Effect of a static magnetic field on the microscopic characteristics of highly efficient oil-removing bacteria
Zhijun Ren, Xiaodong Leng and Qian Liu

ABSTRACT
To better understand the microbial oil removal enhancement process by a magnetic field, the effect of a static magnetic field (SMF) on the microscopic characteristics of highly efficient biodegradation oil-removing bacteria was studied. The Acinetobacter sp. B11 strain with a 53.6% oil removal rate was selected as the reference bacteria. The changes in the microscopic characteristics of Acinetobacter sp. B11 such as the cell surface morphology, cell permeability and cell activity of the bacteria were investigated. The results showed that low-intensity magnetic fields (15–35 mT) improved the ability of Acinetobacter sp. B11 to remove oil by 11.9% at 25 mT compared with that of bacteria with no magnetic field. Without destroying the cell membrane, the low-intensity magnetic fields increased the cell membrane permeability and improved the activity of superoxide dismutase (SOD), which effectively enhanced the oil degradation performance of the bacteria.

Key words | cell membrane permeability, static magnetic field (SMF), superoxide dismutase (SOD) activity, surface morphology

INTRODUCTION
The effects of magnetic fields on biological systems have received considerable experimental and theoretical attention because of their low cost, simple maintenance and low environmental impact (Kim 1976; Hong 1995; Chen et al. 2012). In recent years, the physical, chemical and biological effects of magnetic fields on the microbial degradation process have been widely studied in biologically activated sludge, magnetic biological packing, biological fluidized beds, and magnetically immobilized enzyme water treatment (Ma et al. 2010). Jung et al. (1993) have shown that a magnetic field could enhance the rate of phenol biodegradation in an activated sludge mixed culture by 30%, and a patent for the use of magnetic fields to enhance the bioremediation of hydrocarbon-contaminated soil already exists (Rawls & Provell 1994). Çelebi & Yavuz (2000) found that the organic removal rate was increased by 44% when a magnetic field was added to the activated sludge process. Wang (2010) studied the effects of magnetic field strength on denitrification characteristics and found that there was an optimal magnetic field range (60–90 mT) in which the magnetic treatment could reduce the zeta potential of the sludge particles and correspondingly increase the sludge sedimentation rate. The results of a study by Nong (2001) showed that a magnetic field of 0.15 T inhibited the growth of Candida albicans, but the 0.15 T magnetic field exposure had no significant effect on the growth of Staphylococcus. Masahiro et al. (2000) found that a ferrite magnet caused strength-dependent decreases in the growth rate and maximum growth number of the bacteria S. mutans and S. aureus when cultured under anaerobic conditions, but their growth was not inhibited under aerobic conditions.

The influence of magnetic fields on microorganisms is very complex. Previous studies have focused on evaluating the performance of organic removal or the macroscopic characteristics of microbes with the addition of a magnetic field. In the present study, a highly efficient oil-removing strain (Acinetobacter sp. B11) with a 53.6% oil removal rate was selected as the reference bacteria. The effects of magnetic fields on the microscopic characteristics of Acinetobacter sp. B11 were investigated by measuring the cell surface morphology, cell permeability and cell activity of the bacteria. The effect of magnetic fields on microscopic characteristics of microorganisms is conducive to understanding the microbial biodegradation process and promoting the application of magnetic fields in...
biotransformation, biodegradation, and bioremediation of oil pollution.

MATERIALS AND METHODS

Experimental water samples

The preparation process of oily wastewater was as follows. Crude oil and Tween-80 were mixed at a volume ratio of 1:5. Water was heated to 35 °C and was gradually poured into the crude oil and Tween-80 solution. The emulsified liquid was transferred to a separatory funnel for approximately 30 min; afterwards, the emulsified oil solution was released from the bottom of the separatory funnel, and the upper oil was removed to obtain the stable oily wastewater.

Isolation and cultivation of the bacteria

Twenty-two strains were isolated from the Daqing Oilfield wastewater biochemical treatment plant. Based on the mean 7-day oil removal rate, the strain with the highest removal rate (53.6%) was selected as the reference bacteria (Zhang 2014). The microbial physiology and biochemistry of the strain were tested. The results showed that the colonies of the strain were round with a diameter of 1–2 mm. The surfaces of the colonies were smooth and moist with well-defined edges, and a slight uplift in the center with yellow teeth was observed. The bacteria morphology was composed of short rods that were blunt at the two ends and were usually single or paired and had no spores. The results of the physiological and biochemical identifications showed that the strain was catalase positive, oxidase negative, and gram negative without growth factors.

