

1        **Use of Biosurfactants in Oil Recovery**

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20     **Abstract**

21           Biosurfactant-mediated oil recovery has the potential to recover large amounts of  
22   crude oil that remain entrapped in oil reservoirs after current oil recovery technologies  
23   reach their economic limit. Lipopeptides (surfactins and lichenysins), rhamnolipids,  
24   and other glycolipids generate the low interfacial tensions and the appropriate rock  
25   wettabilities needed to mobilize entrapped oil. Biosurfactants are active over a wide  
26   range of temperatures, pH values, and salinities found in many oil reservoirs and are  
27   effective at low concentrations. A number of laboratory experiments show that  
28   biosurfactant-mediated oil recovery is effective in recovering large amounts of  
29   entrapped oil. Several field trials show that in situ biosurfactant production is possible  
30   and recovers additional oil. Biosurfactant-mediated oil recovery has been difficult to  
31   scale-up to a reservoir-wide technology due to the lack of understanding of how best  
32   to stimulate biosurfactant production in the reservoir. In addition, the relationship  
33   between biosurfactant concentration and oil recovery is still unclear. Ex-situ  
34   biosurfactant-mediated oil recovery where the biosurfactant is added to the injection  
35   fluids has not been implemented on a large scale, most likely due to the high  
36   production costs of biosurfactants. Multidisciplinary approaches are needed to move  
37   biosurfactant-mediated oil recovery from the laboratory to the reservoir.

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39     **Introduction**

40           World economic growth will continue to be strong in the upcoming decades, and

thus, the demand for energy will also be strong (Doman 2016). Total world energy consumption will rise from 580 ExaJoules (EJ) in 2012 to 860 EJ in 2040. An important question is how will we meet the future demand for more energy. Although renewable energy and nuclear power are predicted to be the world's fastest-growing energy sources, it is likely that liquid fuels—mainly petroleum—will remain the largest source of world energy (Doman 2016). Most of the growth in liquid fuel consumption will be in the transportation and industrial sectors where liquid fuels will continue to provide most of the energy consumed (Doman 2016). The demand for liquid fuels by the transportation sector is expected to increase by 62% by 2040. Thus, finding and producing sufficient amounts of petroleum in the future will be critical to sustaining world economic growth.

Petroleum is a non-renewable fossil resource derived from organic matter deposited eons ago in the lithosphere. When a well is drilled into an oil reservoir, oil and water are pushed to the surface by the natural pressure within the reservoir. As reservoir pressure dissipates, pumps are placed on the well to assist in bringing the fluids to the surface. This stage of oil production is called primary production (Youssef et al. 2009). Eventually, additional pressure must be added to the reservoir to continue to recover oil. Often surface water, seawater, or brine from a subterranean formation is injected into the reservoir to push the oil to production wells. This stage of oil production is called waterflooding or secondary oil production (Youssef et al. 2009). When the above exploration strategies reach their economic limits, only about one-third to one-half of the oil originally in place in the reservoir has been extracted,

leaving behind a large amount of oil (known as residual oil) in the reservoir (Hall et al. 2003). The amount of residual oil in reservoirs worldwide ranges from 2 to 4 trillion barrels (0.3 to 0.6 Tm<sup>3</sup>). Thus, there is a large resource of petroleum that could potentially supply future energy needs if technologies can be developed to recover entrapped oil.

Technologies to recover residual oil are called enhanced oil recovery (EOR) technologies. EOR includes three primary techniques: thermal recovery (hot water, steam, combustion), gas injection (N<sub>2</sub>, CO<sub>2</sub>, flue gas), and chemical injection (surfactants, polymers, solvents) (Alvarado and Manrique 2010; Sheng 2010). Over one-half of the EOR-recovered crude oil in the USA is the result of gas injection with CO<sub>2</sub> injection being the most important. The remainder is the result of thermal oil recovery technologies. Chemical-based EOR technologies have been marginally economic due to high chemical costs (Alvarado and Manrique 2010; Sheng, 2010). Another EOR approach is to use microbial technologies to enhance oil recovery (MEOR). Numerous laboratory and field studies have shown that microorganisms produce useful products such as biosurfactants that allow recovery of residual oil (Youssef et al., 2009). Microorganisms can produce these products from inexpensive and renewable nutrients injected into the reservoir. Thus, MEOR technologies have an economic advantage in that they do not consume large amounts of energy as do thermal processes, nor do they depend on expensive chemicals as many chemical processes do (Youssef et al. 2009). In addition, MEOR provides an ecofriendly approach to oil recovery compared to chemical EOR as the products of microbial

85 metabolism are readily degradable (de Cássia et al. 2014).

86 MEOR has been investigated extensively in the laboratory and in the field and a  
87 number of excellent reviews are available (McInerney et al 2009; Youssef et al. 2009;  
88 Harner et al. 2011; Shibulal et al. 2014; Siegert et al. 2014; Patel et al. 2015). In this  
89 chapter, we will discuss the use of biosurfactants for oil recovery. A recent book on  
90 biosurfactants provides an excellent overall resource (Sen, 2010).

91

## 92 **How can biosurfactants help mobilize oil?**

93 Over eighty percent of the production oil wells in the U. S. A. have low  
94 production rates (less than  $1.6 \text{ m}^3$  of crude oil per day) and are at risk of abandonment  
95 due to their marginal economic returns (Youssef et al. 2009). However, oil production  
96 from marginal wells accounts for about 19% of USA domestic production. Thus,  
97 maintaining production in marginal wells will be important to meet future energy  
98 needs. To keep marginal wells economic, one must slow the rate of oil production  
99 decline or increase the rate of oil production in a cost competitive manner. Mobile oil  
100 may be present a short distance from the production well but cannot flow to the well  
101 because drainage channels have been blocked by particulates such as paraffin deposits  
102 or by areas of high water saturation (Youssef et al. 2009) (Figure 1A). Removal of  
103 paraffin deposits and/or changing wettability in the near wellbore region through  
104 biosurfactant production would reconnect regions of high oil saturation, providing  
105 channels for the oil to drain into the well (Figure 1A). There are a number of

Fig 1

commercial microbial technologies where hydrocarbon-degrading bacteria and nutrients are injected into production wells to degrade paraffins and/or produce biosurfactants, which could change near wellbore conditions to allow better oil drainage. In fact, many commercial microbial paraffin removal technologies have been shown to slow the rate of decline in oil production and extend the operational life of marginal oil fields (Youssef et al. 2009). Injection of biosurfactants or their in situ biosurfactant production may also be effective in changing wettability in the near wellbore region (Al-Sulaimani et al. 2012). In fact, the injection of two biosurfactant-producing microorganisms and nutrients into two production wells improved oil production (Youssef et al. 2013).

