AN EXPERIMENTAL STUDY OF MICROBIAL IMPROVED OIL RECOVERY BY USING *RHODOCOCCUS* SP. 094

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ABSTRACT

Core laboratory experiments were conducted on Berea sandstone core plugs to study the mechanisms by which *Rhodococcus* sp. 094 is able to enhance oil recovery during or after waterflooding. To obtain bacterial suspensions with biosurfactant production, the bacterium was grown on media with dodecane as the sole carbon and energy source. Suspensions without biosurfactant producing bacteria were produced by cultivating the bacteria with acetate, instead of dodecane. Cores were then flooded continuously with each type of bacteria, both with and without a previous waterflooding. It was apparent that the most important mechanisms for the bacteria are interfacial tension reduction (through biosurfactants and bacterial activity in the brine-hydrocarbon interface), selective plugging and changes of wettability. Some gas production was observed, but its effect on the final recovery appeared to be very small.

INTRODUCTION

Microbial Improved Oil Recovery (MIOR) processes have been studied since Beckmann introduced the idea in 1926, and ZoBell conducted experiments that showed potential for microbial oil recovery [1]. MIOR has been studied in many different places throughout the world, with varying results, due to the diversity of the methods employed, the differences of the bacteria used, and the uncertainties present in reservoir engineering. In MIOR, microbes are used as a mean to increase the quantity of oil recovered from a reservoir. Some advantages of using MIOR methods over other IOR methods are: (a) lower cost, (b) broader applicability, (c) the ability to produce in-situ the chemicals needed for the process, (d) required materials are widely available and economic.

Some of the suggested mechanisms by which the bacteria enhance the oil recovery are: reduction of interfacial tension (IFT) (lowering capillary pressure), change of wettability, gas production and selective blockage of the more permeable pore channels (changing the flooding pattern).

Laboratory experiments have been carried out injecting *Rhodococcus* sp 094 in Berea core plugs. These experiments consisted in flooding processes using brine followed by bacterial suspensions and bacterial suspensions with no previous brine flooding. Bacterial

suspensions with and without biosurfactant were tested. *Rhodococcus* sp 094 is an alkane oxidizing bacteria, that has been shown to be able to clean oil contaminated environments due to its ability to form extremely stable crude oil in water emulsions [3]. This is one of the main MIOR mechanisms, so combined with its availability and knowledge to the authors, made the use of this bacteria a straightforward choice.

REVIEW OF SUGGESTED MECHANISMS

There are many different mechanisms by which bacteria can increase oil recovery from a reservoir. Usually more than one of these mechanisms will be in action for a given bacteria assuming it has access to nutrients, a carbon source and oxygen (for aerobic bacteria), and that the environmental conditions are favourable for the bacteria's development. Understanding these mechanisms, how they help increase oil recovery, and how the bacteria produce them is the key for developing efficient MIOR technology. Suggested mechanisms are:

Reduction of Interfacial Tension

This effect has been reported in both field [4] and laboratory [5, 6, 7] investigations, and is believed to be one of the main contributors in MIOR processes. The bacteria live in the aqueous phase and need to get access to the oil, which serves as the carbon source. To ease this access, the bacteria need to overcome the interfacial tension (IFT) between these phases. It is well known that some bacteria produce surfactants. Earlier studies with *Rhodococcus* sp 094 have shown that the biosurfactants are associated with the cells i.e. the presence of bacteria at the interface is required for lowering the IFT [3]. Experiments have been conducted where the injection of bacterial metabolites alone give a minor recovery than the combined injection of bacteria and its metabolites [8].

Changes in Wettability

Different studies have found wettability changes both towards more water wet [9] and towards more oil wet [10] as a consequence of bacterial applications, depending on properties of both the rock, the fluids and the metabolites. Changes in wettability will change the drainage / imbibition processes.

Changes in Flow Pattern

Changes in the flow pattern can be caused by bacterial plugging of the pore space and by biopolymers. This effect has been studied thoroughly and it is acknowledged as a very important MIOR mechanism [11, 12, 13, 14, 15]. Some of the experiments that serve as a proof of this mechanism taking place, include: Increase with time of the pressure drop along a core after bacterial inoculation, visualization of plugged fractures with a scanning electron microscope [16], permeability profiles along cores [17], and the movements of bacteria and metabolites by liquid samplings along the core during flooding [8]. At the beginning of the bacterial flooding, the bacteria present in the water phase will flow through the largest pore channels with greatest ease. This is also where nutrients will be most abundant. Therefore, the bacterial growth rate will be directly proportional to the effective rate of water flowing through a given pore channel. Bacterial adhesion and

growth will lead to a reduction of the effective flow area of the pore channel, which will eventually make the water deviate through other previously unswept (or poorly swept) flow paths. Consequently the sweep efficiency will be improved, even in cases where there has been water fingering, making possible the displacement of bypassed oil. Field tracer tests support this theory [18]. Bacterial slime can also contribute to this mechanism [19].

