Biosurfactants can mobilize substantial amounts of entrapped hydrocarbons

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Abstract. Microbially produced biosurfactants may be an economic method to recover residual hydrocarbons since their low critical micelle concentrations indicate that they are effective at low concentrations. However, the recovery of residual hydrocarbon by biosurfactants from model porous systems is often low and inconsistent. We found that a lipopeptide biosurfactant produced by Bacillus mojavensis strain JF-2 mobilized substantial amounts of residual hydrocarbon from sand-packed columns when a viscosifying agent and a low molecular weight alcohol were present. Sand pack columns flooded to residual oil saturation were treated with cell-free supernatant solutions of the culture that contained different concentrations of the biosurfactant. The amount of residual hydrocarbon mobilized depended on the biosurfactant concentration. The injection of one pore volume of cell-free culture fluid with 900 mg l⁻¹ of the biosurfactant, 10 mM 2,3-butanediol and 1000 mg l⁻¹ of partially hydrolyzed polyacrylamide polymer (PHPA) mobilized 82% of the residual hydrocarbon. Even low biosurfactant concentrations (16 mg l⁻¹) mobilized substantial amounts of residual hydrocarbon (29%). Deletion of 2,3-butanediol or PHPA or both from the supernatant solution markedly decreased residual oil recovery to less that 10%. PHPA alone or in combination with 2. 3-butanediol in the absence of the microbially generated, biosurfactant did not recovery any residual oil. Cell-free culture fluids containing the biosurfactant decreased interfacial tensions between crude oil and brine solutions from about 27 mN/m to less that 0.1 mN/m. Significant recovery of residual oil occurred at biosurfactant concentrations 10 to 100-fold less than that used for chemical surfactant flooding. Thus, the lipopeptide, biosurfactant system may be an effective and economic approach for removing hydrocarbon contamination sources in soils and aguifers and for the recovery of entrapped oil from low production oil reservoirs.

Introduction. Previous experiments by other investigators to recover oil using bio-surfactant generated by *Bacillus mojavensis* JF- 2 were not very successful. As in any surfactant flood, the following reasons are the most likely causes for low oil recoveries^{1,2}:

- a) Due to an insufficient concentration of surfactant, interfacial tension between oil and water was not low enough to mobilize oil.
- b) A component or components to work in combination with the surfactant and improve its recovery performance were not present.

The following series of experiments were performed to study the ability of the JF-2 biosurfactant to mobilize residual oil. These experiments were used to identify and explain the role of other components needed in addition to bio-surfactant to mobilize residual oil. Along with the displacement fluid composition, design features of the flooding protocol were tested.

Experimental Procedure. The study was divided into three separate experiments.

Experiment 1. Study the effect of a solution of JF- 2 bio-surfactant, co-surfactant and a viscosifying agent on oil recovery.

Theory. In this experiment, partially hydrolyzed polyacrylamide polymer (PHPA) and 2,3butanediol as co-surfactant were added to JF-2 bio-surfactant solution produced by aerobically grown cells. Under aerobic conditions, JF-2 produces larger quantities of bio-surfactant than under anaerobic conditions, **Table A-1**. Generation of bio-surfactant under aerobic conditions is also more rapid. To be able to conduct a large number of surfactant flooding experiments within a reasonable time, bio-surfactant was produced aerobically and flooded through sand packs that had been water flooded to near residual oil saturation. Under anaerobic conditions, *Bacillus mojavensis* JF-2 also produces an alcohol, 2,3-butanediol⁹. This alcohol is believed to function as a co-surfactant for the anionic bio-surfactant and in the following sections is referred to as co-surfactant for the bio-surfactant. The concentration of 2,3-butanediol anaerobically generated by JF- 2 was 10.0 mM. To replicate the products that JF-2 would make anaerobically in an *in situ* recovery process, 10.0 mM 2,3-butanediol was added to the aerobically prepared bio-surfactant. Polymer addition increased the surfactant solution's viscosity and made the mobility ratio between the oil and displacing surfactant more favorable. The effects of gravity and a viscous preflush before the surfactant flooding on residual oil recoveries were also investigated to develop a surfactant flooding protocol.