With larger, more productive oil fields, increasing the ultimate recovery factor is an important consideration. Large amounts of oil remain entrapped by capillary forces in reservoirs after waterflooding. In water-wet regions of the reservoir, oil will be found as spherical globules in the center of the large pores and as ganglia of oil spanning multiple pores surrounded by water (Armstrong and Wildenschild, 2012a) (Figure 1B). In strongly oil-wet regions of the reservoir, oil will be found in small pores and in large pockets of oil surrounded by water. In both cases, mobilization of the entrapped oil will require an increase in viscous forces, and/or a reduction of capillary forces in the reservoir. The viscous and capillary forces that entrap oil are expressed as a dimensionless ratio called capillary number ( $N_{ca}$ ) (equation 1):

$$N_{ca} = (\mu_w \cdot V_w)/(\gamma) \quad (1)$$

128

129 where  $\mu_w$  is the viscosity of the recovery fluid (aqueous phase),  $V_w$  is the velocity of  
130 the recovery fluid (aqueous phase), and  $\gamma$  is the interfacial tension (IFT) between the  
131 oil and aqueous phases (Gray et al. 2008). Capillary numbers for a mature water-  
132 flooded reservoir are in the order of  $10^{-7}$ - $10^{-6}$  (Gray et al. 2008). Capillary number  
133 needs to be increased 100- to 1000-fold in order to mobilize substantial amounts of  
134 entrapped oil. Typically, capillary number is increased by adding a polymer to  
135 increase the viscosity of the recovery fluid and/or by adding a surfactant or  
136 biosurfactant to reduce the interfacial tension between the oil and aqueous phases.  
137 Biosurfactant activity will mobilize entrapped oil in water-wet pores and will allow  
138 oil to drain from oil-wet regions (Figure 1B) (Armstrong and Wildenschild, 2012a, b).  
139 Many microorganisms produce biopolymers, which will increase capillary number by  
140 increasing the viscous forces. The combination of biopolymer and biosurfactant  
141 production could increase capillary number sufficiently for substantial oil recovery  
142 (Fernandes et al. 2016).

143 Armstrong and Wildenschild (2012a, b) used X-ray computed microtomography  
144 (CMT) to understand the mechanisms of MEOR operative at the pore-scale. Analysis  
145 of CMT images showed that biosurfactant-mediated MEOR altered the oil  
146 morphology, gave more oil-wet curvatures, and decreased the interfacial curvatures.  
147 As a consequence, large oil recoveries ranging from 44 to 80% were observed as a  
148 result of wettability and IFT changes (Armstrong and Wildenschild, 2012a, b).  
149 Sarafzadeh et al. (2013) also found interfacial tension reduction and wettability

alteration by biosurfactants important for oil recovery from carbonate cores. The change in capillary number due to interfacial tension reduction by the biosurfactant explained the observed oil recoveries. However, much lower residual oil saturations than predicted by changes in capillary number alone were observed, when both cells and the biosurfactant were used (Armstrong and Wildenschild, 2012b). Thus, the clogging of pores with cells, which altered flow patterns, has a significant effect on oil recovery beyond that predicted by capillary number (Armstrong and Wildenschild, 2012a, b).

## **Types of biosurfactants**

Diverse microorganisms produce surface-active agents (Youssef et al., 2009; Sen, 2010; Santos et al. 2016). Biosurfactants are classified into five major categories based on their chemical structures: lipopeptides, glycolipids, phospholipids, neutral lipids, and fatty acids (de Cássia et al. 2014; Santos et al. 2016). The most common biosurfactants used in MEOR are lipopeptides (surfactin and lichenysin) and glycolipids (rhamnolipids, sophorolipids and trehalolipids) (McInerney et al 2009; Youssef et al. 2009; Liu et al. 2015; Santos et al. 2016) (Figure 2). The interfacial tension between oil and aqueous phases varies from 20 to 40 mN/m (Gray et al. 2008). A number of biosurfactants reduce oil-water interfacial tension to  $< 1$  mN/m (Table 1), which provides a 100-fold or greater increase in capillary number needed for substantial oil recovery. Some biosurfactant producers are also able to produce

Fig. 2



biopolymers that increase the viscosity of the aqueous phase, which further increases capillary number and oil recovery (Fernandes et al. 2016).

Many biosurfactants, in particular, surfactins and lichenysins, have low critical micelle concentrations (CMC), 10 to 30 mg/L (Table 1). CMC is the concentration at which the biosurfactants form micelles and is the minimum concentration needed to mobilize entrapped oil (Youssef et al. 2009; Sen 2010). Many synthetic surfactants have higher CMC (>100 mg/L) than biosurfactants (Youssef et al. 2007a). Thus, low biosurfactant concentrations can be effective in mobilizing entrapped oil. In fact, microbial cultures where the biosurfactant concentration is at or slightly above the CMC recover large amounts of entrapped oil (Table 1).

Commercial of biosurfactant production is costly due to the low productivity of many biosurfactant-producing strains (Table 1), the use of expensive media components, and high downstream processing costs (Helmy et al. 2011; Banat et al. 2014; Geys et al. 2014). The use of low cost agro-industrial by-products such as whey, molasses, waste oils helps reduce nutrient costs (Banat et al. 2010; Makkar et al. 2011); however, complex substrates may have undesirable components that inhibit production or make downstream processing difficult. A number of investigators have used statistical approaches such as surface response methodology to optimize nutrient composition and operating conditions to improve biosurfactant productivity (Banat et al., 2010; Liu et al. 2015). Rotating disk, biofilm reactors (Chitou et al., 2012), bubble less, membrane-aerated bioreactors (Coutte et al., 2010), and three-phase, inverse fluidized bed reactors (Nikolov et al., 2000) have been developed to provide

adequate aeration without foaming and solid-state fermentation, where the biosurfactant producer is grown on a solid surface such as rice straw, reduces capital costs (Zhu et al., 2013). The combination of ultrafiltration with adsorption and ion exchange chromatography increased the recovery of biosurfactants from fermentation broths (Chen et al., 2010). It should be noted that there are some biosurfactant producers that produce very high concentrations of biosurfactants (Geys et al. 2014). For example, *Pseudomonas aeruginosa* produces 70-120 g/L of rhamnolipids when cultivated on vegetable oil (Giani et al. 1997) and *Starmerella bombicola*, the best studied sophorolipid producer, and produces 400 g/L sophorolipid when grown in a two-stage cultivation process (Daniel et al. 1998).

