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Advances in Microbiology to Enhance Oil Recovery

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ABSTRACT

Microbial experiments to study enhanced oil recovery have generally been conducted using vegetative microorganisms and one-dimensional laboratory models. With the financial support of the Alberta Oil Sands Technology and Research Authority (AOSTRA), we have developed a procedure utilizing microorganisms called ultramicrobacteria (UMB) to assist in oil recovery by virtue of their growth properties. UMB or starved bacteria (.2–.3 μm) have the ability to penetrate deep into model rock cores and respond to nutrient stimulation by effectively plugging these matrices by cell growth and polymer production. Vegetative cells or full-size cells tend to form "skin plugs" at or near the well bore. Initial UMB core studies in 5-cm and 10-cm sandstone cores were one-dimensional with unidirectional flow. To simulate reservoir conditions, UMB experiments were conducted in a pressure vessel, 45 cm in diameter × 28 cm in height. In the first experiment a homogeneous sandpack was used, in the second a heterogeneous sandpack was used. Our experiments to date have shown (1) penetration of UMB, resuscitation upon nutrient stimulation, and reduction in permeability in homogeneous sand packs and (2) that resuscitated UMB preferentially plug the high-permeability zone by virtue of their growth properties and extracellular polysaccharide (polymer) production.

Index Entries: Selective plugging; permeability; ultramicrobacteria (UMB); extracellular polysaccharide (polymer); 45-cm simulator.

INTRODUCTION

Present oil recovery methods characteristically recover 8–30% of the total oil present in the reservoir. Secondary recovery methods, such as

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waterflooding, are used to increase recovery rates. The waterflood follows the path of least resistance, that is, the high-permeability zones, and pushes oil out along that path. These high-permeability barren zones now swept of oil are called "thief zones," since water continues to follow the same course, leaving lower-permeability zones unswept. A process commonly used to block the thief zones is known as selective plugging. Plugging agents currently in use involve the injection of solid or semi-solid fines dispersed in a liquid medium. If the particle size is too large, a skin plug develops at the injection well. A skin plug is a nuisance, because either it prevents injection of water into the unswept formation, or the water channels around the shallow plug and is again lost to the higher-permeability rock strata. Bacteria were first considered as a selective plugging agent by Crawford (*1,2*). Since that time, many research groups have experimented in their laboratories with vegetative microorganisms (*3–5*). Vegetative cells, however, have a tendency to form skin plugs even at permeabilities as high as 8 darcies. These full-size cells stick to surfaces because of their polymer production or are trapped in the pore spaces (*6,7*). To improve the depth of bacterial penetration, we applied a microbial ecology phenomenon first reported by Novitsky and Morita (*8,9*). Vegetative cells deprived of nutrients form small (.2–.3 μm) dormant nonadherent exopolysaccharide-free ultramicrobacteria (UMB). When stimulated with nutrients, these starved cells grow, reproduce, and produce exopolysaccharide (*10*). Our research group has been experimenting with UMB developed from oil-well water isolates as a potential biotechnical process for selective plugging. Initial experiments using scintered glass bead cores, sandpack cores, and sandstone cores of a variety of lengths and permeabilities confirmed uniform penetration, resuscitation, and plugging wth UMB (*11–13*). Although our results were encouraging, they were executed in one-dimensional laboratory models not representative of reservoir conditions. Ideally, a field study would prove our process successful; however, field studies are hindered by the complexity of the system, lack of experimental control, and time required for measurable results. The Alberta Oil Sands Technology and Research Authority (AOSTRA) decided to scale up the process. A project was set up under the AOSTRA Strategic Research Program, to conduct the work in a large scale physical simulator at the Alberta Research Council (ARC). Three advantages of this simulator are:

1. It can be packed and simulated to specific field conditions;
2. It is a three-dimensional model allowing for radial flow; and
3. It contains probes strategically located throughout the pack, which allows the experimenter to follow the pattern of flow.

This paper reports on two studies under simulated reservoir conditions. The first study, Run 1, was designed to establish the pattern of penetration and resuscitation, and the plugging profile of UMB in a fairly homo-

genous sandpack (*14*). As a result of Run 1, a second experiment was designed, Run 2, to study selective plugging in a heterogeneous rock stratum. In this experiment, the test pack consisted of a low-permeability sandstone core embedded in a high-permeability sand pack.