Table A-1. Comparison of bio-surfactant yields under anaerobic and aerobic conditions

Yield of product per kilogram of molasses nutrient (gm/kg)	Aerobic conditions	Anaerobic conditions	
Bio-surfactant	24.9 gm/kg	4.4 gm/kg	
Co-surfactant (2,3- Butanediol)	Negligible	123.5 gm/kg	

Procedure. Bacillus mojavensis strain JF-2 (ATCC 39307) was grown in a phosphate-buffered, mineral salts medium (medium E)⁹ with (in g l^{-1}) 1 g yeast extract, 1 g NaNO₃ and 30 g Proteose Peptone #3 (Difco Laboratories, Inc., Detroit, MI) in 1-liter cultures. The cultures were incubated aerobically at room temperature for 48 hr. with stirring provided by a magnetic stirrer and a stir bar. After incubation, the cells were removed by centrifugation (10,000 x g; 4° C; 20 min). Uninoculated medium served as the control and received the same concentrations of polymer and 2,3-butanediol. The biosurfactant from a 20-ml sample of cell-free culture fluid was collected by acid precipitation. The pH of the cell-free medium was reduced to less than 2 by the addition of 6 N HCI. The acidified, cell-free medium was left at 4°C overnight to precipitate the biosurfactant, which was collected by centrifugation as described above. The pellet containing the biosurfactant was extracted with 2 ml of methanol for 1 min with agitation. The insoluble material was removed by centrifugation as above. The biosurfactant was then quantified by a high-pressure liquid chromotagraph equipped with a C₁₈ column and an ultraviolet detector set at 210 nm. The mobile phase was 70% methanol and 30% of a 10 mM phosphate buffer (pH 6.8). The flow rate was 1 ml/min and the injection volume was 20 µl. Surfactin (Sigma Chemical Co. St. Louis, MO) was used as the standard. The amount of biosurfactant present in cultures was corrected for the percent recovery of known amounts of surfactin that were added to sterile medium and carried through acid precipitation and methanol extraction procedures.

Bio-surfactant concentration used in Experiment 1 was 910 ppm and 10.0 mM 2,3butanediol was added. Sufficient PHPA to give a concentration of 1000 ppm was mixed with the bio-surfactant-2,3-butanediol solution in a low speed blender. The viscosity after mixing was 12.0 cp. An aqueous 1000 ppm PHPA solution of 53.0 cp viscosity was prepared for use as preflush and post-surfactant mobility buffer. Residual oil volumes and saturations of the eight sand packs used in this experiment are tabulated in **Table A-2**. The effective permeability of the sand packs to brine at residual oil saturation ranged from 1.4 and 1.6 Darcies. The brine used for waterflooding contained 5.0% (by weight) NaCl and the oil was a 34^o API oil with 10.0 cp viscosity. The pore volume of all sand packs was approximately 100.0 cc. One pore volume of the bio-surfactant with PHPA and 2,3-butanediol (100.0 cc) or a control solution with the same concentrations of PHPA and 2,3-butanediol in cell-free culture fluid medium without the bio-surfactant was flooded through each pack.

Packs 1.1 and 1.2 were oriented vertically. A 5.00 cc preflush of 1000 ppm PHPA in 5% NaCl brine was first injected into each pack. This was followed by 100.0 cc of surfactant solution containing 910 ppm of the bio-surfactant, 10 mM 2,3-butanediol and 1000 ppm of PHPA. The surfactant solution was followed by 25.0 cc of the 1000 ppm PHPA in 5% NaCl brine as post flush mobility buffer. This was followed by injection of 2.5% NaCl brine. A total of 200.0 cc of fluids was flooded through each pack. Packs 1.3 and 1.4 were flooded similarly, except that no preflush was used. Packs 1.5 and 1.6 were flooded with a protocol identical to Packs 1.1 and 1.2, except that the packs were oriented horizontally. Packs 1.7 and 1.8 served as controls for the experiment. These two packs each received 100.0 cc of the control solution that contained 1000 ppm PHPA and 10.0 mM 2,3-butanediol dissolved in cell-free culture fluid from which the bio-surfactant had been removed by acid precipitation. The control tested whether 2,3-butanediol and polymer alone could recover oil. The inlet pressure for all packs was kept constant at 8.0 psig during the treatment. The effluent from the packs was collected in 50.0 cc tubes.

