

1       **Use of Biosurfactants in Oil Recovery**

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3       Guoqiang Li

4       Department of Microbiology

5       Nankai University

6       94 Weijin Road

7       Tianjin, 3000071

8       P. R. China

9       Email: [gqli@nankai.edu.cn](mailto:gqli@nankai.edu.cn)

10

11       Michael J. McInerney

12       Department of Microbiology and Plant Biology

13       University of Oklahoma

14       770 Van Vleet Oval

15       Norman, OK, USA

16       Email: [mcinerney@ou.edu](mailto:mcinerney@ou.edu)

17       Phone: 1-405-325-6050

18       Fax: 1-405-325-7619

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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.

38

39 **Introduction**

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

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

52 Petroleum is a non-renewable fossil resource derived from organic matter  
53 deposited eons ago in the lithosphere. When a well is drilled into an oil reservoir, oil  
54 and water are pushed to the surface by the natural pressure within the reservoir. As  
55 reservoir pressure dissipates, pumps are placed on the well to assist in bringing the  
56 fluids to the surface. This stage of oil production is called primary production  
57 (Youssef et al. 2009). Eventually, additional pressure must be added to the reservoir  
58 to continue to recover oil. Often surface water, seawater, or brine from a subterranean  
59 formation is injected into the reservoir to push the oil to production wells. This stage  
60 of oil production is called waterflooding or secondary oil production (Youssef et al.  
61 2009). When the above exploration strategies reach their economic limits, only about  
62 one-third to one-half of the oil originally in place in the reservoir has been extracted,

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

68 Technologies to recover residual oil are called enhanced oil recovery (EOR)  
69 technologies. EOR includes three primary techniques: thermal recovery (hot water,  
70 steam, combustion), gas injection (N<sub>2</sub>, CO<sub>2</sub>, flue gas), and chemical injection  
71 (surfactants, polymers, solvents) (Alvarado and Manrique 2010; Sheng 2010). Over  
72 one-half of the EOR-recovered crude oil in the USA is the result of gas injection with  
73 CO<sub>2</sub> injection being the most important. The remainder is the result of thermal oil  
74 recovery technologies. Chemical-based EOR technologies have been marginally  
75 economic due to high chemical costs (Alvarado and Manrique 2010; Sheng, 2010).  
76 Another EOR approach is to use microbial technologies to enhance oil recovery  
77 (MEOR). Numerous laboratory and field studies have shown that microorganisms  
78 produce useful products such as biosurfactants that allow recovery of residual oil  
79 (Youssef et al., 2009). Microorganisms can produce these products from inexpensive  
80 and renewable nutrients injected into the reservoir. Thus, MEOR technologies have an  
81 economic advantage in that they do not consume large amounts of energy as do  
82 thermal processes, nor do they depend on expensive chemicals as many chemical  
83 processes do (Youssef et al. 2009). In addition, MEOR provides an ecofriendly  
84 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

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

116 With larger, more productive oil fields, increasing the ultimate recovery factor is  
117 an important consideration. Large amounts of oil remain entrapped by capillary forces  
118 in reservoirs after waterflooding. In water-wet regions of the reservoir, oil will be found  
119 as spherical globules in the center of the large pores and as ganglia of oil spanning  
120 multiple pores surrounded by water (Armstrong and Wildenschild, 2012a) (Figure  
121 1B). In strongly oil-wet regions of the reservoir, oil will be found in small pores and  
122 in large pockets of oil surrounded by water. In both cases, mobilization of the  
123 entrapped oil will require an increase in viscous forces, and/or a reduction of  
124 capillary forces in the reservoir. The viscous and capillary forces that entrap oil are  
125 expressed as a dimensionless ratio called capillary number ( $N_{ca}$ ) (equation 1):

126

$$127 N_{ca} = (\mu_w \cdot V_w) / (\gamma) \quad (1)$$

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

150 alteration by biosurfactants important for oil recovery from carbonate cores. The  
151 change in capillary number due to interfacial tension reduction by the biosurfactant  
152 explained the observed oil recoveries. However, much lower residual oil saturations  
153 than predicted by changes in capillary number alone were observed, when both cells  
154 and the biosurfactant were used (Armstrong and Wildenschild, 2012b). Thus, the  
155 clogging of pores with cells, which altered flow patterns, has a significant effect on oil  
156 recovery beyond that predicted by capillary number (Armstrong and Wildenschild,  
157 2012a, b).