Genetic sequencing was also conducted by Sangon Biotech (Shanghai) and was compared for sequence homology using the National Center for Biotechnology Information and the ribosomal database (http://rdp.cme.msu.edu/index.jsp). The results of the 16S rDNA sequences were also compared for sequence homology using Blast in GenBank. The comparisons showed a 99.6% similarity to the genetic sequences of Acinetobacter (FJ494703).

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

Magnetic treatment of Acinetobacter sp. B11

To prepare the bacterial suspension, the single colonies taken from the agar slant culture medium were inoculated into a beef extract peptone medium and were placed in a thermostatic shaker at 200 rpm for 24 hours at 30 °C. The bacterial suspension was diluted to an OD600 value of 0.8. Then, 5 mL of the bacterial suspension was placed in a 150 mL conical flask with 50 mL liquid oil medium; the flask was placed in a thermostatic shaker at 100 rpm and 30 °C for 7 days. Subsequently, 50 mL samples of the Acinetobacter sp. B11 cell suspension were exposed to different magnetic fields of 15, 25, 35, 45 or 60 mT. Each experiment was conducted in triplicate, and Acinetobacter sp. B11 that was not exposed to a magnetic field served as the control. Figure 1 shows the experimental setup of the biodegradation process using a static magnetic field (SMF).
A circular ferrite permanent magnet purchased from Changzhou City was used to generate the magnetic field. The diameter and thickness of the magnet were 70 mm and 10 mm, respectively. The SMF intensity was measured using a Tesla meter.

Experimental methods

The oil concentration was determined using an ultraviolet spectrophotometer according to the UV spectrophotometric method for the determination of oil (93-1994 SL). To ensure a good linear relationship between the concentration of the bacterial suspension and the absorbance, the absorbance at a wavelength of 600 nm (OD$_{600}$) was used to characterize the biomass. The surface morphology of the bacteria was monitored using atomic force microscopy (AFM). The xanthine oxidase method (Zhao et al., 2010) was used to determine the superoxide dismutase (SOD) activity. A fluorimunoassay (FIA) fluorescent probe for measuring intracellular calcium was used to determine the concentration of Ca$^{2+}$. Oil concentration and bacterial intracellular enzyme activity were calculated as the average and the average deviation.

RESULTS

Effects of the magnetic field on the oil removal performance of the bacteria

Magnetic field intensities have an important effect on microorganism biodegradation (Zhang et al. 2002). The culture medium was inoculated with Acinetobacter sp. B11. The concentration of the liquid petroleum oil was approximately 200 mg/L, the temperature was set at 30 °C, and the magnetic field intensity was set at 0, 15, 25, 35, 45 or 60 mT. The oil removal performance of Acinetobacter sp. B11 at different magnetic field intensities was measured, and the results are shown in Figure 2.

When no external magnetic field was used for the oil biodegradation process, the concentration of the residual oil was 85.3 mg/L, and the oil degradation rate was 57.5%. When the magnetic field intensity increased from 0 to 25 mT, the oil removal rate also increased. At a magnetic field intensity of 25 mT, the oil removal rate by Acinetobacter sp. B11 reached a maximum value of 66%, and the oil removal rate increased by 11.9% compared with that of the bacteria with no magnetic field treatment (Ren et al. 2016). When the magnetic field intensity was increased to more than 30 mT, the oil degradation by Acinetobacter sp. B11 decreased from its peak value. At a magnetic field intensity of 60 mT, the residual oil concentration was 97.2 mg/L, and the oil removal rate was 10.4% lower than that by the bacteria with no magnetic field treatment.

Effect of the magnetic field on the bacterial surface morphology

AFM is an important research tool used in cell and molecular biology and has an important application in studies on the physiological activity of single cells (Li et al. 2013). Acinetobacter sp. B11 was transferred to a liquid nutrient medium and placed on a shaking bed at 100 rpm and 30 °C for a culture in the presence of the external magnetic fields of 0, 25 or 60 mT. The AFM images of Acinetobacter sp. B11 subjected to different magnetic fields are shown in Figure 3.

In Figure 3(a), it can be observed that the shape of the untreated Acinetobacter sp. B11 was spherical, without flagella, and had a clear boundary and smooth surface with no surface damage; the diameter was approximately 1.2–1.4 μm.

Figure 3(b) shows Acinetobacter sp. B11 that was exposed to a 25 mT magnetic field. There was no obvious damage to the cell, but the surface had a small fold, which indicated that the magnetic field had begun to affect the cells.

Figure 3(c) shows Acinetobacter sp. B11 that was exposed to a 60 mT magnetic field. The AFM images show that the morphology of the cells obviously changed compared with those in the other images. The surface
roughness of these cells increased, the surfaces bulged, and the centers were severely depressed.