Additional Mechanisms

Another mechanism that might be of interest is the CO_2 production, which helps by re-pressurizing the reservoir and reducing oil viscosity [19, 20, 21]. However, it is limited by the amount of oxygen supplied. Other produced gases that help re-pressurize the reservoir have been reported. Additional proposed mechanisms are: acid production, which can increase porosity in carbonate reservoirs [15, 20, 22, 23]; biopolymer production, which can increase the brine viscosity, thus increasing the displacement efficiency [19, 22], modify permeability [20, 24], and cause selective plugging [19, 24]; solvent production [19, 22]; and degradation of long chain hydrocarbons, reducing oil viscosity [22, 25].

MATERIALS AND METHODS

Materials

Core Samples

Berea and Bentheimer porous sandstones were employed initially. However, after the first tests it was decided not to further use Bentheimer due to its very high permeabilities and only Berea was used as it had the desired permeabilities. The porosity of Berea samples varied between 18.1% and 23.5%, and the permeability from 146 md to 288 md. Churcher, *et. al* [2], determined that for this range of permeabilities and porosities, the pore size range would be around 50-125 μm for the low values of porosity and permeability and 50-160 μm for the high values, with an average pore size between 79 and 96 μm, respectively.

Brine and Hydrocarbon

The composition of the brine used for saturating the cores and conducting the waterfloods was the same as that for the dodecane medium omitting dodecane (see Table 1). The hydrocarbon used to saturate the cores was dodecane ($CH_3(CH_2)_{10}CH_3$).

Table 1 Growth media

		Medium concentration		
Component	***Formula***	***Dodecane***	***Acetate***	***Pre-culture***
Ammonium Chloride	NH_4Cl	0,6 g/L	4,6 g/L	4,6 g/L
BICINE		10,0 g/L	10,0 g/L	10,0 g/L
Magnesium sulfate-7-hydrate	$MgSO_4 \times 7H_2O$	0,05 g/L	0,05 g/L	0,05 g/L
Calcium sulfatedihydrate	$CaSO_4 \times 2H_2O$	0,21 g/L	0,21 g/L	0,21 g/L
Potassium Chloride	KCl	0,20 g/L	0,20 g/L	0,20 g/L
Sodium Chloride	NaCl	30,0 g/L	30,0 g/L	
Phosphate stock solution [1]		5 mL/L	5 mL/L	5 mL/L
Trace mineral stock solution [2]		5 mL/L	5 mL/L	5 mL/L
Sodium Acetate	$NaCH_2COOH$		6,83 g/L	6,83 g/L
Dodecane	$CH_3(CH_2)_{10}CH_3$	5,0 g/L		

Core Flooding Apparatus

Three Hassler type cells were arranged inside of an oven to keep the temperature at 30 ^oC (Figure 1). A water driven vacuum pump and a pressurized nitrogen flask with a system of valves were used to control the sleeve pressure. Three dialysis pumps were used to pump the injected fluids, with produced fluids being collected in 500 ml graduated cylinders with a 10 ml pipette inside, allowing a high resolution in the collected dodecane reading.

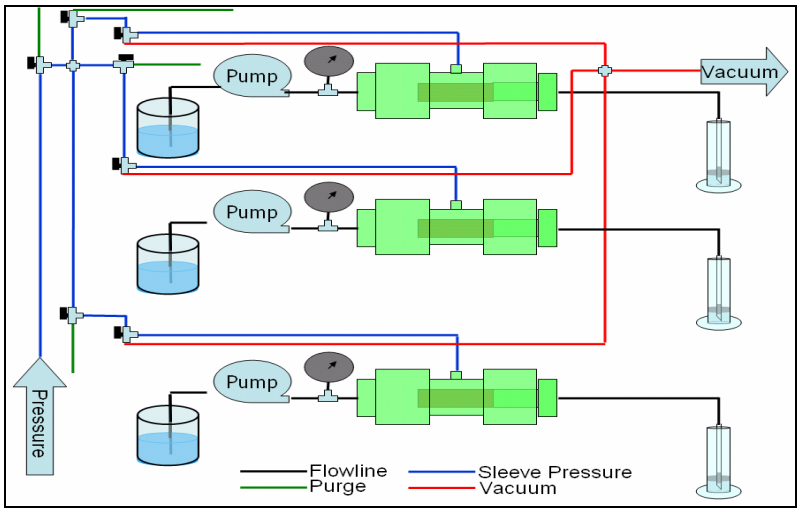

Figure 1 Three test trains experimental setup

[1] The phosphate stock solution contained a mixture of 1M $K_2HPO_4 \cdot 3H_2O$ and 1M KH_2PO_4 in a ratio of 8:1.

[2] The trace mineral stock solution contained ($g \cdot L^{-1}$ distilled water): $ZnSO_4 \cdot 7H_2O$ 0.5; $FeSO_4 \cdot 7H_2O$ 0.5; $MnSO_4 \cdot 5H_2O$ 0.5; and concentrated H_2SO_4 1.0 $mL \cdot L^{-1}$.