158

## 159 **Types of biosurfactants**

160 Diverse microorganisms produce surface-active agents (Youssef et al., 2009; Sen,  
161 2010; Santos et al. 2016). Biosurfactants are classified into five major categories  
162 based on their chemical structures: lipopeptides, glycolipids, phospholipids, neutral  
163 lipids, and fatty acids (de Cássia et al. 2014; Santos et al. 2016). The most common  
164 biosurfactants used in MEOR are lipopeptides (surfactin and lichenysin) and  
165 glycolipids (rhamnolipids, sophorolipids and trehalolipids) (McInerney et al 2009;  
166 Youssef et al. 2009; Liu et al. 2015; Santos et al. 2016) (Figure 2). The interfacial  
167 tension between oil and aqueous phases varies from 20 to 40 mN/m (Gray et al.  
168 2008). A number of biosurfactants reduce oil-water interfacial tension to < 1 mN/m  
169 (Table 1), which provides a 100-fold or greater increase in capillary number needed  
170 for substantial oil recovery. Some biosurfactant producers are also able to produce

Fig. 2

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

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

181 Commercial of biosurfactant production is costly due to the low productivity of  
182 many biosurfactant-producing strains (Table 1), the use of expensive media  
183 components, and high downstream processing costs (Helmy et al. 2011; Banat et al.  
184 2014; Geys et al. 2014). The use of low cost agro-industrial by-products such as  
185 whey, molasses, waste oils helps reduce nutrient costs (Banat et al. 2010; Makkar et  
186 al. 2011); however, complex substrates may have undesirable components that inhibit  
187 production or make downstream processing difficult. A number of investigators have  
188 used statistical approaches such as surface response methodology to optimize nutrient  
189 composition and operating conditions to improve biosurfactant productivity (Banat et  
190 al., 2010; Liu et al. 2015). Rotating disk, biofilm reactors (Chitoui et al., 2012),  
191 bubble less, membrane-aerated bioreactors (Coutte et al., 2010), and three-phase,  
192 inverse fluidized bed reactors (Nikolov et al., 2000) have been developed to provide

193 adequate aeration without foaming and solid-state fermentation, where the  
194 biosurfactant producer is grown on a solid surface such as rice straw, reduces capital  
195 costs (Zhu et al., 2013). The combination of ultrafiltration with adsorption and ion  
196 exchange chromatography increased the recovery of biosurfactants from fermentation  
197 broths (Chen et al., 2010). It should be noted that there are some biosurfactant  
198 producers that produce very high concentrations of biosurfactants (Geys et al. 2014).  
199 For example, *Pseudomonas aeruginosa* produces 70-120 g/L of rhamnolipids when  
200 cultivated on vegetable oil (Giani et al. 1997) and *Starmerella bombicola*, the best  
201 studied sophorolipid producer, and produces 400 g/L sophorolipid when grown in a  
202 two-stage cultivation process (Daniel et al. 1998).  
203

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</i> <i>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-	Shavandi 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>						
Lipopolysach- aride	<i>Alcaligenes</i>	20	<1		9	1.2 ±	Salehizadeh and
	<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>bombycina</i>	0.05					

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

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

207 Oil recovery occurs by the activity of microorganisms and/or their metabolites,  
208 such as biosurfactants, biomass, biopolymers, solvents, acids, gases, etc., which can  
209 be generated ex situ or in situ (Youssef et al. 2009). In ex situ MEOR approaches,  
210 microbes are cultivated in a fermentor on inexpensive nutrients and the microbes  
211 and/or their metabolites are injected into oil reservoir. In situ approaches involve the  
212 growth and metabolism of the indigenous or injected microbes in the reservoir to  
213 produce cells, metabolites, or a particular activity such as hydrocarbon degradation.

214 Thus, there are three main strategies for using biosurfactants for oil recovery (Banat et  
215 al. 2000):

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

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

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

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

224 In addition to generating low interfacial tensions, biosurfactants must maintain  
225 activity under the environmental conditions present in oil reservoirs (Siegert et al.  
226 2014). A number of studies have shown that lipopeptides biosurfactants and

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

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

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

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

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

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

259 ***Injection of nutrients to stimulate indigenous biosurfactant-producing***  
260 ***microorganisms***