204 Table 1. Efficacy of biosurfactants commonly used for microbial oil recovery <sup>a</sup>

Biosurfactant	Microorganism	Lowest surface tension (mM/m)	Lowest interfacial tension (mN/m)	Critical micelle concentration (mg/L)	Additional oil recovery (%)	Yield (g/L)	Reference
Surfactin	<i>Bacillus subtilis</i> or <i>B. mojavensis</i>	28-30	0.006-0.3	10-35	40-80	0.5-1	Lin et al. 1994; Youssef et al. 2007a
Lichenysins	<i>Bacillus licheniformis</i>	28	0.3-0.5	10-19	37	1.1	Joshi et al 2015; Yakimov et al 1999
Lipopeptide	<i>Acinetobacter baylyi</i>	35	15	90	28		Zou et al., 2014
Rhamnolipid	<i>Pseudomonas</i>	25-27	0.2-2	11-120	10-27	0.7-50	Amani et al. 2013; Xia et al. 2012

	<i>aeruginosa</i>						
Glycolipids	<i>Rhodococcus</i>	27-30	1	57	65-86	0.5-12.9	Shavandi et al., 2011; Zheng et al., 2012
	sp.						
Glycolipids	<i>Enterobacter</i>	31	0.6-3.2		27-48	1.5-1.7	Darvishi et al. 2011; Rabiei et al. 2013; Sarafzadeh et al. 2013
	<i>cloacae</i> and <i>E. hormaechei</i>						
Lipopolysacharide	<i>Alcaligenes</i>	20	<1		9	1.2 ± 0.05	Salehizadeh and Mohammadizad 2009
	<i>faecalis</i>						
Sucrose lipid	<i>Serratia</i>				90		Pruthi and Cameotra 2000
	<i>marcescens</i>						
Sophorolipid	<i>Candida</i>	33 ±	1.6 ± 0.3		27		Elshafie et al. 2015
	<i>bombicola</i>	0.05					

205 <sup>a</sup> The values differ depending on the strains, growth conditions, oils and porous media used in different experiments.

## **Strategies for biosurfactant-mediated oil recovery**

Oil recovery occurs by the activity of microorganisms and/or their metabolites, such as biosurfactants, biomass, biopolymers, solvents, acids, gases, etc., which can be generated ex situ or in situ (Youssef et al. 2009). In ex situ MEOR approaches, microbes are cultivated in a fermentor on inexpensive nutrients and the microbes and/or their metabolites are injected into oil reservoir. In situ approaches involve the growth and metabolism of the indigenous or injected microbes in the reservoir to produce cells, metabolites, or a particular activity such as hydrocarbon degradation. Thus, there are three main strategies for using biosurfactants for oil recovery (Banat et al. 2000):

(1) Production of biosurfactants in batch or continuous culture under industrial conditions, followed by their addition to the reservoir.

(2) Production of biosurfactant-producing microorganisms in batch or continuous culture under industrial conditions, followed by the injection of cells and nutrients into the reservoir.

(3) Injection of nutrients into a reservoir to stimulate the growth of indigenous biosurfactant-producing microorganisms.

### ***Injection of ex situ-produced biosurfactants***

In addition to generating low interfacial tensions, biosurfactants must maintain activity under the environmental conditions present in oil reservoirs (Siegert et al. 2014). A number of studies have shown that lipopeptides biosurfactants and

rhamnolipids are effective over a wide range of environmental conditions such as temperatures up to 80°C, NaCl concentrations up to 15% and pH values from 5 to 10 (Youssef et al., 2009; Amani et al., 2013; Al-Wahaibi et al., 2014; Joshi et al., 2015).

Although many biosurfactants exhibit extraordinary interfacial properties, commercialization of biosurfactant-mediated oil recovery remains difficult and costly (Banat et al. 2014). The maximum concentrations produced during cultivation tend to be low (<2 g/L) (Table 1) although higher concentrations have been reported (Joshi et al. 2008; Xia et al. 2012). To our knowledge, there are still not any reports of ex situ field trial applications of biosurfactants. A promising approach is the use biosurfactants in conjunction with synthetic surfactants to reduce the amount of synthetic surfactants needed, providing cost savings (Youssef et al. 2007a; Al-Sulaimani et al. 2012).

### ***Injection biosurfactant-producing microorganisms and nutrients***

If the biosurfactant-producing microorganisms or their activities are absent, then inoculation of the reservoir with exogenous biosurfactant-producing microorganism is needed. The use of large concentrations of exogenous microorganisms may also be an effective way to establish the appropriate activity quickly in the reservoir. The foremost consideration would be whether the exogenous biosurfactant-producing microorganism would grow under the environmental conditions present in the reservoir in presence of competing indigenous population. However, many known biosurfactant-producing microorganisms grow under the environmental conditions

present in many oil reservoirs (Youssef et al. 2009).

Another important critical factor is the transport abilities of the exogenous microorganism. Ideally, the injected microorganisms should migrate freely in the reservoir formation and have minimal adsorption to reservoir rock material. A field pilot conducted at Guan 69 Unit in Dagang Oilfield indicated that exogenous biosurfactant-producing bacteria migrated through the reservoir matrix at a speed about 1.7 to 4.2 meters per day (Liu et al. 2005). The use of starved cells or spores could facilitate the migration of exogenous microorganisms (Youssef et al. 2009; Shibulal et al. 2014). While it may be problematic to inject microorganism large distances into the reservoir, it is possible to treat the near wellbore region with exogenous biosurfactant-producing *Bacillus* species (Youssef et al. 2007b, 2013).

#### ***Injection of nutrients to stimulate indigenous biosurfactant-producing microorganisms***

To choose this strategy, one must first determine if the biosurfactant-producing microorganisms or their activities are present and then decide on how to stimulate these microbes and their activities. Often, this decision is based on the analysis 16S ribosomal RNA gene sequences or other genes with phylogenetic information (Kryachko et al. 2016; Li et al. 2014). In one field trial, phylotypes related to known biosurfactant producers in genera such as *Pseudomonas*, *Alcaligenes*, and *Rhodococcus*, were detected and their concentration in production liquids was closely related to the increase oil production and oil emulsification (Li et al. 2014). While

phylogenetic analysis shows the types of microorganisms present, it can be difficult to infer metabolic function from phylogeny. The use target genes involved in biosurfactant synthesis such as *srfA* for surfactin, *licA* for lichenysin, *rhIR* for rhamnolipid production would provide direct information on the potential for biosurfactant production in an oil reservoir. Such an approach showed that lipopeptide biosurfactant-producing *Bacillus* species, but not rhamnolipid-producing microorganisms, were present in Oklahoma reservoirs with a wide range of salinities (Simpson et al. 2011). Whether it can be concluded that biosurfactant producers are routinely present in oil reservoirs worldwide remains to be determined.