Methods

Bacteria Cultivation and determination of residual dodecane

Cell suspensions containing 20% glycerol and stored at -80ºC were used to inoculate a sterile pre-culture medium (Table 1). Pre-cultures were incubated overnight to the stationary growth phase. Bacteria used for flooding were grown on either acetate or dodecane as sole carbon and energy source. The compositions are given in Table 1. Cells grown on acetate and dodecane were incubated for 48 and 72 hours, respectively. Stationary phase cells were diluted 1:3 with brine. Viable cell counts of the diluted bacterial suspensions were obtained on agar plates. All media were sterilized by autoclaving and the pH was adjusted to 8.2. Bacteria were grown in 500mL shake flasks containing 100 mL medium, which were placed on a reciprocating shaking machine with 180 movements·min^{-1} and an amplitude of 5 cm. The incubation temperature was 30ºC.

Residue alkane in bacterial suspensions grown on dodecane was determined by hexane extraction and gas chromatography. Triplicate samples (1mL) were shaken vigorously with 1mL hexane for 4 hours at 30ºC. After 20 hours of static storage the hexane phase was transferred to glass vials, and analyzed using a gas chromatograph.

Core Preparations

Before conducting the flooding experiments the cores were cleaned in a soxhlet apparatus, using methanol and toluene. Then the cores were dried at 60 ^{o}C for at least 24 hours. The core dimensions, dry weights, porosity and Klinkenberg corrected absolute permeabilities were determined. The cores were then saturated with brine and the brine saturated porosity was determined. Liquid permeability and injectivity were determined. Dodecane was injected at increasing rates. Irreducible water saturation (S_{wi}) values of 35% and 40% were established by injecting at maximum rates of 50 ml/min. The produced volumes were recorded together with the corresponding time and pressure. The saturation was corroborated by weighing the dodecane and brine saturated core.

Core flooding

These operations constitute the central part of the study. The cores have been subjected to 3 different processes, once for the surfactant producing (SP) bacteria, and once for the non surfactant producing (NSP) bacteria:

- Waterflooding (WF), conducted until no more oil was recovered from the core.
- MIOR after WF process ($MIOR_{WF}$), the core was bacterially flooded (BF) starting at the residual oil saturation after waterflooding (So_{rWF}).
- MIOR without previous WF ($MIOR_0$) the core was BF from initial oil saturation (S_{oi}).

For each of these tests the core was placed inside the coreholder and the displacing fluid (water or bacterial suspension) was injected at a rate of 0.1 ml/min. The produced volumes and pressures were measured regularly. After each flooding the core was weighed again. In the case of the $MIOR_{WF}$ process the BF was started immediately after the WF. After the MIOR processes (both $MIOR_0$ and $MIOR_{WF}$) the cores were cleaned and dried for reuse.

Interfacial Tension and Wettability

A digital ring tensiometer was used for measuring the surface tension of the brine, dodecane and bacterial solutions, and the IFT between the brine and dodecane and between the bacterial solutions and dodecane. The contact angle and wettability for the same systems on quartz blocks were determined by the contact angle imaging method.

RESULTS AND DISCUSSION

The density for dodecane at the studied temperatures was 0.75 kg/l, and its viscosity 1.47 cp. There was not measurable variation in density and viscosity for the different aqueous media used as Brine, the SP bacteria suspension and the NSP bacteria suspension had densities of 1.03 kg/l and viscosities of 1.04 cp. The IFT and wettability of the studied systems are shown in Table 2. These systems are: brine-dodecane (Brine-C_{12}), SP bacteria suspension-dodecane (SPB-C_{12}) and NSP bacteria suspension-dodecane (NSPB-C_{12})

Table 2 Interfacial Tension and Wettability Data

	Interfacial Tension [mN/m]			Time [s]	Contact Angle [^{o}]		
System	Initial reading	Slope [s^{-1}]	Final reading		Instant	After 15 hours	Wettability
Brine – C_{12}	41.7	$-4x10^{-4}$	41.4	570	62	62	Water wet
SPB – C_{12}	22.6	$-6x10^{-4}$	19.7	4555	80	Lens	Intermediate
NSPB – C_{12}	24.3	$-4x10^{-4}$	23.2	2722	74	79	Intermediate

As expected the IFT is lower for the SP bacteria - dodecane system, than that of both the brine and the NSP bacteria in contact with dodecane. The continuous measurement of IFT shows that it decreases with time, and that the rate of reduction of the IFT through time is higher for the SP bacteria than for the NSP bacteria. However, the formation of a stable emulsion for the bacterial solution, points towards even lower IFTs. It is expected that the obtained values would continue to decrease, as the bacterial activities at the interface increase. Due to instrument availability limitations, these tests were not conducted for long enough times (while the core floodings last for up to one month, the IFT measurements could not be conducted for much more than one hour), and they continued to show a decreasing IFT slope when the tests were ended. For these values to be similar to the IFTs achieved in the coreflooding experiments, the times of the tests would have to be similar.

The wettability measured in quartz plaques changed towards less water wet when in presence of both NSP and SP bacteria. The change was slightly stronger on the SP bacteria. This made the wettability more neutrally wet, which could be a contributing factor to the increased oil recovery, and it serves as evidence that this is one of the mechanisms that can be triggered by *Rhodococcus* sp 094. However, as the used cores are strongly water wet, the effect of this mechanism is believed to be low.