Observations and Discussions. Observations and results are tabulated in Table A-2. Packs 1.1 and 1.2 that received a preflush and were flooded vertically had the highest residual oil recovery; about 80.0% of the residual oil was recovered from each pack. About 70% of residual oil was recovered from each of pack (1.3 and 1.4) that did not receive a polymer preflush. Sixty three percent of residual oil was recovered from each pack (1.5 and 1.6) that received a preflush but were flooded horizontally. Only 1.0% residual oil was recovered from each control pack (1.7 and 1.8) that did not receive the bio-surfactant. Oil produced with the first 50.0 cc sample of effluent was excluded from the recovery calculations since it was oil trapped at the discharge end of the sand packs at the end of waterflooding and was displaced the moment chemical injection started. The higher recovery of residual oil from packs 1.1 and 1.2 compared with recovery from packs 1.3 and 1.4 suggested that the viscous preflush improved sweep and recovery efficiency. This can be seen from the cumulative percentage oil recovery plot, Figure A-1. On the oil production plot, Figure A-2, a peak in oil production followed by a decline indicates the oil bank production. The figure shows smaller peaks for packs 1.3 and 1.4 compared to packs 1.1 and 1.2. Given that packs 1.3 and 1.4 contained nearly the same amount of residual oil as packs 1.1 and 1.2, this confirmed that the PHPA solution preflush in packs 1.1 and 1.2 helped mobilize more oil. The preflush displaces saline water and lowers the surfactant mobility by reducing the permeability of the porous medium^{3,4}. The surfactant behind the mobilized oil is not affected by the resident saline brine and sweeps the porous medium of oil more efficiently. Experiments were not done to study the relationship between salinity and degradation of bio-surfactant, but Meyers and Salter⁵ have reported the

deleterious effects of salinity on surfactant. Since a complete pore volume of the bio-surfactant was injected into each pack, production of mobilized oil continued until 200.0 cc of fluids were flooded through or until the surfactant was displaced from the pack. This is shown by a steady increase in cumulative percentage recovery in Figure A-1 and in oil production in Figure A-2 until 200.0 cc was recovered. A 12.5% decrease in oil recovery from each of packs 1.5 and 1.6 compared to the oil recoveries from packs 1.1 and 1.2 recoveries showed that surfactant flooding against gravity improved oil recovery. Gravity helped stabilize the flood front and improved the bio-surfactant solution's sweep efficiency. Oil production in the horizontal packs. 1.5 and 1.6 until the 200.0 cc mark was a combination of flooding a pore volume of surfactant and stable portion of the surfactant front. Due to this, oil production continued until 200.0 cc of fluids was injected into the pack. Negligible oil recoveries from the control packs, 1.7 and 1.8 confirmed that 2,3-butanediol and PHPA alone did not mobilize oil. Addition of water soluble alcohols to anionic surfactant solutions increases the optimum salinity and optimum interfacial tension of the surfactant solution⁶. 2,3-Butanediol, being a water-soluble alcohol, may have increased the tolerance of the surfactant solution to the saline environment and in combination with the preflush that reduces salinity may have improved recovery. Gravity was shown to improve oil recovery in the experiments, but will not offer any advantage in thick reservoirs. The adsorption and resulting degradation of the bio-surfactant are unknown factors that may make the tertiary recovery process uneconomic.

Pack #	Orientation of pack	Volume Res. Oil, V _{OR,wf}	Residual Saturation , S _{OR,wf}	Pre-flush volume	Surfactant volume	Post-flush volume	Recovered oil	Recovery*
1.1	Vertical	20.0	20.6	5	100.0	25	15.5	77.5
1.2	Vertical	18.0	19.2	5	100.0	25	15.5	85.6
1.3	Vertical	19.0	21.0	0	100.0	25	12.9	67.9
1.4	Vertical	18.0	19.8	0	100.0	25	13.3	73.9
1.5	Horizontal	22.0	25.4	5	100.0	25	14.1	64.1
1.6	Horizontal	20.0	21.9	5	100.0	25	12.7	63.5
1.7	Vertical	25.0	26.7	5	100.0 (Control)	25	0.3	1.20
1.8	Vertical	19.0	21.7	5	100.0 (Control)	25	0.2	1.10

Table A-2. Results	of Exp	eriment 1
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Recovery* (%) = <u>Recovered oil (cc) x 100</u> Residual oil volume (cc)







Figure A-2. Oil production vs. Cumulative volume flooded through each pack. Experiment 1

Experiment 2. Study the relation between oil recovery and volume of bio-surfactant flooded through sand packs.