Once it is known that the indigenous biosurfactant-producing microorganisms are present, further tests are needed to confirm biosurfactant production and to develop a nutrient mixture to stimulate biosurfactant production selectively. The use of complex substrates such as molasses may provide a cost advantage over using more refined ingredients. However, the use of complex substrates makes it hard to control the process in situ. Systematic adjustment of C, N and P ratios and concentrations of other nutrients is a proven approach to stimulate biosurfactant production (Sen, 2010). A simple, direct approach to stimulate in situ biosurfactant production in oil reservoirs has yet to be developed.

## **Success of field trails**

Although a number of laboratory studies show the efficacy of biosurfactant



production on oil recovery (Table 1) (Youssef et al. 2009), large-scale applications of biosurfactant-mediated oil recovery are rare due to the high cost of the biosurfactant or difficulties in controlling biosurfactant production within the reservoir. Sporadic reports of biosurfactant-mediated oil recovery have appeared in the literature. Earlier field trials have been extensively reviewed (Youssef et al. 2009); here, we summarize more recent field trials results (Table 2).

In the past two decades, a number of field trials of MEOR have been implemented in Chinese oil fields, including Dagang Oilfield, Daqing Oilfield, Huabei Oilfield, Shengli Oilfield, and Xinjiang Oilfield, (Liu et al. 2005; Huang et al. 2014; Li et al. 2014; Chai et al. 2015; Le et al. 2015; Li et al. 2015). A well-documented trial involving hydrocarbon-degrading and biosurfactant-producing bacteria was implemented in a sandstone oil reservoir (Guan 69 Unit of the Dagang Oilfield in Hebei Province, China) (Liu et al. 2005). The injected, exogenous bacteria were detected in 4 of 7 production wells after several months of injection. A slight decrease in the surface tension of the production liquids was observed and oil production increased over a six months period following the microbial treatment. About 9120 m<sup>3</sup> of additional oil was produced (Table 2). In another trial, a biosurfactant-producing, *Pseudomonas aeruginosa* P-1, and its metabolic products were injected into more than 60 oil-producing wells in Daqing oilfield, China (Li et al. 2002). About 80% of injected wells showed a significant decrease in the amount of water produced with a corresponding increase in oil produced.

311 Table 2. Recent field trials involving biosurfactant-producing microorganisms.

Mechanism	Microorganisms	Approach	Oil recovery  (m <sup>3</sup> )	Comments	Reference
Stimulate in situ hydrocarbon production	Indigenous	Treat injection wells with	2200	Emulsification	Chai et al. 2015
	<i>Pseudomonas</i> sp.	air and nutrients			
	Indigenous hydrocarbon degraders	Treat injection wells with H <sub>2</sub> O <sub>2</sub> or oxygenated water with N and P	4420	Emulsification; interfacial tension reduction	Nazina et al. 2008
	Indigenous hydrocarbon degraders	Treat injection wells with air-saturated brine and minerals	16,200	Reduction in interfacial tension	Nazina et al. 2007

	Oil-degrading and biosurfactant-producing microorganisms	Repetitive treatment of injection wells with nutrients and inoculum	9122	All seven wells had increased oil production	Liu et al. 2005
	<i>Pseudomonas aeruginosa</i> and its metabolic products	Not disclosed	7-14 m <sup>3</sup> per well	80 % of wells had increased production	Li et al. 2002
Stimulate biosurfactant production	<i>Bacillus</i> sp. RS-1 and <i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	Treat producing wells with glucose-nitrate-metals and inoculum	53	20-28 mg/L of biosurfactant	Youssef et al. 2013

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We implemented two successful tests of biosurfactant-mediated oil recovery in a Viola limestone oil formation in Oklahoma (Youssef et al., 2007b, 2013). The first test showed that inoculation of oil wells with exogenous, biosurfactant-producing microorganisms is possible and in situ biosurfactant production was detected (Youssef et al. 2007b). The second test involved larger volumes of materials (10-fold greater quantities than the first test) to determine if in situ biosurfactant production simulated oil production (Youssef et al. 2013). Lipopeptide biosurfactants were detected in produced fluids of the two inoculated wells (20 and 28 mg/L, respectively) and the increase in microbial products in the production fluids corresponded directly with an increase in oil recovery. About 52.5 m<sup>3</sup> of additional oil (net cumulative increase) occurred during the first 60 days.

One of the more common approaches to MEOR is to stimulate hydrocarbon degradation by the controlled injection of air or H<sub>2</sub>O<sub>2</sub> along with other nutrients (Liu et al. 2005; Nazina et al. 2007, 2008; Huang et al. 2014; Li et al. 2014; Chai et al. 2015; Le et al. 2015; Li et al., 2015). Hydrocarbon metabolism often results in biosurfactant production. After the microbial process was initiated, products of microbial metabolism including biosurfactants and hydrocarbon-degrading microorganisms were detected in production fluids (Nazina et al, 2007 and 2008). The water content of production liquids decreased and the oil content increased, resulting in large amounts of additional oil (Table 2).

## Research Needs

Research to date shows that biosurfactant-mediated oil recovery is technically feasible. That is, microorganisms produce biosurfactants that generate low interfacial tensions and recover large amounts of oils. Limited studies indicate that biosurfactant producers are likely present in oil reservoirs. Much more work is needed to understand how to control biosurfactant production in the reservoir in order for biosurfactant-mediated oil recovery to become a successful commercial approach to oil recovery.

(1) More work is needed in media design and fermentation approaches to reduce nutrient costs and increase final biosurfactant concentrations. Very little work has been done to increase biosurfactant concentration or activity by genetic manipulation.

(2) A greater understanding of the pore-level processes that occur during biosurfactant-mediated oil recovery is needed to understand how biosurfactants influence capillary forces and wettability and how multiple microbial mechanisms operate to enhance oil recovery.

