentration (CMC) is the threshold concentration for surfactants to form micelles and is an important characteristic of a surfactant. In our experiment, surfactants extracted from the B11 solutions cultivated in 0-mT and 25-mT magnetic fields were prepared as sample solutions with mass concentrations ranging from 10 mg/L to 100 mg/L. The analysis showed that the surface tensions of the sample solutions decreased with the increase in the solution mass concentration until it stabilized at a certain value. An intersection point can be found when we extend the straight line parts to both sides of the inflection point of the surface tension curve. The mass concentration value of the intersection point is the CMC of the surfactants. Our experimental results showed that when the magnetic field intensity was 0mT, the CMC of the bio-surfactants was 57.79 mg/L and the lowest surface tension was 33.06 mN/m. The CMC value was significantly different from those of commonly used chemical surfactants (e.g., the CMC of cetyltrimethyl ammonium bromide is 397 mN/m), indicating a better performance of the bio-surfactant. When the magnetic field intensity was 25 mT, the CMC of the bio-surfactants extracted from the bacterial solution was 57.68 mg/L, which was very similar to the surfactant CMC without an external magnetic field.

4. Conclusion

Through studying the effects of the magnetic field on oil-degrading bacteria (i.e., the bioactivity, cell membrane permeability, hydrophobicity, oil-spreading diameter, surface tension and bio-surfactants), this effort provides a more in-depth understanding of the impact of magnetic field effects on oil biodegradation by bacteria. A few highlights are worth noting:

Lower magnetic field strength (15–25 mT) increased the degradation of n-alkanes by microorganisms, especially for short-chain hydrocarbon removal.

Under the premise of not destroying the cell membrane, lower magnetic fields enhanced the permeability of the cell membrane and improved the activity of the oil-degrading bacteria.

The magnetic field stimulated oil-degrading bacteria to release more surfactants into their surroundings but did not change the properties of the extracted surfactants, which increased the surface hydrophobicity and decreased the surface tension. Thus, the microorganisms had better physical contact with the oil pollutants in the wastewater, which improved the degradation of hydrocarbons.

Acknowledgments

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