Dispersants were globally applied to physico-chemically enhance the dispersion of oil in water and were assumed to stimulate oil biodegradation by indigenous microorganisms and to reduce the environmental impact of oil spills. Since the 1960s, chemical dispersants have been applied as an emergency response to oil spills in marine ecosystems, and have showed effectiveness at removing oil slicks from the coast. However, most of the chemically synthesized dispersants are inherently toxic to various aquatic species and hardly biodegradable in the natural environment. The application of chemical dispersants in the 2010 Gulf of Mexico oil spill also raised concerns regarding the toxicity and the potential environmental impact, and caused a debate about the effectiveness of chemical dispersants on the rates of oil biodegradation. Biosurfactants are promising dispersants in oil-spill remediation, owing to their environmentally friendly and biodegradable properties. Chemical surfactants could be replaced with biosurfactants and this change would diminish the environmental impact of traditional dispersants. Lipopeptide produced by microorganisms is one of the representative biosurfactants and has showed great potential applications in food, medicine, microbial enhanced oil recovery, and other fields. Nevertheless, the knowledge about the application of biosurfactant lipopeptide in marine oil-spill remediation is still limited.

In the present work, the dispersion effectiveness, aquatic toxicity, biodegradability and environmental compatibility of the biosurfactant lipopeptide were determined using recognized standardized methods and the biosurfactant lipopeptide used as a bio-dispersant for marine oil-spill remediation were studied, which is, to the best of our knowledge, the first report about biosurfactant lipopeptide used in oil-spill remediation.

The lipopeptide samples were isolated from cell-free broth of B. subtilis HSO121 at our laboratory. The typical chemical structure of the lipopeptide used in the study was shown in Fig. 1 and its critical micelle concentration (CMC) was $8.69 \times 10^{-5}$ mol L$^{-1}$.

Dispersion effectiveness (DE) of lipopeptides was examined at different surfactant-to-oil ratios (SORs), temperatures, pH values, and salinities. It indicated in Fig. 2 that DE of lipopeptides reached 70.23% at SORs of 1 : 10 (w/w) at 25°C, pH 7 and the present of 3% NaCl (w/v). It should be noticed that the DE of lipopeptides was almost kept when SORs dropped to 1 : 250 w/w. The increase in DE with increasing SORs can be attributed to the generation of emulsions with smaller droplets and lower rising velocity. Sharp drop off in DE was observed for lipopeptides when SORs below 1 : 500 (w/w), and DE value was 36.45% at an extreme SORs of 1 : 1250 (w/w). It had been reported that the abrupt decline for 80 : 20 lecithin : Tween 80 (w/w) surfactant happened when SORs below 1 : 100 v/v, from 77% (SORs 1 : 100 v/v) to 15% (SORs 1 : 200 v/v), indicating a lower
SOR in lipopeptides usage could reach its maximum effectiveness. Lipopeptides exhibited >70% DE values with temperatures ranged from 15 °C to 25 °C, and an increasing DE values when pH values raised, the largest DE was 77.45% at pH 11. DE of lipopeptides increased from 56.12% to 71.14% with increase in salinity. Higher DE at higher salinity was observed for anionic surfactants, which can be attributed to the electrostatic repulsion between polar head groups reduced by ions, and a close-packed arrangement of surfactant molecules at the oil-water interface were formed.7

Mortalities of zebrafish under different concentrations of different surfactants were shown in Fig. 3. It was evident that the toxicity of lipopeptides was far less than those of sodium dodecyl sulfate (SDS) and 3-(N,N-dimethyl palmyl) ammonio propane sulfonate (Betaine). The 24 h median lethal concentration (LC50) values were calculated and showed in Table 1. The 24 h LC50 values of lipopeptides, SDS, and Betaine for zebrafish were 1145 mg L−1, 8.25 mg L−1, and 0.872 mg L−1, respectively. The toxicity of SDS was comparable to the study by Edwards et al.,28 in which an 96 h LC50 of 1.9 mg L−1 for Cyprinodon variegatus was reported. Low toxicities of lipopeptides on whiteleg shrimp and copepods were also evaluated that the 96 h LC50 values were 1145 mg L−1, 1174 mg L−1, respectively.27

Table 1  Ecotoxicity of tested surfactants to the zebrafish

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>LC50 (mg L−1)</th>
<th>95% confidence intervals (mg L−1)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipopeptide</td>
<td>1145</td>
<td>1090–1229</td>
<td>0.981</td>
</tr>
<tr>
<td>SDS</td>
<td>8.25</td>
<td>7.75–8.77</td>
<td>0.998</td>
</tr>
<tr>
<td>Betaine</td>
<td>0.872</td>
<td>0.853–0.890</td>
<td>0.988</td>
</tr>
</tbody>
</table>

Fig. 2 The dispersion effectiveness (DE) of lipopeptides under different SORs (ⅰ), temperatures (ⅱ), pH values (ⅲ) and salinities (ⅳ).

Fig. 3 Mortality of zebrafish (M3) after a 24 h exposure to Betaine, SDS or lipopeptides.

Fig. 4 Effect of lipopeptides and SDS on growth concentrations (c) of Pseudokirchneriella subcapitata during 72 hours of incubation.
Lipopeptides showed higher biodegradability as 94.01% even increasing the initial concentration to 300 mg L⁻¹. Lima et al.²⁹ studied the biodegradability of surfactants and observed the lowest decrease (24.8%) in SDS, the highest decrease in lipopeptides (69.1%) and glycolipid (73.4%). Biosurfactants were seemed to be more biodegradable than synthetic surfactants.

The degradation rates of aliphatic fractions (n-C₁₁–C₂₉) in crude oil (Xinjiang oilfield, P. R. China) were analysis using GC-MS and the results were illustrated in Fig. 6. Significant degradation rates of n-alkanes were observed in presence of biosurfactant lipopeptides, while n-alkanes dispersed by commercial dispersant degraded similar to that in control group (in absence of any surfactant). It was well known that the degradation rates of alkanes decreased and finally vanished with increase in the chain length of hydrocarbon. In the present study, lipopeptides showed excellent activity in accelerating degradation of long-chain hydrocarbons after 2 days. The alkanes degradation rates treated by lipopeptides after 1 day and 2 days were 38.78% (Fig. 6a) and 71.45% (Fig. 6b), respectively, which were much higher than those of commercial dispersant-treated group (9.16% and 34.16% after 1 day and 2 days treatment) and control group (13.26% and 33.55% after 1 day and 2 days treatment). It was reported that commercial chemical dispersants such as Corexit 9500A and GM-2 made no enhancement to the degradation of the petroleum hydrocarbons, whereas biosurfactants such as rhamnolipids enhanced the degradation.²²,³³ Degradation stimulation by lipopeptides could be attributed to their good dispersion activities and biocompatible. Dispersed oil droplets formed and considerable interfacial area was available to the microorganisms followed by microbial bioremediation. In addition, microorganisms could utilize the nutrients derived from culture broth as an excellent substrate for growth.³⁴

Conclusions
In the present study, lipopeptides secreted by Bacillus subtilis HSO121 showed good dispersion effectiveness on crude oil (Xinjiang oilfield, P. R. China) at low SOR and kept this activity against temperature, pH values, and salinity. Comparing with SDS and Betaine, lipopeptides exhibited much lower toxicity, significant biocompatible, and higher activity in stimulating in microbial biodegradation of crude oil. In conclusion, lipopeptides acted effectively at dispersing oil and performed excellently at stimulating microbial oil biodegradation, which indicated its application in oil spill cleaning.

Conflicts of interest
There are no conflicts of interest to declare.

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Notes and references