MATERIALS

UMB and Nutrient

A *Pseudomonas* species (FC3) was isolated from oil-well water and identified by standard microbiological methods. The organism was grown to stationary phase in sodium citrate medium (SCM) containing (/L distilled water); $NaC_6H_8O_7$, 6.45 g; $(NH_4)_2SO_4$, 0.198 g; KH_2PO_4, 2.72 g; K_2HPO_4, 5.23 g; $MgSO_4$, 0.12 g; $FeCl_3$, 0.008 g; pH 7.0. The cells were harvested by centrifugation (10,000×*g*, 15 min, 4°C) washed 3x in a phosphate-buffered salts solution (PBS) containing (/L distilled water) NaCl, 8.5 g; KH_2PO_4, 0.42 g; K_2PO_4, 1.23 g; pH 7.0, resuspended, and starved for 46 d. The final UMB solution contained 6.8×10^6 cells/mL. The nutrient injected to resuscitate the UMB was SCM.

Large volumes of UMB and SCM solutions were held in sterile carboys until injection. Equipment and/or solutions not sterilized in an autoclave (120°C at 15 psi for 20–35 min.) were sterilized in a gas sterilizer (Steri-Vac 202, 3M, St. Paul, Minnesota) with ethylene oxide gas for 4 h. On-site sterilization consisted of flushing with 5% (v/v) bleach followed by a distilled water rinse.

Equipment

The test simulator is a 45 cm×38 cm steel cylinder (Fig. 1) capped at each end with a blind flange. A piston located on top is used to apply a 400 psi (2758 kpa) overburden pressure and is separated from the test bed by a wax layer and metal sheets. A saturation distributor located at the bottom is used to saturate the test pack. The injection fluid is injected through a single injection point located at the top center. Eight sampling probes are located at various points in the pack (Fig. 2). Probe locations are such that probes 1 and 2 are closest to the effluent production point, probes 3, 4, and 5 are in the middle, and probes 6, 7, and 8 are closest to the injection point. Differential pressure between the injector and producer is measured using differential pressure transducers calibrated with nitrogen. The pressure and flow rate data are recorded on a Hewlett Packard HP86B computer. The injection fluid is pumped from the reservoir to the test pack with a positive displacement pump (Fig. 3) at a flow rate of 120 cc/min. Flow rates are periodically determined from effluent production collection rates. Effluent travels through the producer and is collected in a reservoir, where it is sterilized prior to disposal. Effluent samples taken from the production end are labeled as Probe 9 in the results section.