The first core flooding experiments were conducted to determine whether there was a measurable effect of flooding with the bacteria and if it was limited by the permeability

(mostly as a consequence of the pore size). The Hassler cell was placed horizontally, and the experiments were run at room temperature. The cores used in this test were Berea 6 and 20. Their properties are shown in Table 3, and the results in Table 4 and 5. These cores were WF and then BF ($MIOR_{WF}$). Then the cores were cleaned and resaturated and were BF ($MIOR_0$). Only surfactant producing (SP) bacteria were used.

Table 3 Properties of studied cores.

Core	Diameter [cm]	Length [cm]	Pore Volume [cm^3]	Porosity [%]	Permeability [mD]
Berea 6	3.76	7.62	19.91	23.5	279
Berea 20	3.71	6.15	13.80	20.8	288
Berea B1	3.71	11.95	24.18	18.7	146
Berea B2	3.71	11.75	23.02	18.1	148
Berea B3	3.71	14.14	28.85	18.9	160
Berea B5	3.71	11.59	24.29	19.4	144
Berea B6	3.71	14.04	28.79	18.9	177
Berea B7	3.71	11.54	23.75	19.0	154

Table 4 Results for $MIOR_{WF}$ bacterial flooding for first group of cores

	Waterflooding				Bacterial Flooding after Waterflooding					
	OOIP [cm^3]	PVi	Recovery		OIP_{WF} [cm^3]	PVi	Recovery			
Core	(So_i)		cm^3	%	(So_{rWF})		cm^3	% OIP_{WF}	% OOIP	% $_{WF+BF}$
Berea 6	13.3 (0.67)	5.3	5.5	41	7.8 (0.39)	12.3	0.4	5	3	44
Berea 20	8.8 (0.63)	7.2	5.1	57	3.7 (0.27)	10.1	0.3	7	3	60

OOIP = Original oil in place OIP_{WF} = Oil in place after waterflooding PV_i = Injected pore volumes

So_i = Initial Oil saturation S_{orWF} = Residual oil saturation after waterflooding

Table 5 Results for $MIOR_0$ bacterial flooding for first group of cores.

Core	OOIP [cm^3] (Soi)	PVi	Recovery [cm^3]	Recovery [%]
Berea 6	11.3 (0.57)	18.9	9.1	81
Berea 20	8.8 (0.64)	18.8	7.0	79

OOIP = Original oil in place

The next experiments were conducted in longer cores, sequentially named Berea B1 to B7. These tests were performed at 30 ^oC. Surfactant producing (SP) bacteria were tested against non-surfactant producing (NSP) bacteria. Up to 6 different flooding processes were carried out on each core: One water flooding followed by one bacterial flooding with SP bacteria ($MIOR_{WF}$), one bacterial flooding with SP bacteria without previous WF ($MIOR_0$), and the same processes for NSP bacteria. The coreholders were now held in vertical position, so that the process would resemble more a reservoir injection process.

The cores studied in the second group were: Berea B1, B2 and B3 (Table 3) the results are in Tables 6 and 7 and Figures 2, 3 and 4. During the microbial runs in this setup, the cores were flooded with approximately 600 ml of bacterial suspension (both for the $MIOR_0$ and $MIOR_{WF}$ processes), and afterwards with brine (indicated with circles in the figures), which might have limited the oil recovery. Corefloodings were held for much longer times. The results for these tests show that both the SP bacteria and the NSP

bacteria allow to recover much higher volumes of oil than standard WF, with fewer injected pore volumes, i.e., there is an increased sweep efficiency. This indicates that microbial improved oil recovery is not only due to biosurfactants.

Table 6 Results for $MIOR_{WF}$ bacterial flooding for second group of cores

	Waterflooding				Bacterial Flooding after Waterflooding						
Core	OOIP [cm^3] (So_i)	PVi	Recovery		OIP_{WF} [cm^3] (So_{rWF})	C_B [N^o/ml]	PVi	Recovery			
			cm^3	%				cm^3	% OIP_{WF}	% OOIP	% $_{WF+BF}$
B1 (SP)	14.9 (0.62)	21.3	8.5	57	6.4 (0.27)	$1x10^7$	5.7	0.5	8	3	60
B1 (NSP)	13.7 (0.57)	44.4	8.7	64	5.0 (0.21)	$4x10^8$	38.9	0.7	14	5	69
B2 (SP)	12.8 (0.55)	29.7	5.0	44	7.8 (0.33)	$9x10^7$	17.5	0.4	5	3	47
B3 (NSP)	12.4 (0.43)	16.2	8.1	65	4.3 (0.15)	-	47.9	1.2	28	10	75

OOIP = Original oil in place OIP_{WF} = Oil in place after waterflooding PV_i = Injected pore volumes
So_i = Initial Oil saturation S_{orWF} = Residual oil saturation after waterflooding
C_B = Concentration of bacteria

Table 7 Results for $MIOR_0$ bacterial flooding for second group of cores.

