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Isolation, identification and characterization of *Bacillus amyloliquefaciens* BZ-6, a bacterial isolate for enhancing oil recovery from oily sludge

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ABSTRACT

Over 100 biosurfactant-producing microorganisms were isolated from oily sludge and petroleum-contaminated soil from Shengli oil field in north China. Sixteen of the bacterial isolates produced biosurfactants and reduced the surface tension of the growth medium from 71 to <30 mN m^{-1} after 72 h of growth. These bacteria were used to treat oily sludge and the recovery efficiencies of oil from oily sludge were determined. The oil recovery efficiencies of different isolates ranged from 39% to 88%. Bacterial isolate BZ-6 was found to be the most efficient strain and the three phases (oil, water and sediment) were separated automatically after the sludge was treated with the culture medium of BZ-6. Based on morphological, physiological characteristics and molecular identification, isolate BZ-6 was identified as *Bacillus amyloliquefaciens*. The biosurfactant produced by isolate BZ-6 was purified and analyzed by high performance liquid chromatography–electrospray ionization tandem mass spectrometry. There were four ion peaks representing four different fengycin A homologues.

1. Introduction

Oil refineries and petrochemical industries generate large amounts of solid waste. Of special concern is the oily sludge that accumulates at the bottom of crude oil storage tanks or is generated in water–oil separation systems (Mait et al., 2008). Oily sludge contains large amounts of benzene, phenol and polycyclic aromatic hydrocarbons which have highly toxic, mutagenic and carcinogenic effects on humans and pollute the environment. They are therefore classified as priority environmental pollutants by the US Environmental Protection Agency (USEPA, 1986) and are also on the List of Dangerous Solid Wastes in China. Improper disposal of oily sludge may contaminate soils and pose a serious threat to groundwater.

Landfill, coking treatment, solvent extraction and incineration are currently the main methods for disposal of oily sludge. However these methods are considered expensive or inadequate to meet current and future regulations (Conaway, 1999). The amount of oily sludge produced in Chinese refineries and oil fields has been estimated to be 450 kt yr^{-1} (Deng et al., 2007). Oily sludge contains various fractions of petroleum hydrocarbons (typically 10–50 wt%), solids (6–10 wt%) and water and is of widely varying composition. Recycling is the most desirable environmental option for handling oily sludge as the supply of oil declines and concern about environmental pollution increases. Separation and reclamation of oil from the sludge can minimize the disposal of pollutants and minimize environmental pollution.

Biosurfactants are complex biopolymers produced by microorganisms and they have desirable characteristics such as biodegradability, low toxicity, ecological acceptability and the capacity to be produced from renewable and cheaper substrates than many synthetic surfactants for oil industry applications (Banat et al., 2000). There has been a growing interest in biosurfactant applications in environmental remediation (Banat, 1995; Barathi and Vasudevan, 2001; Urum and Pekdemir, 2004; Bordoloi and Konwar, 2009; Joseph and Joseph, 2009). The potential application of biosurfactants in stimulating indigenous microorganisms for enhanced bioremediation of diesel-contaminated soil has been confirmed (Bordoloi and Konwar, 2009). Banat et al. (1991) described the application of microbial biosurfactants for the clean-up of oil storage tanks and the successful removal of oil from the bottom of the tanks.

Although a number of studies have been carried out on soil bioremediation and tank cleaning using biosurfactants, few assessments of their performance in recovering oil from oily sludge have been reported. The purpose of the present study was to isolate

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biosurfactant-producing bacteria and evaluate their oil recovery efficiency from sludge. Furthermore, surface-active substances produced by the bacterial isolate with the highest oil recovery capacity were purified and identified.

2. Materials and methods

2.1. Media and oily sludge

Minimal medium (MM) used throughout the study contained (in g L^{-1}) $(NH_4)_2SO_4$ 5, glucose 2, KCl 1.1, NaCl 1.1, $FeSO_4$ 0.028, KH_2PO_4 1.5, K_2HPO_4 1.5, $MgSO_4$ 0.5 and 5 mL of trace element solution (TES). TES contained (in g L^{-1}) $ZnSO_4$ 0.2, $CaCl_2$ 0.24, $CuSO_4$ 0.25, and $MgSO_4$ 0.17. Solid MM plates were prepared by adding 20 g agar to 1000 mL MM. Blood agar was prepared with sheep's blood (5%) and LB medium. LB medium contained (in g L^{-1}) tryptone 10, yeast extract 5, and NaCl 10 and was adjusted pH to 7. Blood was added prior to pouring and the plates were allowed to solidify. Oily sludge used in this study was collected from Bingyi Collection Facility at Shengli oil field, Shandong province, China. The oil concentration of the sludge was 31% by weight.