**Effect of the magnetic field on the cell membrane permeability**

The cell membrane, a key component of the cell structure, separates the cytoplasm from the outside environment. Cell permeability is a direct measurement of how easily an extracellular material can move in and out of the cytoplasm through the cell membrane. In the present study, the effect of a magnetic field on the membrane permeability was investigated by examining the Ca\(^{2+}\) concentration. Ca\(^{2+}\) is a messenger in the cell signaling of living cells, and the concentration of intracellular Ca\(^{2+}\) is much less than that of extracellular Ca\(^{2+}\). When the cell membrane is damaged, the cell membrane permeability is increased, and Ca\(^{2+}\) from the external environment can enter the cell; thus, the Ca\(^{2+}\) concentration in the microorganism is increased. As a universal Ca\(^{2+}\) fluorescence indicator, Fluo3-AM has a proportional relationship with Ca\(^{2+}\) concentration, and the use of FIA as a fluorescent probe for calcium is commonly used to characterize the permeability of the cell membrane. *Acinetobacter* sp. B11 was transferred to a liquid nutrient

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**Figure 3** | AFM microscopic images of *Acinetobacter* sp. B11 after exposure to the different magnetic fields. (a) AFM microscopic images of the untreated *Acinetobacter* sp. (b) AFM microscopic images of *Acinetobacter* sp. after exposure to the 25 mT magnetic field. (c) AFM microscopic images of *Acinetobacter* sp. after exposure to the 60 mT magnetic field.
medium, placed on a shaking bed at 100 rpm and 30 °C to culture, and exposed to external magnetic fields of 0, 15, 25, 35, 45 or 60 mT. Three parallel samples were cultured overnight at each magnetic field strength. The effects of the different magnetic field intensities on the cell permeability of *Acinetobacter* sp. B11 are shown in Figure 4.

The fluorescence intensity of the intracellular calcium concentration was 96.1 A.U. at 528 nm when *Acinetobacter* sp. B11 was not exposed to a magnetic field, and the fluorescence intensity increased with SMF exposure within the experimental range of 15–60 mT. The fluorescence intensity of the intracellular calcium concentrations reached a maximum of 498.7 A.U. at the magnetic field strength of 35 mT. When the magnetic field strength was 60 mT, the increasing rate of the intracellular calcium fluorescence intensity in the cells was the smallest, and the fluorescence intensity of the intracellular calcium concentration was 266.7 A.U.; this value was higher than that with a 0 mT exposure.

**Effect of the magnetic fields on the bacterial enzyme activity**

The SODs are a group of metalloenzymes that catalyze the conversion of reactive superoxide anions into hydrogen peroxide. *Acinetobacter* sp. B11 was transferred to a liquid nutrient medium on a shaker at 100 rpm and 30 °C for culture and exposed to 0, 15, 25, 35, 45, or 60 mT magnetic fields. For each magnetic field intensity, three parallel samples were used to measure the SOD activity of the B11 strain at 0, 2, 4, 6, 8, 10 and 12 hours. The effects of the magnetic field strength on the SOD activity of the B11 strains are shown in Figure 5.

Figure 5 shows that the activity of SOD without magnetic field treatment was stable at approximately 29.1 U/L. After exposure to the low-intensity SMFs (15 mT, 25 mT and 35 mT), the activity of SOD increased with an increase of the culture time. For the 25 mT magnetic field intensity, the maximum SOD activity of 32.71 U/L occurred at 8 hours, which was 12.3% higher than the SOD activity with no magnetic field exposure. However, further increases in the magnetic field exposure times did not cause increases in the enzyme activities. This result is similar to that of Çelik et al. (2009). At higher magnetic field intensities, the SOD activity gradually decreased with an increase in the culture time. At 60 mT, the activity of SOD at 12 hours was 22.5 U/L, which was 24.7% less than that with no magnetic field.

**DISCUSSION**

We can see from Figure 2 that low-intensity magnetic fields (15–35 mT) improved the ability of *Acinetobacter* sp. B11 to remove oil by 11.9% at 25 mT compared with that of bacteria with no magnetic field. The results were similar to Han & Shao (2002), who studied the effect of magnetic fields on activated sludge used for oily wastewater treatment; the activity of the activated sludge was enhanced, and the removal rate of the chemical oxygen demand (CODCr) was also increased when the intensity of the magnetic field was 6.4–58 mT. In Figure 3, the results showed that the cell surface morphology was affected by the magnetic field. When the magnetic field intensity was 25 mT,
small pits, folds and other minor changes appeared on the cell surface. When the magnetic field intensity was further increased to 60 mT, the cell surface was rougher, with marked depressions and out folding. The damage to the cell surface increased, ultimately forming microperforations that led to holes in the structure, resulting in irreversible damage to the cell membrane and decreased cell activity (Liu 2011). Li et al. (2015) studied the magnetic field treatment of the newly isolated Paenibacillus sp.; this study also found that, at 300 mT, the cells became thin and longer, while at 500 mT, the cell membrane ruptured and the cytoplasm was released, leading to the death of the Paenibacillus sp. bacteria. These results revealed that a suitable processing time with a specific intensity can promote the growth of Paenibacillus sp., but a higher intensity and longer magnetic exposure time inhibited the growth and led to the death of Paenibacillus sp.