Core	OOIP [cm^3] (Soi)	PVi	Recovery [cm^3]	Recovery [%]	C_B [N^o/ml]
B1 (SP)	13.2 (0.55)	38.9	9.4	74	$9x10^7$
B1 (NSP)	12.4 (0.51)	64.2	8.4	68	$5x10^8$
B2 (SP)	12.8 (0.55)	43.8	7.3	57	$1x10^7$
B3 (NSP)	14.4 (0.50)	67.9	8.6	60	$4x10^8$

OOIP = Original oil in place PV_i = Injected pore volumes
So_i = Initial Oil saturation C_B = Concentration of bacteria

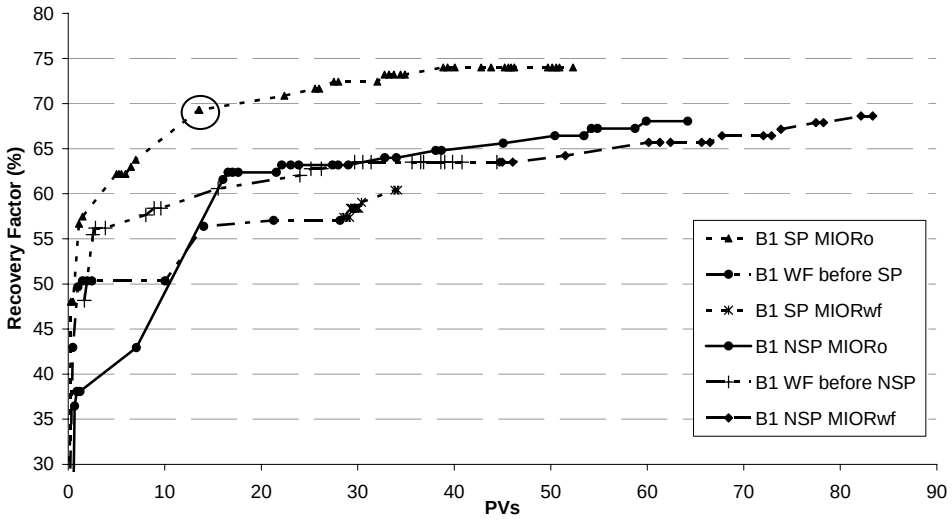

Figure 2 Recovery vs. Injected pore volumes for Berea B1

Berea B1 was the only core in the second group of cores having all the 6 possible tests. It shows a very clear superiority for the SP $MIOR_0$ process, with NSP $MIOR_0$ performing

slower than WF before NSP during the first PVi, but performing slightly better (also compared with NSP $MIOR_{WF}$) after30 PVs. This could be explained by the high concentrations of the bacterial suspensions used, the differences in saturations achieved, and the possibility of a deficiency in some of the cleanings.

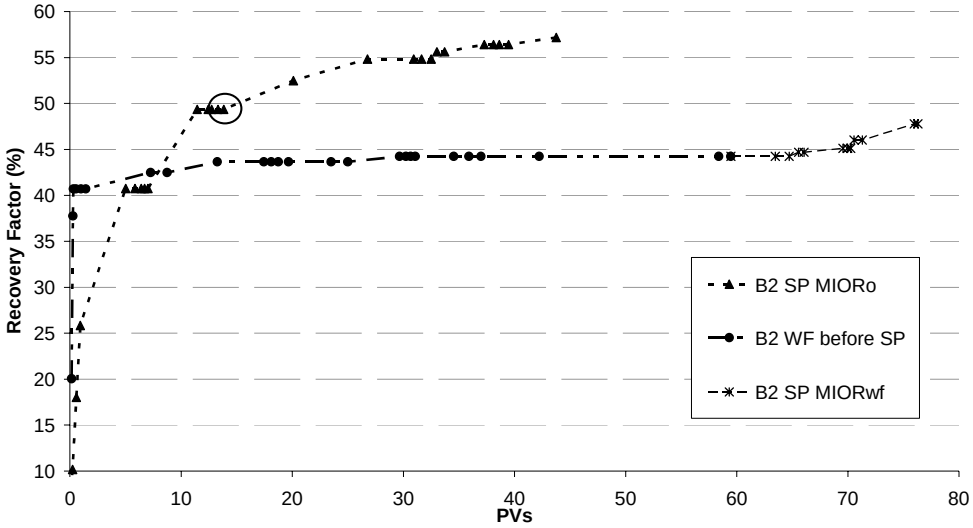

Figure 3 Recovery vs. injected pore volumes for Berea B2

Berea B2 indicates an SP $MIOR_0$ BF that is initially slower than WF, but that rapidly outperformed it and the subsequent SP $MIOR_{WF}$. Berea B3 showed an anomalous behaviour, with WF outperforming NSP $MIOR_0$ and the tests will have to be repeated under more standardized conditions