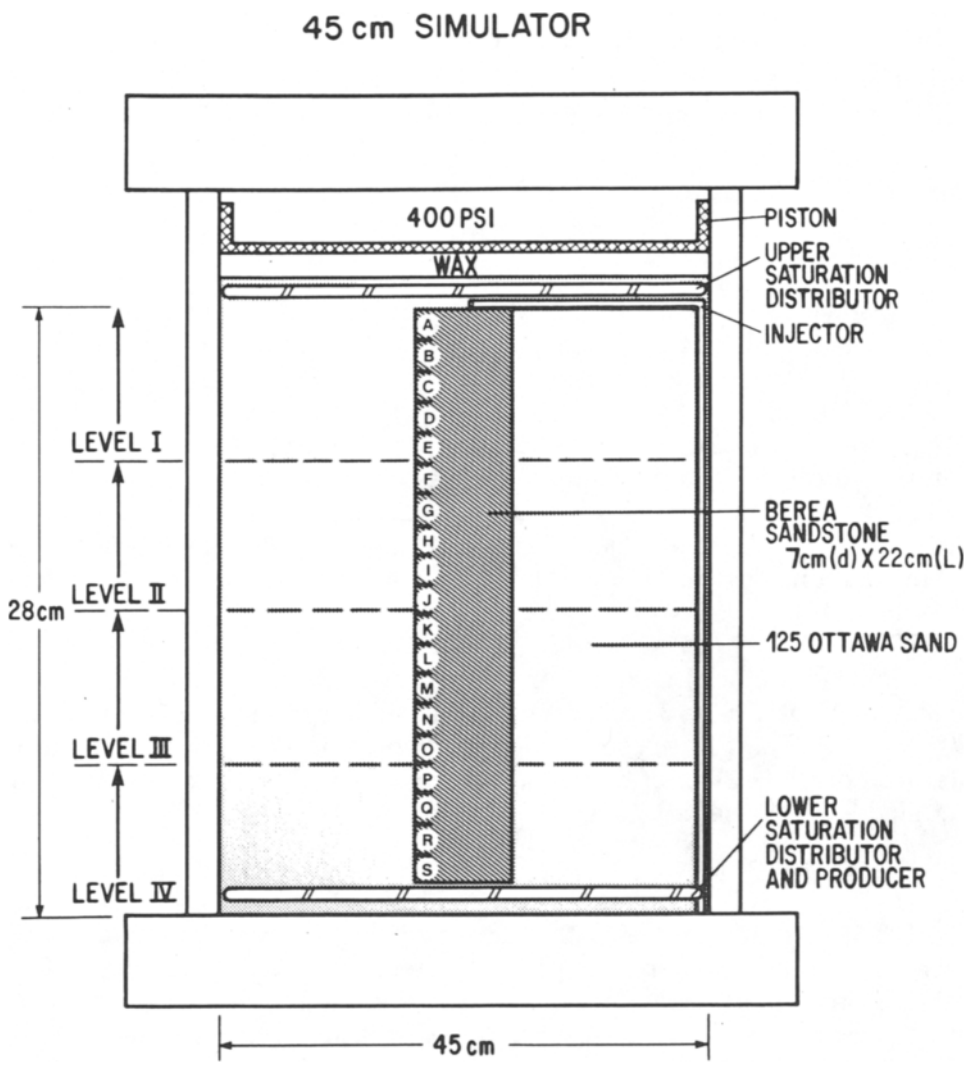

Fig. 1. Sandstone core and sand pack in the 45-cm simulator. A–Q are locations of sandstone samples.

Test Packs

In Run 1, the test pack is 65 kg of +125 mesh Ottawa sand. Porosity is 40% and permeability 3.8 darcies. In Run 2, the test pack is made up of a Berea sandstone core (Cleveland Quarries, Ohio) measuring 7 cm in diameter × 22 cm in length and embedded in +125 mesh Ottawa sand (Fig. 1). Porosity of the sand is 40% and permeability is 3.8 darcies; porosity of the sandstone core is 22% and permeability is 400 millidarcies. Both test packs are under an overburden pressure of 400 psi (2758 kpa). The test packs are saturated with filtered sterilized water (.45-μ millipore filter) under vacuum to stabilize mobile fines and to remove air pockets.

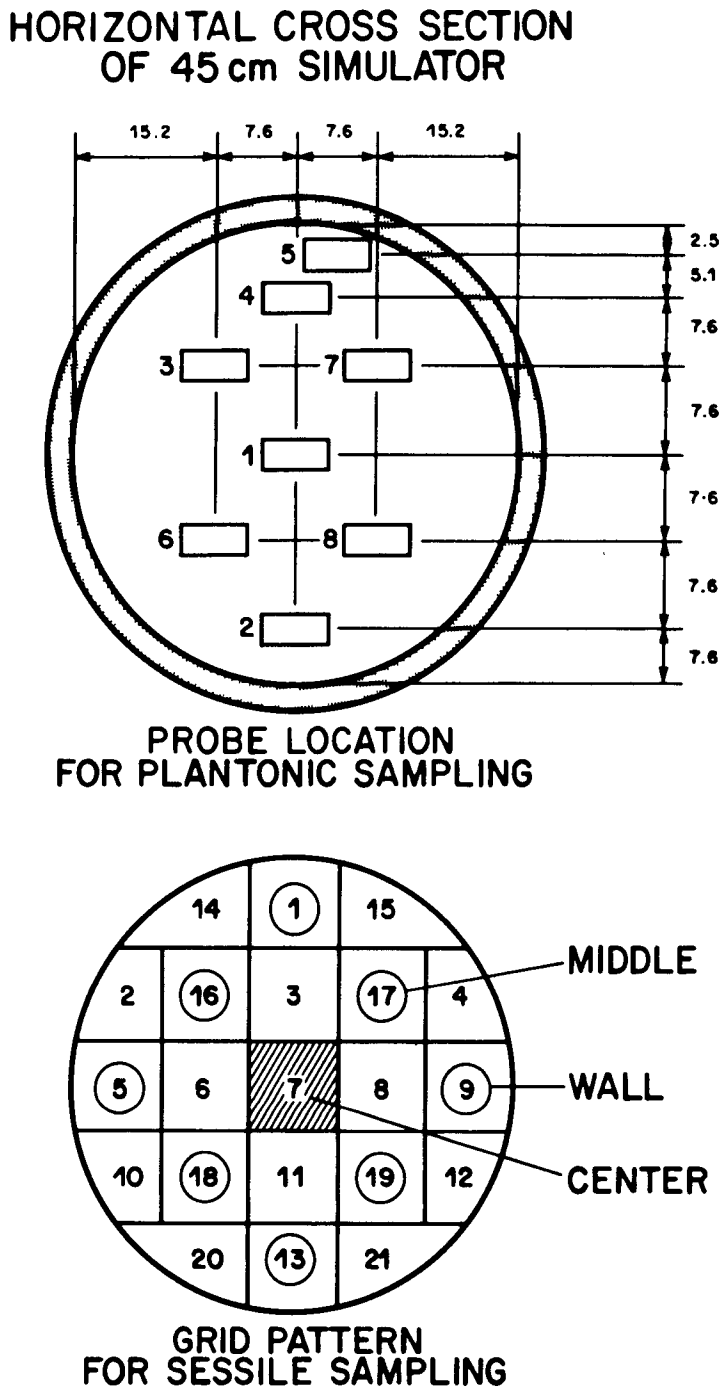

Fig. 2. A cross-sectional view of the 3-dimensional sand pack showing the planktonic sample probe locations and the grid pattern for sessile sampling.