(3) More work is needed to develop nutrient and injection regimes to stimulate in situ biosurfactant production reproducibly. Fundamental information on the ecology of biosurfactant-producing microorganisms in oil reservoirs is critically needed as are the tools needed to monitor changes of biosurfactant concentration and metabolic activity of biosurfactant producers.

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## References

- Al-Sulaimani H, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S, Ayatollahi S (2012) Residual-oil recovery through injection of biosurfactant chemical surfactant, and mixtures of both under reservoir temperatures: Induced-wettability and interfacial-tension effects. *SPE Reservoir Eval Eng* 15:210–217
- Alvarado V, Manrique E (2010) Enhanced oil recovery: An Update Review. *Energies* 3:1529-1575
- Al-Wahaibi Y, Joshi S, Al-Bahry S, Elshafie A, Al-Bemani A, Shibulal B (2014) Biosurfactant production by *Bacillus subtilis* B30 and its application in enhancing oil recovery. *Colloids Surf B Biointerfaces* 114:324–333
- Amani H, Müller MM, Syldatk C, Hausmann R (2013) Production of microbial rhamnolipid by *Pseudomonas aeruginosa* MM1011 for ex situ enhanced oil recovery. *Appl Biochem Biotechnol* 170: 1080-1093.
- Armstrong RT, Wildenschild D (2012a) Microbial enhanced oil recovery in fractional-wet systems: A pore-scale investigation. *Transport Porous Med* 92:819-835

Armstrong RT, Wildenschild D (2012b) Investigating the pore-scale mechanisms of microbial enhanced oil recovery. *J Petr Sci Engin* 94-95:155-164

Banat IM, Makkar RS, Cameotra SS (2000) Potential commercial applications of microbial surfactants. *Appl Microbiol Biotechnol* 53:495–508

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R (2010) Microbial biosurfactants production, applications and future potential. *App Microbiol Biotechnol* 87: 427-444

Banat IM, Satpute SK, Cameotra SS, Patil R, Nyayanit NV (2014) Cost effective technologies and renewable substrates for biosurfactants' production. *Front Microbiol* 5:697. doi: 10.3389/fmicb.2014.00697

Chai L, Zhang F, She Y, Banat IM, Hou D (2015) Impact of a microbial-enhanced oil recovery field trial on microbial communities in a low-temperature heavy oil reservoir. *Nat Environ Pollut Technol* 14:455-462

Chitoui O, Dimitrov K, Gancel F, Dhulster P, Nikov I (2012) Rotating discs bioreactor, a new tool for lipopeptides production. *Proc Biochem* 47: 2020-2024

Chen HL, Chen YS, Juang RS (2008) Recovery of surfactin from fermentation broths by a hydrid salting-out and membrane filtration process. *Sep Puri Technol* 59: 244-252

Coutte F, Lecouturier D, Yahia, Leclère V, Béchet M, Jacques P, Dhulster P, (2010) Production of surfactin and fengycin by *Bacillus subtilis* in a bubbleless membrane bioreactor. *Appl Microbiol Biotechnol* 87: 499-507

Daniel HJ, Reuss M, Sylдатk C (1998) Production of sophorolipids in high

concentration from deproteinized whey and rapeseed oil in a two stage fed batch process using *Candida bombicola* ATCC 22214 and *Cryptococcus curvatus* ATCC 20509. Biotechnol Lett 20:1153-1156

Darvishi P, Ayatollahi S, Mowla D, Niazi A (2011) Biosurfactant production under extreme environmental conditions by an efficient microbial consortium, ERCPPI-2. Colloids Surf B Biointerfaces 84:292-300

de Cássia FSSR, Darne G, Almeida DG, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2014) Applications of biosurfactants in the petroleum industry and the remediation of oil spills. Int J Mol Sci 15:12523-12542

Doman LE (2016) International energy outlook 2016, DOE/EIA-0484, U. S. Energy Information Administration. Washington, DC

Elshafie AE, Joshi SJ, Al-Wahaibi YM, Al-Bemani AS, Al-Bahry SN, Al-Maqbali D and Banat IM (2015) Sophorolipids production by *Candida bombicola* ATCC 22214 and its potential application in microbial enhanced oil recovery. Front Microbiol 6:1324. doi: 10.3389/fmicb.2015.01324

Fernandes PL, Rodriques EM, Paiva FR, Ayupe BAL, McInerney MJ, Tótola MR (2016) Biosurfactant, solvents, and polymer production by *Bacillus subtilis* RI4914 and their applications for enhanced oil recovery. Fuel 180:551-567

Geys R, Soetaert W, Van Bogaert I (2014) Biotechnological opportunities in biosurfactant production. Curr Opin Biotech 30: 66-72

Giani C, Meiwes J, Rothert R, Wullbrandt D (1996) *Pseudomonas aeruginosa* and its use in a process for the biotechnological preparation of L-rhamnose. US



421 Patent 5,501,966

422 Gray M, Yeung A, Foght J, Yarranton, HW (2008) Potential microbial  
 423 enhanced oil recovery processes: a critical analysis. In: Proceeding of the SPE  
 424 Annual Technical Conference and Exhibition, Denver, Colorado, USA. SPE-  
 425 114676-MS.

426 Hall, C., Tharakan, P., Hallock, J., Cleveland, C., and Jefferson, M. (2003).  
 427 Hydrocarbons and the evolution of human culture. *Nature* 426:318-322

428 Harner NK, Richardson TL, Thompson KA, Best RJ, Best AS, Trevors JT  
 429 (2011) Microbial processes in the Athabasca Oil Sands and their potential  
 430 applications in microbial enhanced oil recovery. *J Ind Microbiol Biotechnol*  
 431 38:1761-1775

432 Helmy Q, Kardena E, Funamizu N, Wisjnuprpto (2011) Strategies toward  
 433 commercial scale of biosurfactant production as potential substitute for it's  
 434 chemically counterparts. *Inter J Biotechnol* 12: 66-86

435 Huang L, Yu L, Luo Z, Song S, Bo H, Zheng C (2014) A microbial-enhanced  
 436 oil recovery trial in Huabei Oilfield in China. *Petrol Sci Technol* 32:584-592

437 Joshi S, Bharucha C, Jha S, Yadav S, Nerurkar A, Desai AJ (2008)  
 438 Biosurfactant production using molasses and whey under thermophilic conditions.  
 439 *Bioresour Technol* 99: 195-199.