Theory. In surfactant floods, the surfactant often constitutes the largest component of the costs. A relationship between oil recovery and surfactant consumption must be established to estimate the minimum amount of surfactant required for economic oil recovery. Though laboratory scale results differ from field scale flood results, well designed experiments can define basic parameters needed to design a tertiary recovery project.

In this experiment, decreasing volumes of the bio-surfactant solution were flooded through sand packs to establish a relationship between the oil recovery and surfactant consumed. A 50.0 cc post flush mobility buffer fluid was used here instead of 25.0 cc used in Experiment 1 to avoid fingering of post flush brine through the mobility buffer solution. The polymer concentration was decreased in a stepwise manner to ensure a favorable mobility ratio at the surfactant- mobility buffer interface at the front and a polymer solution mobility as close as possible to the post flush brine mobility at the rear⁸. Hence, an attempt was made to maintain favorable mobility ratios across the length of the pack and reduce polymer requirement.

Procedure. The viscosified bio-surfactant solution had 10 mM 2,3-butanediol, 283 ppm of the bio-surfactant, and 1000 ppm of PHPA (viscosity of 12.0 cp). Eight sand packs, numbered 2.1 to 2.8, were water flooded to residual oil saturation with 2.5% NaCl brine. Brine salinity was lowered from 5.0 % NaCl in Experiment 1 to 2.5% NaCl in order to reduce the deleterious effects of a saline environment on the bio-surfactant and polymer solution. The sand packs had an average pore volume of 100.0 cc. Residual oil saturations are shown in **Table A-3**. The effective permeability of the packs to brine at residual water saturation was between 1.4 and 1.7 Darcies. The oil was a 34.5⁰ API oil and had a viscosity of 11.5 cp. The graded post flush mobility buffer solutions consisted of 1000 ppm PHPA and 700 ppm PHPA solutions dissolved

in 2.5% NaCl brine. The viscosities of the 1000 ppm and 700 ppm PHPA solutions were 53.0 cp and 17.0 cp respectively. Four pairs of two packs each were flooded with 100.0 cc, 80.0 cc, 60.0 cc and 40.0 cc of bio-surfactant solution, respectively. All packs were oriented vertically and flooded from the bottom. The bio-surfactant solution was preceded by 5 c.c. of 1000 ppm PHPA in 2.5% NaCl brine as a preflush and followed by 25.0 cc of each post flush mobility buffer solution. After the two post flush injections, 2.5% NaCl brine was then injected until a total of 200.0 cc of fluid was injected into each pack. Effluent was collected in 50.0 cc samples. Plot of oil production against cumulative volume flooded is shown in **Figure A-3**. Oil produced with the first 50.0 cc of effluent was excluded from recovery calculations.

Observations and Discussion. **Table A-3** summarizes the experimental results. Packs 2.1 and 2.2 that received with 100.0 cc of bio-surfactant solution had the highest recovery, each with nearly 50% residual oil recovery. The lowest residual oil recovery (30%) was from packs 2.7 and 2.8, each. Each was flooded with 40.0 cc of the bio-surfactant solution. The residual oil recovery from packs 2.7 and 2.8 is significant because nearly 30% of the residual oil was recovered from each pack with less than one-half of a pore volume of the bio-surfactant solution.