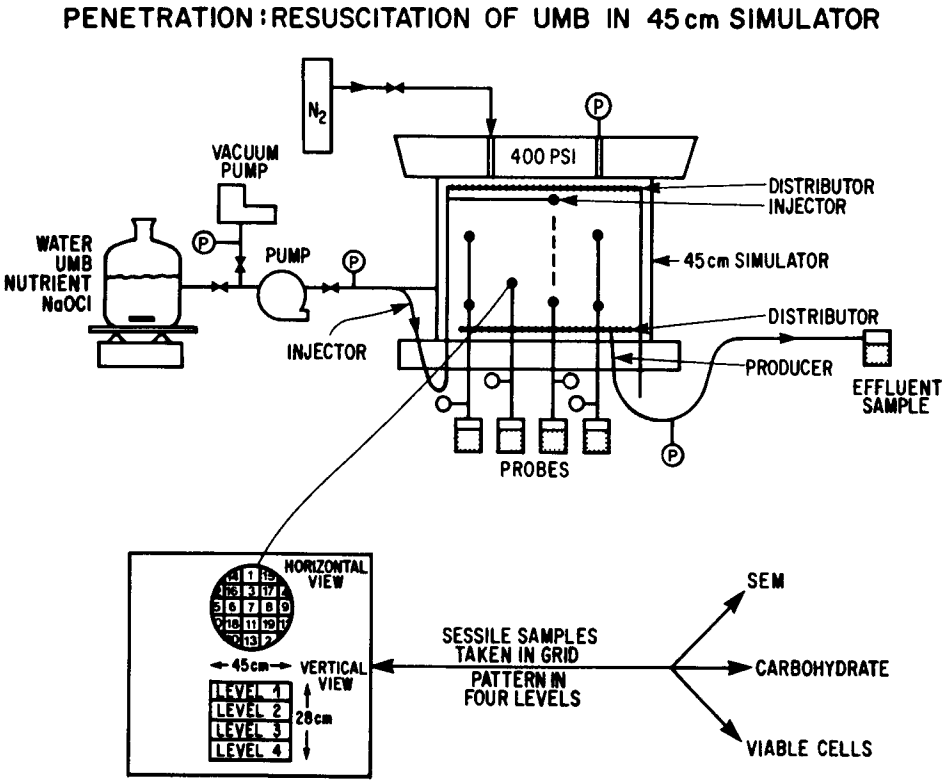

Fig. 3. A schematic diagram of the equipment used in Runs 1 and 2.

METHODS

In both experiments, 1.5 pore volumes (PV) of UMB followed by 1.0 PV of nutrient is injected from the reservoir to the test pack through the central injector on top of the simulator (Fig. 3). Additional nutrient is injected according to the schedule shown in Table 1. Planktonic samples are taken from all probes and the producer (effluent) during UMB and nutrient injection. Each sample is analyzed for viable cell numbers by counting colony-forming units (CFU), determined by spread plate dilutions from PBS onto Brain Heart Infusion agar (Difco) incubated at 22°C for 48 h. At 24 d in Run 1 and 42 d in Run 2, the test packs are dismantled in four vertical zones. Random sessile samples from each level are removed with a sterile scoop following the grid pattern in Fig. 2. The sandstone core in Run 2 is cut into smaller cores with a drill and bit (Ruska) approximately 5 cm in length. Each small core is cut into smaller sample pieces (A–S in Fig. 1), as described by MacLeod et al. (*11*). All samples are weighed and prepared for the determination of viable cell counts and extracellular polymer production (carbohydrate), and for scanning electron microscopy (SEM). Sessile viable cell counts are prepared as for planktonic samples.