440 Joshi SJ, Geetha SJ, Desai AJ (2015) Characterization and application of  
 441 biosurfactant produced by *Bacillus licheniformis* R2. *Appl Biochem Biotechnol*  
 442 177: 346-361

443 Kryachko Y, Semler D, Vogrinetz J, Lemke M, Links MG, McCarthy EL,  
 444 Haung B, Hemmingsen SM (2016) Enrichment and identification of biosurfactant-  
 445 producing oil field microbiota utilizing electron acceptors other than oxygen and  
 446 nitrate. J Biotechnol 231:9-15

447 Le JJ, Wu XL, Wang R, Zhang JY, Bai LL, Hou ZW (2015) Progress in pilot  
 448 testing of microbial-enhanced oil recovery in the Daqing oilfield of north China.  
 449 Int Biodeter Biodegr 97:188-194

450 Li CF, Li Y, Li XM, Cao YB, Song YT (2015) The application of microbial  
 451 enhanced oil recovery technology in Shengli Oilfield. Petrol Sci Technol 33: 556-  
 452 560

453 Li G, Gao P, Wu Y, Tian H, Dai X, Wang Y, Cui Q, Zhang H, Pan X, Dong  
 454 H, Ma T (2014) Microbial abundance and community composition influence  
 455 production performance in a low-temperature petroleum reservoir. Environ Sci  
 456 Technol 48:5336-5344.

457 Li Q, Kang C, Wang H, Liu C, Zhang C (2002) Application of microbial  
 458 enhanced oil recovery technique to Daqing Oilfield. Biochem Eng J 11:197-199

459 Lin SC, Minton MA, Sharma MM, Georgiou G (1994) Structural and  
 460 immunological characterization of a biosurfactant produced by *Bacillus*  
 461 *licheniformis* JF-2. Appl Environ Microbiol 60:31-38

462 Liu, J, Lijun M, Mu B, Liu R, Ni F, Zhou J (2005) The field pilot of microbial  
 463 enhanced oil recovery in a high temperature petroleum reservoir. J Petrol Sci Eng  
 464 48:265-271

Liu JH, Chen YT, Li H, Jia YP, Xu RD, Wang J (2015) Optimization of  
 fermentation conditions for biosurfactant production by *Bacillus subtilis* strains  
 CTCC M201163 from oilfield wastewater. *Environ Prog Sust Energy* 34: 548-554

Makkar RS, Cameotra SS, Banat IM (2011) Advances in utilization of  
 renewable substrates for biosurfactant production. *AMB Express*, 1: 5

McInerney MJ, Youssef N, Nagle DP (2009) Lipopeptide biosurfactants and  
 their use in oil recovery. In: Ashby R, Solaiman D, Kitamoto D (eds), *Bio-based  
 surfactants and detergents: synthesis, properties, and applications*, American Oil  
 Chemists Society, Urbana, IL, pp 129-153

Nazina TN, Pavlova NK, Ni F, Shestakova NM, Ivoilov VS, Feng Q.,  
 Dongyun Z, Prusakova TS, Belyaev SS, Ivanov MV (2008) Regulation of  
 geochemical activity of microorganisms in a petroleum reservoir by injection of  
 H<sub>2</sub>O<sub>2</sub> or water-air mixture. *Microbiology* 77:324-333

Nazina TN, Grigor'yan AA, Feng Q, Shestakova NM, Babich TL, Pavlova  
 NK, Ivoilov VS, Ni F, Wang J, She Y, Xiang T, Mei B, Luo Z, Belyaev SS,  
 Ivanov MV (2007) Microbiological and production characteristics of the high-  
 temperature Kongdian petroleum reservoir revealed during field trial of  
 biotechnology for the enhancement of oil recovery. *Microbiology* 76:297-309

Nikolov V, Farag I, Nikov I (2000) Gas-liquid mass transfer in bioreactor  
 with TPIFB. *Bioprocess Eng* 23 (427-429)

Patel J, Borgohain S, Kumar M, Rangarajan V, Somasundaran P, Sen R  
 (2015) Recent developments in microbial enhanced oil recovery. *Renew Sust*

Energy Rev 52:1539-1558

Pruthi V, Cameotra SS (2000) Novel sucrose lipid produced by *Serratia marcescens* and its application in enhanced oil recovery. J Surf Deter 3:533-537

Rabiei A, Sharifinik M, Niazi A, Hashemi A, Ayatollahi S (2013) Core flooding tests to investigate the effects of IFT reduction and wettability alteration on oil recovery during MEOR process in an Iranian oil reservoir. Appl Microbiol Biotechnol 97: 5979-5991

Salehizadeh H, Mohammadizad S (2009) Microbial enhanced oil recovery using biosurfactant produced by *Alcaligenes faecalis*. Iranian J Biotechnol 7:216-223

Santos DKF, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2016) Biosurfactants: multifunctional biomolecules of the 21st Century. Int J Mol Sci 17: 401. doi:10.3390/ijms17030401

Sarafzadeh P, Hezave AZ, Ravanbakhsh M, Niazi A, Ayatollahi S (2013) *Enterobacter cloacae* as biosurfactant producing bacterium: Differentiating its effects on interfacial tension and wettability alteration mechanisms for oil recovery during MEOR process. Colloids Surf B Biointerfaces 105:223–229

Sen, R. (ed.) Biosurfactants. Landes Bioscience and Springer Science+Business Media. New York.

Shavandi M, Mohebbi G, Haddadi A, Shakarami H, Nuhi A (2011) Emulsification potential of a newly isolated biosurfactant-producing bacterium, *Rhodococcus* sp. strain TA6. Colloids Surf B Biointerfaces 82:477-482

509 Sheng J (2010) Modern chemical enhanced oil recovery: theory and practice.  
 510 Gulf Professional Publishing, Burlington, MA

511 Shibulal B, Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Joshi  
 512 SJ (2014) Microbial enhanced heavy oil recovery by the aid of inhabitant spore-  
 513 forming bacteria: an insight review. The Scientific World J, 2014. Article ID  
 514 309159, 12 pages. doi:10.1155/2014/309159

515 Siegert M, Sitte J, Galushko A, Krüger M (2014) Starting up microbial  
 516 enhanced oil recovery. Adv Biochem Eng Biotechnol 142:1-94

517 Simpson DR, N. Natraj, M. J. McInerney, K. E. Duncan. 2011. Biosurfactant-  
 518 producing *Bacillus* spp. are present in produced brines from Oklahoma oil  
 519 reservoirs with a wide range of salinities. Appl Microbiol Biotechnol 91:1083-  
 520 1093