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

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

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

287

## 288 **Success of field trials**

289 Although a number of laboratory studies show the efficacy of biosurfactant

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

296 In the past two decades, a number of field trials of MEOR have been  
297 implemented in Chinese oil fields, including Dagang Oilfield, Daqing Oilfield,  
298 Huabei Oilfield, Shengli Oilfield, and Xinjiang Oilfield, (Liu et al. 2005; Huang et al.  
299 2014; Li et al. 2014; Chai et al. 2015; Le et al. 2015; Li et al. 2015). A well-  
300 documented trial involving hydrocarbon-degrading and biosurfactant-producing  
301 bacteria was implemented in a sandstone oil reservoir (Guan 69 Unit of the Dagang  
302 Oilfield in Hebei Province, China) (Liu et al. 2005). The injected, exogenous bacteria  
303 were detected in 4 of 7 production wells after several months of injection. A slight  
304 decrease in the surface tension of the production liquids was observed and oil  
305 production increased over a six months period following the microbial treatment.  
306 About 9120 m<sup>3</sup> of additional oil was produced (Table 2). In another trial, a  
307 biosurfactant-producing, *Pseudomonas aeruginosa* P-1, and its metabolic products  
308 were injected into more than 60 oil-producing wells in Daqing oilfield, China (Li et  
309 al. 2002). About 80% of injected wells showed a significant decrease in the amount of  
310 water produced with a corresponding increase in oil produced.

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 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	20-28 mg/L of biosurfactant	Youssef et al. 2013

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

324        One of the more common approaches to MEOR is to stimulate hydrocarbon  
325        degradation by the controlled injection of air or H<sub>2</sub>O<sub>2</sub> along with other nutrients (Liu  
326        et al. 2005; Nazina et al. 2007, 2008; Huang et al. 2014; Li et al. 2014; Chai et al.  
327        2015; Le et al. 2015; Li et al., 2015). Hydrocarbon metabolism often results in  
328        biosurfactant production. After the microbial process was initiated, products of  
329        microbial metabolism including and biosurfactants and hydrocarbon-degrading  
330        microorganisms were detected in production fluids (Nazina et al, 2007 and 2008). The  
331        water content of production liquids decreased and the oil content increased, resulting  
332        in large amounts of additional oil (Table 2).