2.2. Isolation of biosurfactant-producing bacteria

Biosurfactant-producing microorganisms were isolated from oily sludge and petroleum-contaminated soil from Shengli oil field using blood agar lysis and surface tension measurements (McInerney et al., 1990; Youssef et al., 2004). Briefly, an aliquot of 5 g of soil (sludge) sample was inoculated into 50 mL of MM medium containing 0.5 g crude oil and incubated aerobically at 25 °C on a reciprocal shaker at 150 rpm for 72 h. After incubation the medium was serially diluted with sterile water and plated on blood agar. The plates were incubated at 37 °C and observed after 48 h. Colonies producing biosurfactant (hemolytic zone around the colonies) were picked up. Isolation and purification procedures were carried out on LB plates by conventional spread plate techniques. The isolated strains were inoculated into 50 mL of LB medium and incubated at 25 °C with shaking at 180 rpm for 24 h. Then the surface tension of the culture medium of each strain was measured by the ring method at 25 °C using a Model ZL-2 digital tensiometer (Boshan Tongye Analytical Instrument, Shandong, China).

2.3. Oily sludge treatment experiment

Each isolated strain was inoculated into a 250 mL flask with 100 ml LB medium at 25 °C and incubated aerobically on a reciprocal shaker at 150 rpm for 48 h. The LB broth was then used in the following procedure. An aliquot of 10 g oily sludge sample was added to each LB broth and shaken at 25 °C and 180 rpm for 24 h. Unamended LB medium was also used as a control. The broth was allowed to settle for several hours and oil separation was observed visually. Oil was extracted and measured gravimetrically following the procedure recommended by USEPA test method 418 (USEPA, 1986). The oil recovery efficiency was estimated as follows:

$$\text{Oil recovery efficiency} = \frac{\text{Oil from liquid extraction}}{\text{Oil from liquid extraction} + \text{Oil from sludge}}$$

Fig. 1. The three phases separate of sludge after treated by the culture medium of BZ-6.

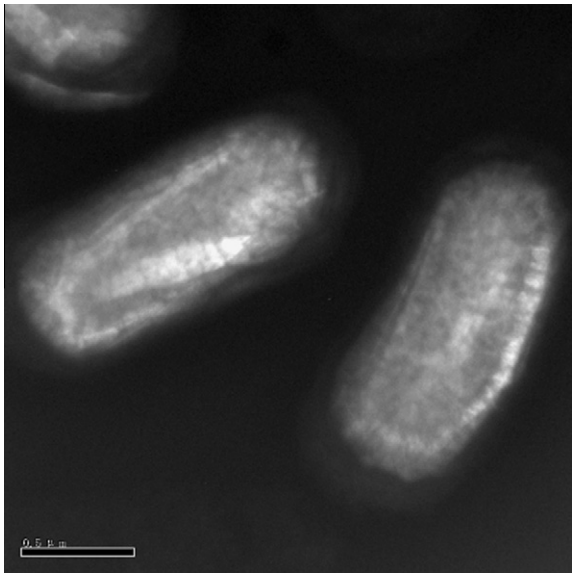

Fig. 2. Electron micrograph (TEM) of BZ-6.

Table 1
Surface tension and oil recovery efficiency of the 16 bacterial isolates.

Isolate	Surface tension (mN m^{-1})	Recovery efficiencies (%)	Isolate	Surface tension (mN m^{-1})	Recovery efficiencies (%)
SB-5	27.3	70	SO-4	29.6	50
SB-11	28.3	55	SO-9	29.3	48
SB-13	27.8	69	SO-12	28.2	56
SB-18	28.2	51	SO-18	29.4	42
SB-21	28.0	75	BZ-3	29.2	51
SS-8	29.7	39	BZ-6	28.5	88
SS-12	28.7	56	BZ-12	29.0	48
SS-15	27.4	78	BZ-14	30.0	42