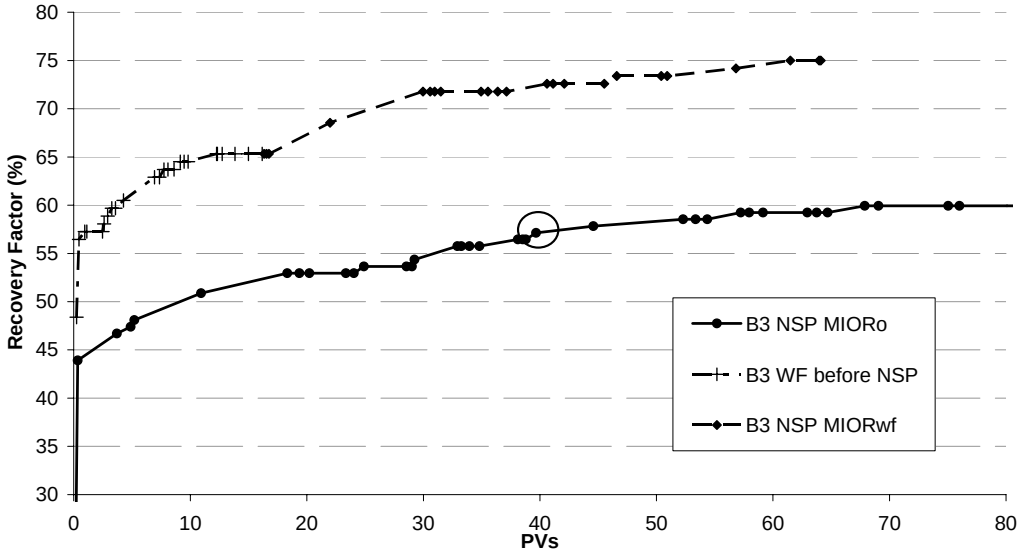

Figure 4 Recovery vs. injected pore volumes for Berea B3

The last setup, applied to cores Berea B5, B6 and B7 was made so that the cores under the bacterial flooding processes received fresh bacteria during the whole process. Another change is that by a change in the oil saturation method, the S_{wi} were established between 35% and 40%, so the initial conditions in the cores are more equal. The duration of the floodings was also reduced to 30 PVs. The properties of these cores are in Table 3, and the results are shown in Tables 8 and 9 and Figure 5, 6 and 7.

Table 8 Results from $MIOR_{WF}$ bacterial flooding for third group of cores

	Waterflooding				Bacterial Flooding after Waterflooding						
Core	OOIP [cm^3] (So_i)	PVi	Recovery		OIP_{WF} [cm^3] (So_{rWF})	C_B [N^o/ml]	PVi	Recovery			
			cm^3	%				cm^3	% OIP_{WF}	% OOIP	% $_{WF+BF}$
B5 (NSP)	15.2 (0.62)	26.6	6.9	46	8.3 (0.34)	$7x10^8$	9.6	0	0	0	46
B6 (NSP)	15.9 (0.55)	27.8	8.6	54	7.3 (0.23)	$9x10^8$	14.4	1.6	19	9	63
B7 (NSP)	14.8 (0.62)	42.2	11.4	77	3.4 (0.14)	$7x10^8$	76.6	3.1	91	21	98

OOIP = Original oil in place OIP_{WF} = Oil in place after waterflooding PV_i = Injected pore volumes
So_i = Initial Oil saturation S_{orWF} = Residual oil saturation after waterflooding
C_B = Concentration of bacteria

Table 9 Results from $MIOR_0$ bacterial flooding for third group of cores

Core	OOIP [cm^3] (Soi)	PVi	Recovery [cm^3]	Recovery %	C_B [N^o/ml]
B5 (NSP)	15.6 (0.64)	32.6	10.7	69	$9x10^8$
B5 (SP)	16.6 (0.68)	40.2	10.0	60	$1x10^9$
B6 (NSP)	18.0 (0.62)	123.0	16.2	90	$5x10^8$
B6 (SP)	17.2 (0.60)	53.0	14.5	84	$1x10^9$
B7 (NSP)	15.5 (0.65)	34.3	9.3	60	$9x10^8$
B7 (SP)	16.1 (0.67)	29.8	10.6	66	$1x10^9$

OOIP = Original oil in place PV_i = Injected pore volumes
So_i = Initial Oil saturation C_B = Concentration of bacteria