333

334     **Research Needs**

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

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

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

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

355

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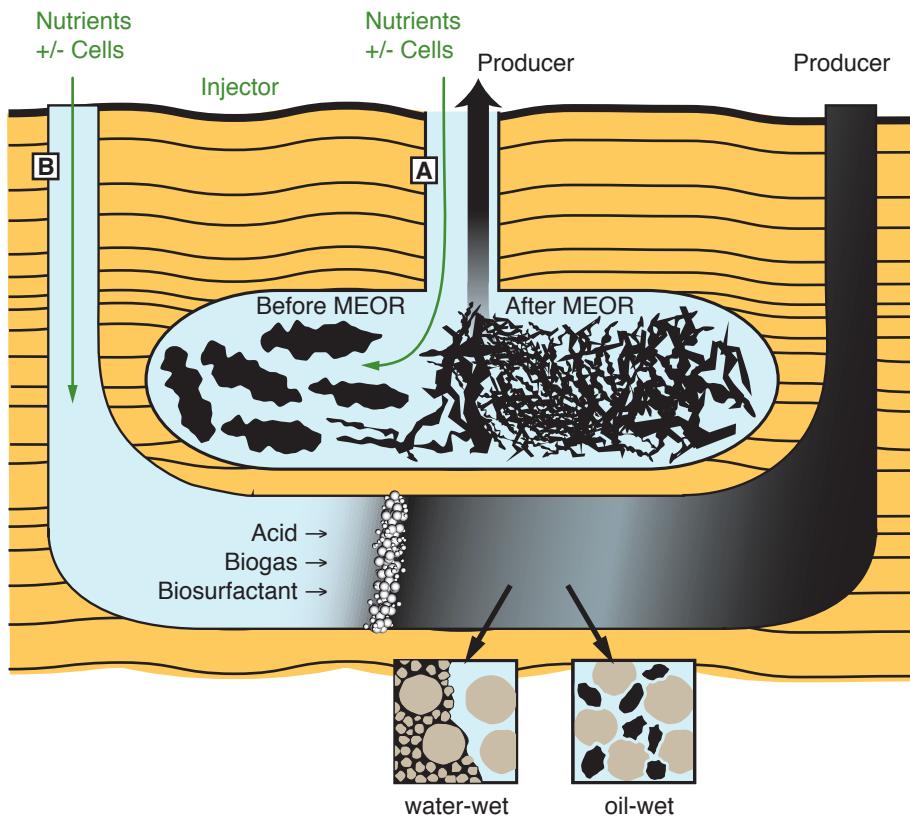
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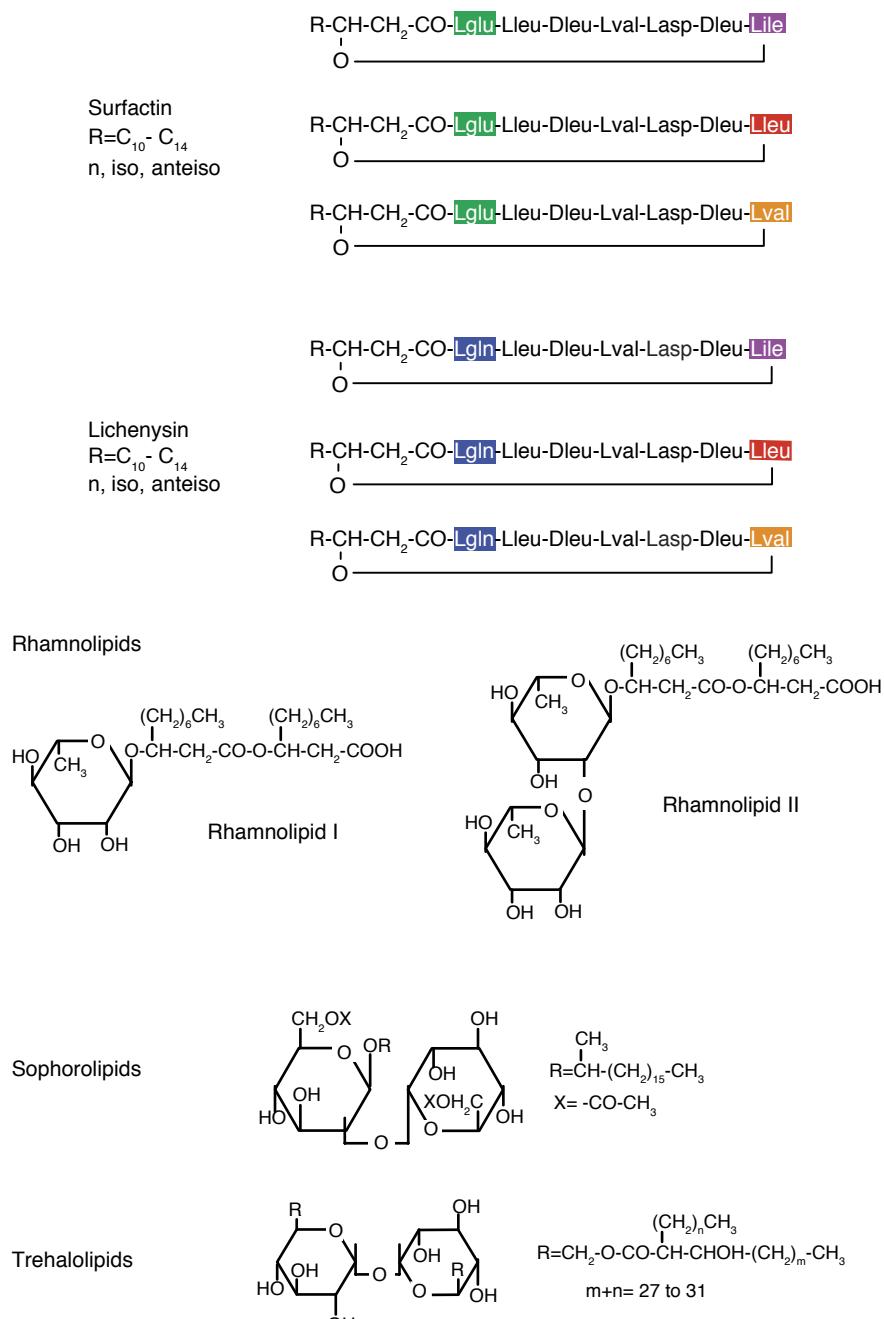
550 **Figures**

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



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



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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</i> <i>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			

<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 metabolic products		per well increased production
Stimulate biosurfactant production	<i>Bacillus</i> sp. RS-1 and <i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	Treat producing wells 53 20-28 mg/L of Youssef et al. 2013 biosurfactant metals and inoculum

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