Table 1
Schedule of Nutrient Injection and Pressure Readings

Run 1				Run 2			
Day	Nutrient, L	Injected PV[a]	DP[b] KPA	Day	Nutrient, L	Injected PV	DP, KPA
1	30	2.00	16	2	15	1	338
2	2	0.13	17	7	15	1	303
5	2	0.13	43	14	15	1	-
8	20	1.30	31	21	15	1	605[c]
10	2	0.13	137	35	15	1	152
11	2	0.13	149	42	15	1	165
12	2	0.13	133				
17	2	0.13	98				
18	30	2.00	110				
24	2	0.13	143				

[a]PV = Pore volume.
[b]DP = Differential pressure.
[c]High pressure is caused by plugging of the injection lines. This was cleared prior to incubation.

Samples are fixed in 5% (v/v) glutaraldehyde, dehydrated in ethanol (30%–100%) and freon (30%–100%), and then coated with gold palladium for SEM. Extracellular polymer production is measured against a glucose standard following methods described by Dubois et al. (*15*). Samples not processed on site are placed in sterile whirlpack bags (Nasco) and refrigerated until analysis.

Samples of clean sand and sand inoculated with UMB only were processed for SEM as controls for comparison purposes (Fig. 4).

RESULTS

Run 1

Pressure

Differential pressure (DP) readings increased as the bacteria grew and produced extracellular polymer. Initial DP was 8 kPa and increased to a maximum of 149 kPa on d 11, two days after the second nutrient injection of 1.5 PV into the test pack (Table 1). DP decreased to 98 kPa on d 17, then increased to 143 kPa on d 24. An increase in DP is directly proportional to a reduction in permeability. Fluctuations in DP were attributed to flow channeling.

Planktonic Samples

Samples taken during UMB and nutrient injections demonstrate uniform penetration and resuscitation of UMB through the entire test pack.

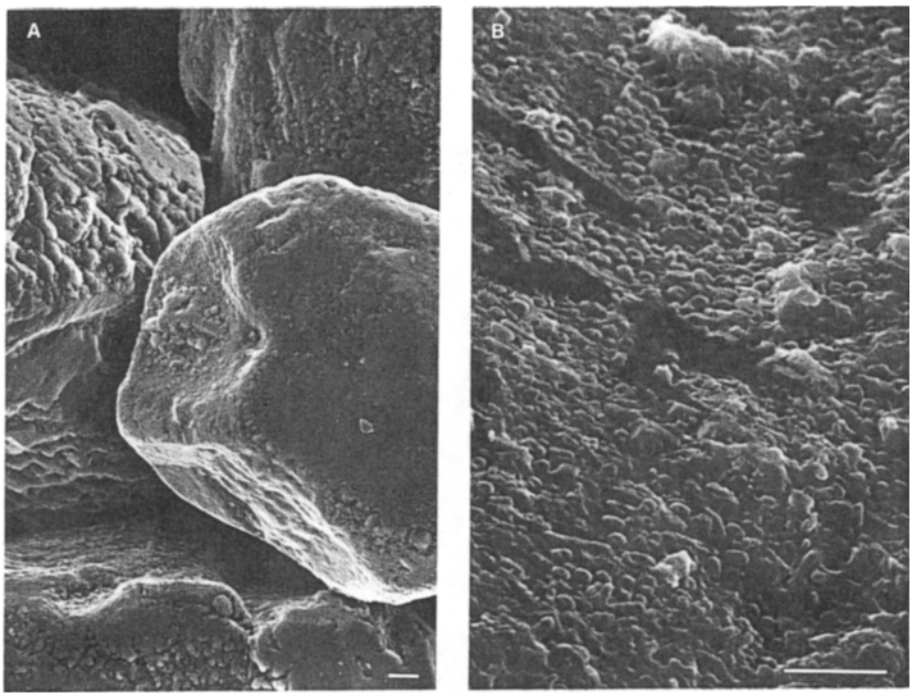

Fig. 4. Scanning electron microscopy of (A) clean sand, and (B) UMB trapped in the sand. Scale bar represents 5.0 μm.

Cell counts of 10^5 CFU/mL were detected in all fluid samples taken from the eight probes and the producer at 1 PV of UMB injection (Table 2). Viable cell counts increased from 10^5 CFU/mL to 10^9 CFU/mL over the 24-d test period when nutrient was injected.

Sessile Samples

Healthy vegetative bacterial growth and polymer production throughout the test bed is confirmed in data presented in Fig. 5A and B. Carbohydrate production was uniform in all levels, whereas viable cell numbers were highest in levels 1 and 2 with counts ranging from 1.0×10^6 CFU/gram to 5.0×10^8 CFU/gram. These results suggest that maximum CFU counts are not required to yield maximum carbohydrate production. SEM photographs show healthy cells encased in extracellular polymer in all four levels of the test pack.