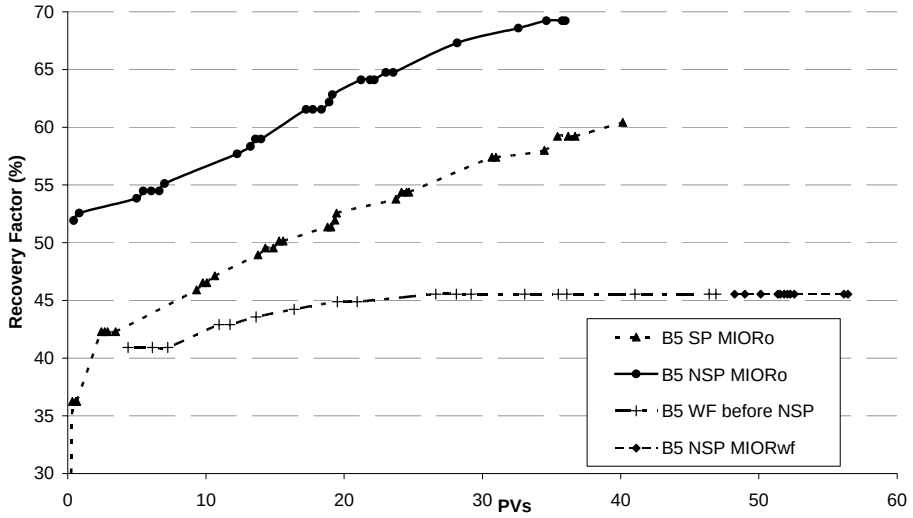

Figure 5 Recovery vs. injected pore volumes for Berea B5

Berea B5 shows NSP $MIOR_0$ as the most efficient method, over SP $MIOR_0$ and WF and NSP $MIOR_{WF.}$ However, the latter seemed to reach a peak production rapidly and did not respond to BF, so it would have to be repeated. The results seem to indicate there are cases where NSP bacteria can outperform SP bacteria, but further testing is necessary.

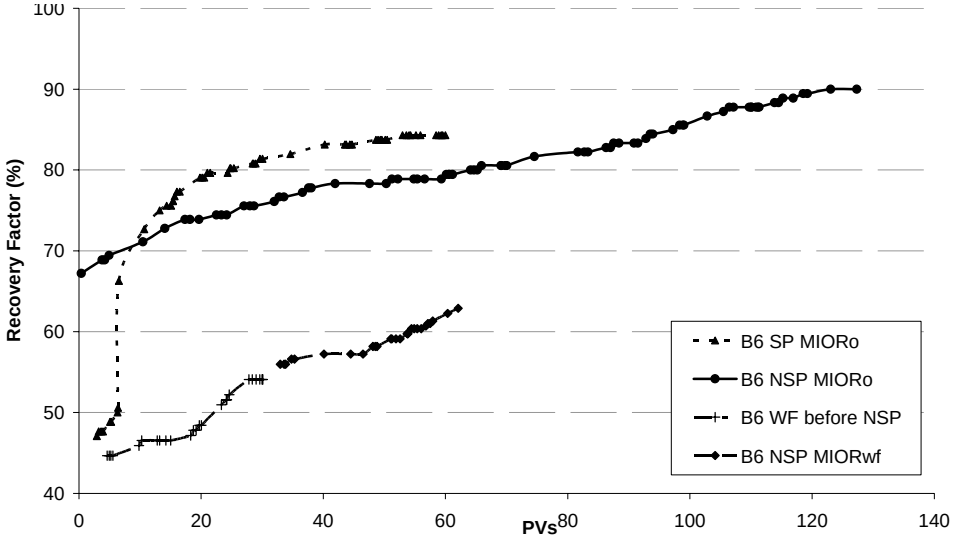

Figure 6 Recovery vs. injected pore volumes for Berea B6

Berea B6 showed an initially less efficient SP $MIOR_0$ that rapidly became more efficient than NSP $MIOR_0$. WF and NSP $MIOR_{WF}$ were the less efficient methods. Berea B7 shows a very similar initial production for both SP and NSP $MIOR_0$, but after 20 PVi SP becomes the most efficient of the two. However WF was far more efficient than both of them. This could be due to the high concentration of the bacteria causing clogging, or deficiencies in the cleaning process. Repetitions would be necessary.

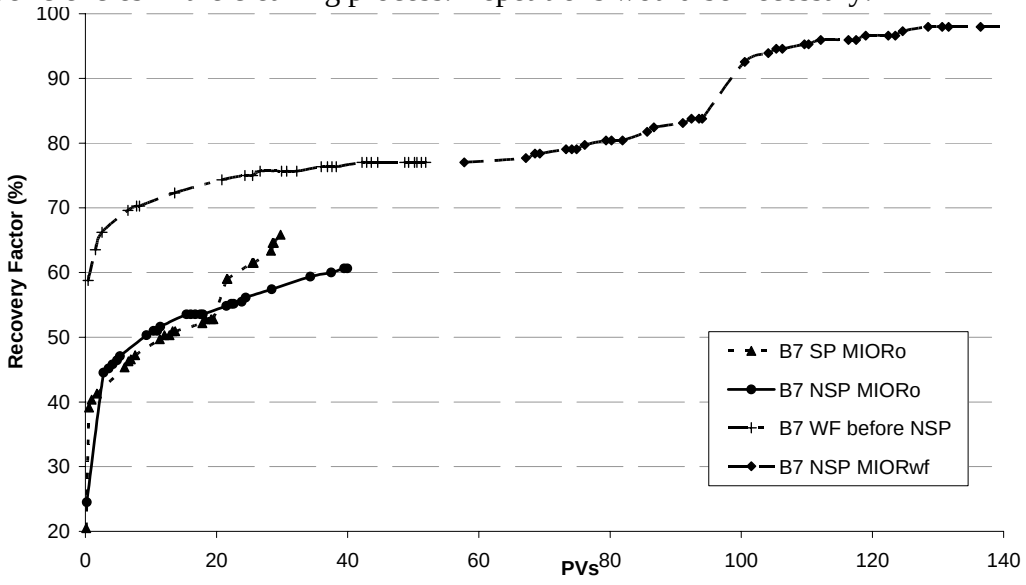

Figure 7 Recovery vs. injected pore volumes for Berea B7

The productions seemed to no longer reach a peak, as is evidenced on the NSP $MIOR_{WF}$ BF for core Berea B7, and on the NSP $MIOR_0$ BF for core Berea B6. However there was the previously mentioned exception with Berea B5. The other flooding experiments were interrupted not because there was no more production, but because it was decided that the experiments would run for about 30 PVi due to time limitations.