The effects of the external magnetic field on cell permeability can cause the fluorescence intensity of the intracellular calcium concentration to increase, and the improved cell permeability can enhance the catalytic efficiency of the cells, which is shown in Figure 4. According to the analysis of the effect of the magnetic field on the surface morphology of Acinetobacter sp. B11 shown in Figure 3, the 25–35 mT magnetic field caused a depression of the cell surface, but the overall shape of the cells was not damaged; thus, the cells could maintain normal activity. At the 25–35 mT magnetic field exposure, the Ca$^{2+}$ in the cell completely combined with the Fluo3 because of the increased permeability of the cells, as indicated by the fluorescence intensity. Aoki et al. (1990) studied the effects of magnetic fields on the plasma membrane permeability in living cells using flow cytometric techniques to measure the accumulation and efflux of adriamycin (ADR) in a cell line. The amount of ADR that accumulated in the cells after 15 min of exposure to a SMF (0.4 T) was less than 5–10% compared with that in the control cells without exposure to the magnetic field within a temperature range of 41–46 ºC, corresponding to the phase transition temperature. With exposure to the 60 mT magnetic field, there was a severe depression in the middle of the cell, and the cell membrane surface might have had multiple perforations, causing the cytoplasm to leak out. In a study of cell membrane permeability after exposure to an intense pulsed electric field, Zhang et al. (2007) found that the cell membrane permeability was increased, which caused the intracellular fluid to leak out and seriously affected the cell activity. When the magnetic field strength was 60 mT, the cell membrane of Acinetobacter sp. B11 was destroyed, the cytoplasm leaked out, and the cell activity was decreased; these changes caused the Ca$^{2+}$ concentration that had increased because of the cell membrane permeability to decrease, leading to a decrease in the fluorescence intensity. Shen et al. (2007) evaluated the effects of moderate-intensity SMFs on two types of voltage-gated potassium channel (VGPC) currents; I (K, A) and I (K, V); whole-cell patch-clamp experiments were conducted using acute dissociated rat trigeminal root ganglion (TRG) neurons. The results demonstrated that a 125 mT SMF could influence the inactivation kinetics of the two VGPC currents by altering the inactivation rate and velocity. No significant change was observed in the activation properties. These findings supported the hypothesis that biological membranes are deformed in a moderate-intensity SMF, and the physiological characteristics of the ion channels of the membrane are influenced.

The effects of the magnetic field strength on the SOD activity (in Figure 5) showed that low-intensity SMFs (15 mT, 25 mT and 35 mT) had a positive effect on the activity of SOD and high-intensity SMFs (45 mT and 60mT) had a negative effect on the activity of SOD. There are two theories about the influence of magnetic fields on microbial enzyme activity. One theory is that magnetic fields can affect some active centers, which are the enzymes or enzymes with auxiliary groups that contain trace metal atoms or ions; these metal atoms and ions exposed to the dynamic magnetic field are rearranged by the Lorentz force to affect the enzyme activity. The enzymatic reactions are usually paired reactions between the electrons produced and the reactant substances, and the magnetic field can produce a magnetic force on these unpaired electrons, affecting the enzymatic reactions (Frankel & Liburdy 1996). A study by Sato et al. (1992) showed that SOD contains magnetotactic metal elements such as copper, which are released when SOD is destroyed and inactivated. It can be inferred that the active center, the metal-containing coenzyme and auxiliary elements of SOD, are affected by a change in the magnetic field from the original state, causing the SOD activity to change. If the magnetic field intensity is too great, then the SOD activity may decrease or even be 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 for protection against a harsh environment. The second theory is that SOD is involved in the catalysis of superoxide anion free radicals (O$_2^-$) in the body, resulting in a large number of free electrons in the reaction process. These electrons may affect the enzyme
reaction system under the influence of the magnetic field, thereby affecting the activity of SOD (Mao et al. 1993; Mannerling et al. 2010).

In present research, the results show that addition of low-intensity SMFs is an effective method for enhancing the oil degradation by Acinetobacter sp. B11. The optimum magnetic field strength was 25 mT, which increased the oil removal rate by 11.9% compared with that of the bacteria exposed to no magnetic field. By increasing the cell membrane permeability and the SOD activity without destruction of the cell membrane, the low-intensity magnetic field could effectively enhance the oil degradation performance of the bacteria.

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