Run 2

Pressure

DP readings increased dramatically from 7.68 kPa to 438.8 kPa during UMB injection. The high-pressure reading is likely a direct result of UMB being pushed through the low-permeability sandstone core. Injection of

Table 2
Planktonic Samples, CFU/mL

Run 1	Sample probe	UMB 1 PV	SCM 1 PV	SCM 2 PV	SCM 6 PV
	1	4.5×10^5	2.1×10^6	7.1×10^7	2.9×10^8
	2	5.0×10^5	3.3×10^6	1.6×10^8	7.3×10^8
	3	2.7×10^5	3.2×10^6	2.0×10^7	2.3×10^8
	4	1.8×10^5	2.2×10^6	4.2×10^7	7.3×10^9
	5	6.5×10^5	3.0×10^6	1.2×10^7	3.1×10^8
	6	6.5×10^5	2.7×10^6	4.4×10^7	7.3×10^9
	7	7.0×10^5	2.8×10^6	9.1×10^7	7.3×10^9
	8	4.0×10^5	2.9×10^6	5.1×10^7	7.3×10^9
	9	2.4×10^5	2.6×10^6	4.0×10^7	3.5×10^8
Run 2	Sample probe	UMB 1 PV	SCM 1 PV	SCM 2 PV	SCM 6 PV
	1	9.0×10^4	4.4×10^6	3.2×10^8	1.1×10^9
	2	3.0×10^5	1.5×10^6	3.2×10^8	1.1×10^9
	3	3.2×10^5	2.5×10^6	2.7×10^8	6.6×10^9
	4	2.6×10^5	2.5×10^6	2.9×10^8	1.6×10^9
	5	2.1×10^5	1.5×10^6	3.7×10^8	1.1×10^9
	6	3.2×10^5	1.6×10^6	3.5×10^8	2.3×10^9
	7	5.4×10^5	2.7×10^6	3.8×10^8	1.3×10^9
	8	2.4×10^5	1.8×10^6	3.2×10^8	2.3×10^9
	9	2.0×10^5	1.9×10^6	3.5×10^8	2.6×10^9

UMB = Ultramicrobacteria.
SCM = Sodium citrate media.
Sample No. 9 is effluent from producer.

the first slug of nutrient reduced the DP to 338 kPa as some of the UMB were dislodged from the test pack. DP readings remained high over the first 4 wks, then dropped to 165 kPa over the final 2 wk (Table 1).

Planktonic Samples

Viable cells were found in high numbers in all samples taken from each probe. Table 2 shows cell counts of 10^5 CFU/mL after injection of 1.0 PV of UMB increased to 10^9 CFU/mL after injection of 6 PV of nutrient over a 6-wk period. Data suggest that viable cells are located in the high- and low-permeability zones in the test bed.

Sessile Samples

Sand and sandstone samples analyzed for viable cell counts and carbohydrate production (Figs. 5C, 5D, and 6) indicate preferential plugging in the highly permeable sand, since the carbohydrate results are much higher in this zone. Cells deposited in the low permeability sandstone survived,