Oil production from packs 2.5 to 2.8 sharply increased and then declined as shown in the oil production plot, Figure A-3. The rise in production signified the breakthrough of the mobilized oil bank. Oil production declined as bio-surfactant started was displaced out of the pack by the pos flush mobility buffer as seen by the decline in oil production after 100.0 cc of cumulative fluid production in Figure A-3. It can be observed in Figure A-3 that packs 2.1 to 2.4 displayed unexpected oil recovery profiles. The four packs did not show a peak in oil production followed by a decline. Also, packs 2.3 and 2.4, flooded with 80.0 cc of surfactant solution, had lower oil recoveries than packs 2.5 and 2.6 that were flooded with 60.0 cc of surfactant. The anomalous behavior may be due to a large time interval from the end of water flooding to the start of bio-surfactant flooding for packs 2.3 and 2.4. This may have resulted in oil and water near the effluent end of each pack that was produced at the instant that the preflush was injected and resulting in the premature production of oil that would normally have been produced as part of the bio-surfactant mobilized oil bank. Since oil produced with the first 50.0 cc of effluent was discarded from the recovery calculations, this recovered oil was not included in our calculations resulting in a lower oil recovery values for packs 2.3 and 2.4. This problem was corrected in packs 2.5 to 2.8. The larger volume of post flush mobility buffer prevented brine from fingering through the buffer solution before a major fraction the oil and surfactant was displaced.

Pack #	Residual Oil, V _{OR,wf}	Residual oil saturation S _{OR,wf}	Preflush Volume	Surfactant solution Volume	Volume of post surfactant flood mobility buffer solution		Recovered Oil	Recovery*
	(CC)	(%)	(CC)	(CC)	(CC)	(cc)	(CC)	(%)
2.1	15.0	15.8	5.00	100.0	25.0	25.0	8.9	52.7
2.2	15.0	16.7	5.00	100.0	25.0	25.0	8.0	48.1
2.3	18.0	19.4	5.00	80.0	25.0	25.0	6.5	33.1
2.4	21.0	22.8	5.00	80.0	25.0	25.0	7.0	32.7
2.5	19.0	20.8	5.00	60.0	25.0	25.0	7.5	36.1
2.6	19.0	20.7	5.00	60.0	25.0	25.0	8.9	40.6
2.7	23.0	26.5	5.00	40.0	25.0	25.0	7.2	30.4
2.8	20.0	22.6	5.00	40.0	25.0	25.0	6.3	30.1

Table A-3. Results of Experiment 2



Figure A-3 Oil production vs. Cumulative Volume flooded through each pack. Experiment 2

Experiment 3. Confirm that JF-2 bio-surfactant works best in combination with a co-surfactant and a viscosifying agent.

Theory. In Experiment 1, JF-2 bio-surfactant along with 2,3-butanediol and PHPA polymer was identified as a system that recovered residual oil. Experiments were not conducted to study whether the bio-surfactant could recover oil without 2,3-butanediol or PHPA. Since separately added polymer and co-surfactant increase the cost of a micellar polymer flood, understanding their impact on oil recovery is an important consideration in surfactant-polymer flood design.

Procedure. Three separate bio-surfactant solutions were prepared, each containing 43 ppm of the biosurfactant. Solution A was 10.0 mM of 2,3-butanediol dissolved in bio-surfactant solution, Solution B contained 1000 ppm PHPA dissolved in the bio-surfactant solution, and Solution C contained the bio-surfactant solution with 10.0 mM 2,3-butanediol and 1000 ppm PHPA. A fourth solution, Solution D, contained only 10.0 mM2,3-butanediol and 1000 ppm PHPA dissolved in cell-free culture fluid that had the bio-surfactant removed by acid precipitation (see above). PHPA solutions with concentrations of 1000 ppm and 700 ppm in

2.5% NaCl brine were used as post flush mobility buffer solutions. The viscosity of the viscosified bio-surfactant solution was 12.0 cp. The viscosities of the 1000 ppm and 700 ppm PHPA solutions were 53.0 cp and 17.0 cp, respectively. Eight sand packs, numbered 3.1 to 3.8 were water flooded with 2.5% NaCl brine to near residual oil saturation. The effective permeabilities of the packs to water at residual oil saturation were between 1.6-1.8 Darcies. The residual saturations are listed in **Table A-4**. The oil was 34.5⁰ API oil and had a viscosity of 11.5 cp. The sand packs had an average pore volume of 100.0 cc. All the packs were oriented vertically and flooded from bottom. The inlet pressures were kept constant at 9.0 psig during the flooding. Each of the four solutions was flooded through two packs. First, 5.00 cc of preflush 1000 ppm PHPA solution was injected into each pack. Then, 100.0 cc of Solution D was injected through packs 3.1 and 3.2, 100.0 cc of Solution A through packs 3.3 and 3.4, 100.0 cc of Solution B through packs 3.5 and 3.6 and 100.0 cc of Solution C through packs 3.7 and 3.8. Then 25 c.c. of each post flush mobility buffer was injected. Sufficient 2.5% NaCl brine was injected after the post flush solutions until a total of 200.0 cc of fluid was recovered from each pack. Effluent from the packs was collected in 50.0 cc samples. Oil produced with the first 50.0 cc of effluent was excluded from the recovery calculations.