When these cores were flooded with NSP bacteria, the recovery was faster initially, but the recovery curves of the NSP and SP processes intersect, so that in the long run SP processes achieve higher recoveries. This can be seen clearly on cores B6 and B7 where the SP bacteria $MIOR_0$ floodings clearly outperforms the NSP bacteria, at the finishing PVi for the SP bacteria. This also shows that additional mechanisms are produced by the NSP bacteria or are stronger on the NSP bacteria than on the SP bacteria. These mechanisms seem to be more effective at the beginning of the process, but as oil saturations decrease, the biosurfactant becomes a more important factor, so that ultimately the SP bacteria would allow a higher recovery. The most likely mechanism to explain this would be selective plugging (pressure measurements during production in all the BF show a continuous increase with time, which seems to confirm the presence of this mechanism), which would sweep ever decreasing pore channels, until eventually the differential pressure available for the displacement would not be enough for this mechanism to displace the oil. Then the SP bacteria would have the advantage, as the lower IFT means a lower pressure differential is needed to displace the oil. However these tests were not long or numerous enough to reach conclusions, but there is a tendency that needs further investigation.

The highest recovery was achieved in the NSP $MIOR_{WF}$ BF in Berea B7 (Residual oil saturation, So_r, was reduced to 1%), but very close to the results given by the NSP $MIOR_0$ BF in Berea B6 (So_r was reduced to 6%). However these floodings endured more than 120 PVi, and the SP $MIOR_0$ BF for Berea B6 had a higher recovery than the NSP one until it was finished. As the results of Berea B7 contradict the rest of the experiments, it would have to be repeated, but these extremely low oil saturations appears to show that as long as there is still oil in place and bacteria with favourable conditions, it will be possible to continue producing. The bacteria drive a continuous process that is more efficient than regular IOR processes, because it occurs exactly where it is needed, in the interface between the oil and the water.

Most of the experiments for the three groups were consistent in that higher recoveries were achieved with the $MIOR_0$ BF than with WF or $MIOR_{WF}$ BF, and for a given number of PVi, more dodecane could be expected to be recovered by the $MIOR_0$ process than by the $MIOR_{WF}$, i.e. a faster recovery. An analysis of the So_r reduction throughout the process for the second and the third group of cores also suggests that the most efficient method appears to be $MIOR_0$ injection of the SP bacteria. However too few experiments have been conducted, there were some exceptions, and some experiments will have to be repeated.

The residual dodecane in the SP bacterial suspensions was used to determine how much the recoveries for SP BFs could have been exaggerated. This residual dodecane is emulsified in the bacterial suspension, and it is unknown if it will be produced with the dodecane as it flows through the core, or if it will remain in emulsion in the produced water, not affecting the recovery. The worst case was $MIOR_0$ BF for Berea B6 where the final recovery would have been reduced by 6%. The residual dodecane was 0.8 ml/l bacterial solution. All the other recovery reductions were below 3%, so residual dodecane in the bacterial suspension, if it has any effect, does not change the general results in comparison with the NSP process for the same cores.

During the bacterial processes the oil production was intermittent. This leads to believe that the mechanisms in action act continuously on the dodecane trapped in the unswept areas of the core, releasing it slowly, forming a bank of dodecane that eventually exceeds the critical dodecane saturation so that a small quantity of dodecane is produced. A similar mechanism was described by Yonebayashi and Ono [21] for the bacteria TRC-4118.

After the BFs bacterial cakes were observed in the intake of the cores. This must have increased the injection pressures, and as in drilling mud filtercakes, it must have filtered the injected suspension. No relationship between the concentrations of the bacteria and the ultimate recovery was found in the studied range from $1x10^7$ to $1x10^9$ cells/ml. This would be an expected consequence of the cake filtering the bacterial suspension effectively entering the core. An optimal concentration value is an important factor to determine in future investigations, to allow the highest recovery, without using more oxygen and nutrients than necessary, and also reducing or preventing the cake formation.

CONCLUSIONS

1. *Rhodococcus* sp. 094 has been successfully tested and applied as a model organism for both biosurfactant and non-biosurfactant aided microbial enhanced oil recovery.
2. The main production mechanisms triggered by *Rhodococcus* sp. 094 are IFT reduction, wettability changes and selective plugging. CO_2 production can be a minor mechanism.
3. Oil saturations were reduced to values as low as 1%, when given enough time, showing that MIOR processes are continuous processes that act on the oil interface and does not have the same limitations as other IOR methods.
4. An IFT reducing mechanism is also in action with the NSP Bacteria.

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