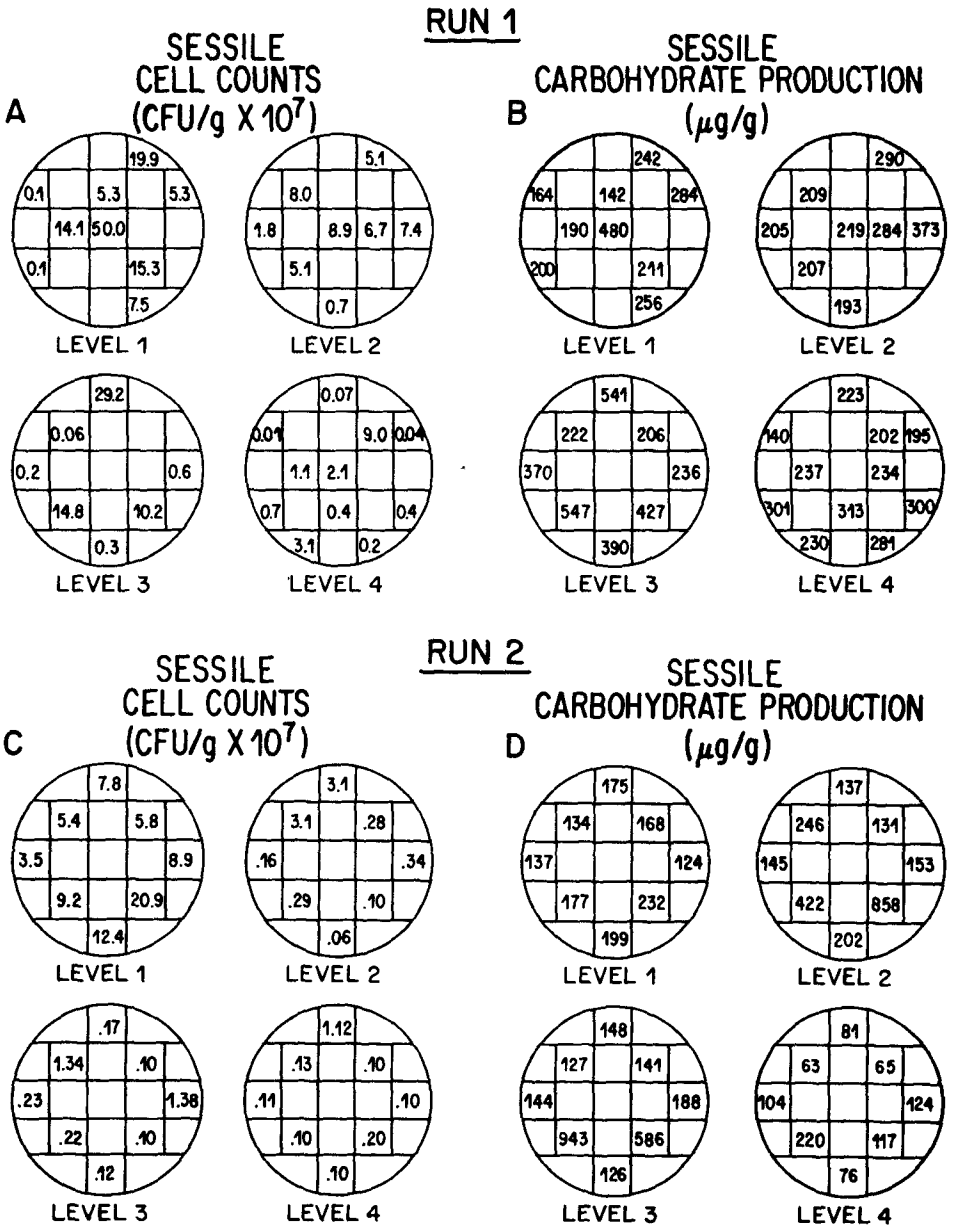

Fig. 5. Diagrammatic representation of viable cell counts and carbohydrate production of the attached sessile population in the homogenous test pack (Run 1) and in the heterogeneous test pack (Run 2).

but did not produce large amounts of polymer. The nutrient followed the path of least resistance through the high-permeability sand, where it was utilized by the cells in the sand to produce polymer. Micrographs (Fig. 7) show cells and extracellular polymer in all four levels of the highly permeable sand. Few cells and no polymer are visible in the low-permeability sandstone.

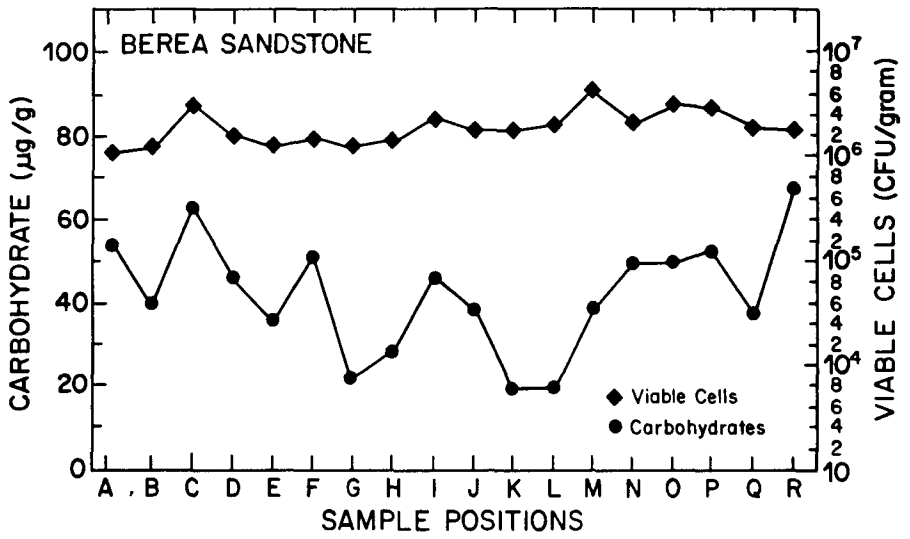

Fig. 6. Graphic representation of viable cell counts (Δ) and carbohydrate production (o) of the sandstone sections in Run 2.