521 Xia WJ, Luo ZB, Dong HP, Yu L, Cui QF, Bi YQ (2012) Synthesis,  
 522 characterization, and oil recovery application of biosurfactant produced by  
 523 indigenous *Pseudomonas aeruginosa* WJ-1 using waste vegetable oils. Appl  
 524 Biochem Biotechnol 166:1148-1166

525 Yakimov MM, Abraham WR, Meyer H, Giuliano L, Golyshin PN (1999)  
 526 Structural characterization of lichenysin A components by fast atom bombardment  
 527 tandem mass spectrometry. Biochim Biophys Acta 1438:273-280

528 Youssef N, Simpson DR, McInerney MJ, Duncan KE (2013) In-situ  
 529 lipopeptide biosurfactant production by *Bacillus* strains correlates with improved  
 530 oil recovery in two oil wells approaching their economic limit of production. Int

531 Biodeter Biodegr 81:127-132.

532 Youssef N, Elshahed MS, McInerney MJ (2009) Microbial processes in oil  
533 fields: culprits, problems, and opportunities. Adv Appl Microbiol 66:141-251

534 Youssef N, Nguyen T, Sabatini DA, McInerney MJ (2007a). Basis for  
535 formulating biosurfactant mixtures to achieve ultra low interfacial tension values  
536 against hydrocarbons. J Ind Microbiol Biotechnol 34:497–507

537 Youssef N, Simpson DR, Duncan KE, McInerney MJ, Folmsbee M, Fincher  
538 T, Knapp RM. (2007b) In situ biosurfactant production by *Bacillus* strains injected  
539 into a limestone petroleum reservoir. Appl Environ Microbiol 73:1239-1247

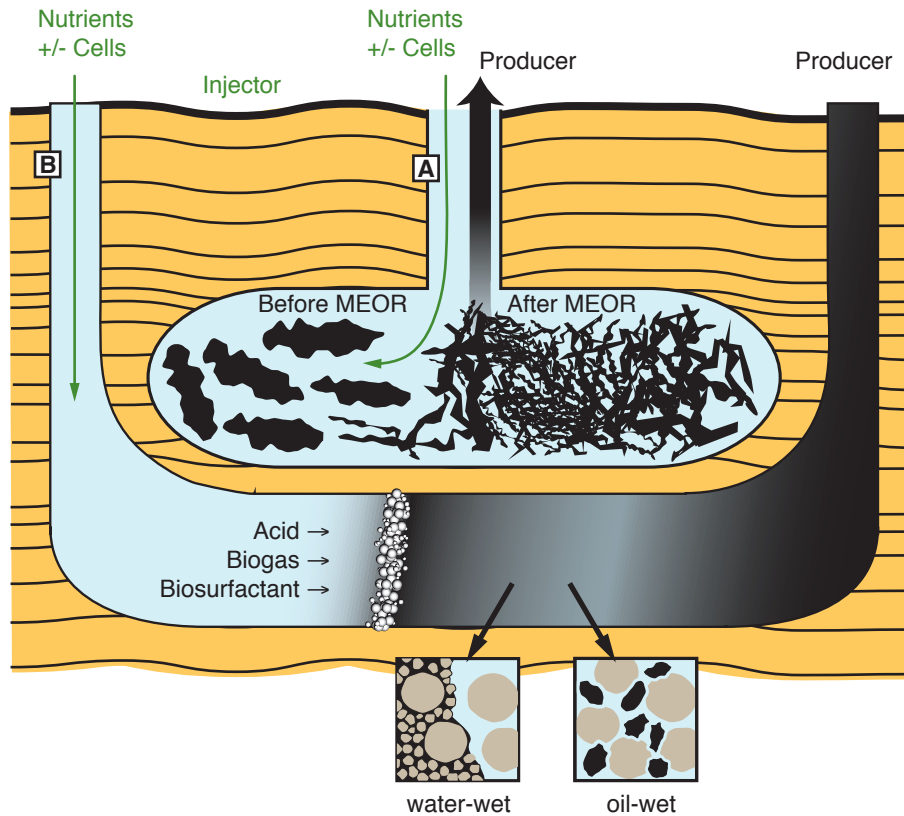
540 Zheng C, Yu L, Huang L, Xiu J, Huang Z (2012) Investigation of a  
541 hydrocarbon-degrading strain, *Rhodococcus ruber* Z25, for the potential of  
542 microbial enhanced oil recovery. J Petrol Sci Eng 81:49-56

543 Zhu Z, Zhang F, Wei Z, Ran W, Shen Q (2013) The usage of rice straw as a  
544 major substrate for the production of surfactin by *Bacillus amyloliquefaciens* XZ-  
545 173 in solid-state fermentation. J Environ Manage 127: 96-102