Observations and Discussion. The experiment results are summarized in **Table A**-**4**.Cumulative percentage oil recovery against cumulative volume flooded for each pack is plotted in **Figure A-4**. About 1.0 % of residual oil was recovered from packs 3.1 and 3.2 that were treated with Solution D, which did not contain the bio-surfactant. Solution A injected into packs 3.3 and 3.4 recovered approximately 12% of residual oil, Solution B in injected into packs 3.5 and 3.6 recovered 17% of residual oil, and Solution C injected into packs 3.7 and 3.8 recovered about 22.0% of residual oil.

Negligible oil recovery from packs 3.1 and 3.2 confirmed that 2,3-butanediol and PHPA only assisted the JF-2 bio-surfactant in recovering oil. In the absence of the bio-surfactant, these two chemicals did not recover oil. Due to negligible oil recovery, the production profiles for packs 3.1 and 3.2 were not plotted in Fig A-4. For packs 3.3 and 3.4 treated with Solution A, an increase in oil recovery occurred when 100.0 and 150.0 cc of fluid was recovered (Fig.A-4), suggesting that the recovery fluid fingered through the polymer preflush solution and that the mobilized oil was not recovered until the post flush injection began. Oil banks formed in packs 3.5 and 3.6 that were treated with Solution B, containing the bio-surfactant and PHPA. The breakthrough of the bank is shown by the large increase in cumulative recovery when 100.0 cc of fluid had been recovered from each pack (Figure A-4). The oil production decreased behind the oil bank and is indicated by the flattening of the cumulative recovery curves for the two packs.

Packs 3.7 and 3.8, flooded with Solution C had the highest recoveries at 22.0 % of residual oil each. PHPA made the mobility ratio between the surfactant and displaced oil favorable^{7,8} and the co-surfactant increased the optimal salinity of the surfactant⁶. It is significant that 22% of the residual oil was recovered with a very low bio-surfactant concentration, 0.0043 % by weight (43 ppm). The small difference in oil recoveries between packs 3.5 and 3.6 treated with Solution B and packs 3.7 and 3.8 can lead the reader to infer that 2,3-butanediol is not needed and bio-surfactant acting in treated with Solution C, suggested that 2,3-butanediol addition may not be critical. However, the presence of a water-soluble alcohol is known to raise the optimum salinity of the surfactant solution and bring it closer to the brine salinity⁵. The combination of a viscous preflush and the co-surfactant may

have helped the bio-surfactant solution approach its optimum IFT at the salinity inside the sand packs.

Pack	Solution	Residual	Residual oil	Recovered	Recovery*
	Турс	V _{O,Rwf}	Sor,wf		
#	(100.0 cc)	(cc)	(%)	(cc)	(%)
3.1	Solution D	25.0	26.7	0.3	1.20
3.2	Solution D	19.0	21.7	0.2	1.05
3.3	Solution A	19.0	19.8	3.0	11.1
3.4	Solution A	17.0	18.2	2.9	13.0
3.5	Solution B	21.0	23.4	4.1	15.5
3.6	Solution B	18.0	23.4	4.2	18.8
3.7	Solution C	23.0	24.7	6.2	21.9
3.8	Solution C	22.0	25.3	4.8	21.8

 Table A-4. Results of Experiment 3





Conclusions.

- 1. It has been shown that *Bacillus mojavensis* JF-2 bio-surfactant requires a co-surfactant (2,3-butanediol) and a viscosity modifier (PHPA) to recover oil from a saline environment.
- 2. A very low bio-surfactant concentration, 43 ppm (0.0043%), recovered nearly 22% of the residual oil.
- 3. The viscosifying agent improved sweep and recovery efficiency.
- 4. The co-surfactant, 2,3-butanediol, helped the surfactant approach its optimum IFT at a given salinity.

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