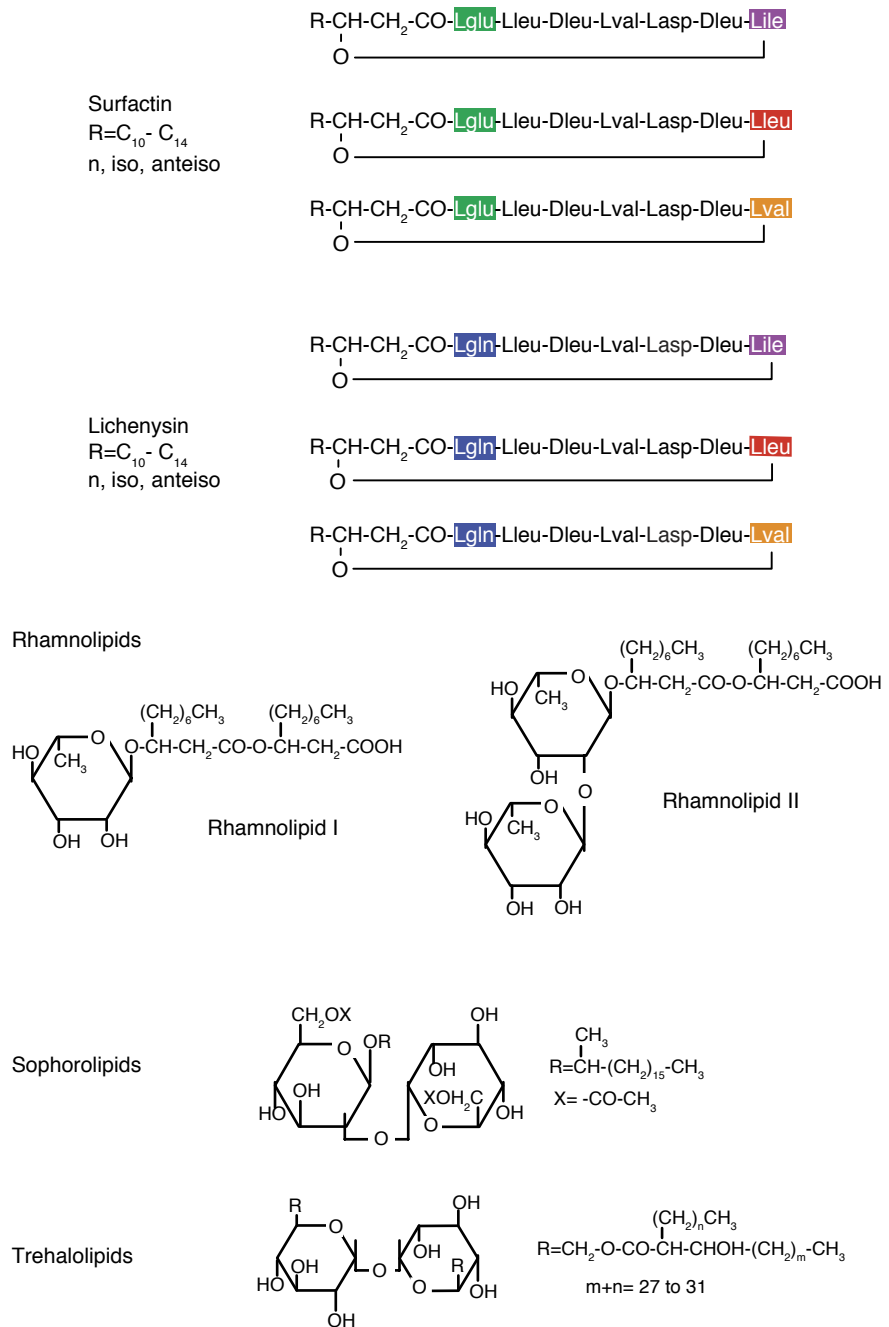
546 Zou C, Wang M, Xing Y, Lan G, Ge T, Yan X, Gu T (2014) Characterization  
547 and optimization of biosurfactants produced by *Acinetobacter baylyi* ZJ2 isolated  
548 from crude oil-contaminated soil sample toward microbial enhanced oil recovery  
549 applications. Biochem Eng J 90:49-58

## Figures

Figure 1. Biosurfactant-mediated oil recovery. A. The change in wettability by biosurfactants near the production well reconnects oil ganglia and increases oil drainage. B. Biosurfactant production during waterflooding mobilizes entrapped oil. Insets: After waterflooding, large globules of oil exist large pores in water-wet regions and oil is found in small pores or in large pockets surrounded by water in oil-wet regions.



558 Figure 2. Structures of lipopeptide, rhamnolipid, sophorolipid, and trehalolipid  
 559 biosurfactants. Boxes highlight variations in amino acid sequence of lipopeptides.



560



Table 1. Efficacy of biosurfactants commonly used for microbial oil recovery <sup>a</sup>

Biosurfactant	Microorganism	Lowest surface tension (mM/m)	Lowest interfacial tension (mN/m)	Critical micelle concentration (mg/L)	Additional oil recovery (%)	Yield (g/L)	Reference
Surfactin	<i>Bacillus subtilis</i> or <i>B. mojavensis</i>	28-30	0.006-0.3	10-35	40-80	0.5-1	Lin et al. 1993; Youssef et al. 2007a
Lichenysins	<i>Bacillus licheniformis</i>	28	0.3-0.5	10-19	37	1.1	Joshi et al 2015; Yakimov et al 1999
Lipopeptide	<i>Acinetobacter baylyi</i>	35	15	90	28	ND	Zou et al., 2014
Rhamnolipid	<i>Pseudomonas aeruginosa</i>	25-27	0.2-2	11-120	10-27	0.7-50	Amani et al. 2013; Xia et al. 2012
Glycolipids	<i>Rhodococcus</i>	27-30	1	57	65-86	0.5-	Shavandia et al., 2011; Zheng et

	sp.				12.9	al., 2012
Glycolipids	<i>Enterobacter</i>	31	0.6-3.2	27-48	1.5-1.7	Darvishi et al. 2011; Rabiei et al.
	<i>cloacae</i> and <i>E.</i>					2013; Sarafzadeh et al. 2013
	<i>hormaechei</i>					
Lipo-	<i>Alcaligenes</i>	20	<1	9	1.2 ±	Salehizadeh and
polysacharide	<i>faecalis</i>				0.05	Mohammadizad 2009
Sucrose lipid	<i>Serratia</i>			90		Pruthi and Cameotra 2000
	<i>marcescens</i>					
Sophorolipid	<i>Candida</i>	33	± 1.6 ± 0.3	27		Elshafie et al. 2015
	<i>bombicola</i>	0.05				

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<sup>a</sup> The values differ depending on the strains, growth conditions, oils and porous media used in different experiments.

Table 2. Recent field trials involving biosurfactant-producing microorganisms.

Mechanism	Microorganisms	Approach	Oil recovery (m <sup>3</sup> )	Comments	Reference
Stimulate in situ hydrocarbon production	Indigenous <i>Pseudomonas</i> sp.	Treat injection wells with air and nutrients	2200	Emulsification	Chai et al. 2015
	Indigenous hydrocarbon degraders	Treat injection wells with H <sub>2</sub> O <sub>2</sub> or oxygenated water with N and P	4420	Emulsification; interfacial tension reduction	Nazina et al. 2008
	Indigenous hydrocarbon degraders	Treat injection wells with air-saturated brine and minerals	16,200	Reduction in interfacial tension	Nazina et al. 2007
	Oil-degrading and biosurfactant-producing	Repetitive treatment of injection wells with	9122	All seven wells had increased oil	Liu et al. 2005

	microorganisms	nutrients and inoculum		production	
	<i>Pseudomonas</i>	Not disclosed	7-14 m <sup>3</sup>	80 % of wells had	Li et al. 2002
	<i>aeruginosa</i> and its		per well	increased production	
	metabolic products				
Stimulate	<i>Bacillus</i> sp. RS-1 and	Treat producing wells	53	20-28 mg/L of	Youssef et al. 2013
biosurfactant	<i>Bacillus subtilis</i> subsp.	with glucose-nitrate-		biosurfactant	
production	<i>spizizenii</i>	metals and inoculum			

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