DISCUSSION

Microorganisms have been studied as potential plugging agents because of their ability to produce stable extracellular polysaccharide slimes. For successful microbial-enhanced oil recovery, a deep plug is required. Moses (*16*) thought it necessary to eliminate polymer formation until the bacteria were in the target zone. Penetration of bacteria into the reservoir rock matrix can be achieved with UMB. A nutrient injection will "wake up" the UMB, and *in situ* plugging will result. Initial studies in our laboratory with UMB were executed in unidirectional model cores. Bubela (*17,18*) expressed the importance of reservoir modeling because field studies are limited by a complexity of systems, and laboratory studies are sometimes limited by oversimplification.

Results of experiments presented in this paper were acquired in a three-dimensional oil-field simulator designed by the Alberta Research Council. The experimental protocol was developed to use *in situ* nutrient resuscitation of UMB to produce deep bacterial plugging under simulated reservoir conditions. In Run 1, the unconsolidated sand pack, results support our previous laboratory studies. The UMB penetrate the entire three-dimensional area, as shown in the samples taken from the strategically positioned probes during UMB injection. Nutrient stimulation resuscitated the cells, which resulted in cell growth and extracellular polysaccharide production. The effect was a reduction in permeability throughout the sand pack without the formation of a skin plug. In Run 2, the heterogeneous test pack of high-permeability unconsolidated sand and low-permeability sandstone, UMB penetrated through the entire test pack, resulting

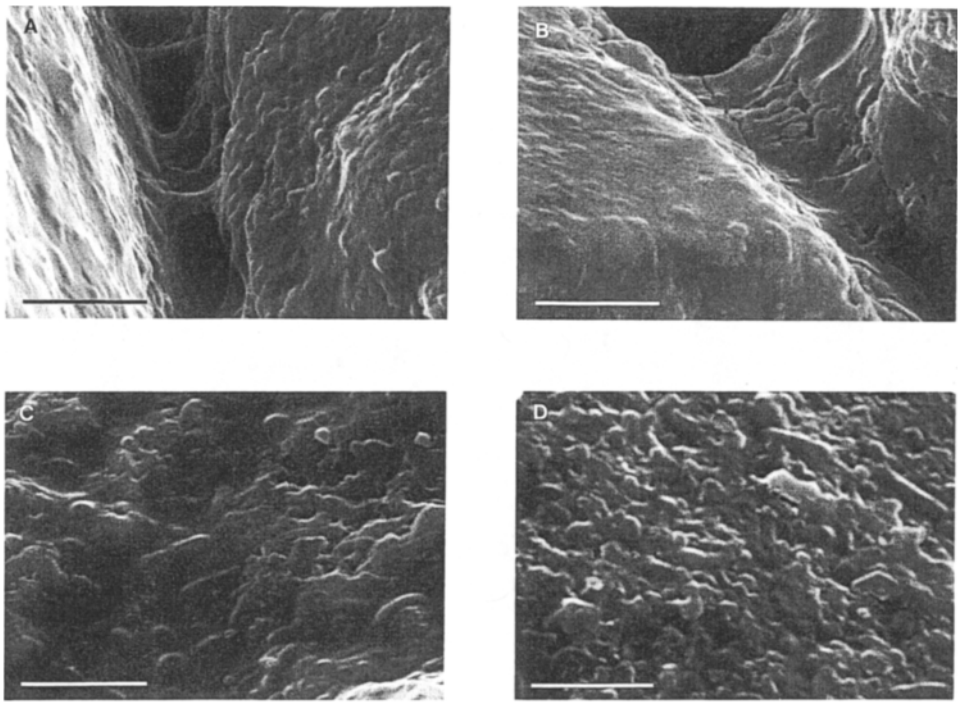

Fig. 7. Scanning electron micrograph of four samples taken from the sand pack (A–D) from Run 2. Bacterial polymer is found in each of the high-permeability sand-pack sections, but not in the low-permeability sandstone sections, showing selective plugging of the high-permeability zones. Scale bar represents 5 μm.

in a sharp increase in pressure. Most of the injected nutrients followed the path of least resistance, as polymer production was much higher in the high-permeability sand pack (10–100-fold). An interesting observation is that a skin plug did not form on the surface of the low-permeability sandstone. In conclusion, experiments under simulated reservoir conditions proved that the use of UMB was feasible for the production of selective plugging. Their small size enables them to penetrate as far as the injection fluid carries them. Plugging occurs *in situ* when the UMB are nutrient-stimulated. In a heterogeneous formation, the high-permeability zones are preferentially plugged. Our work with the simulating model has opened the doors for field studies scheduled to begin in the near future.

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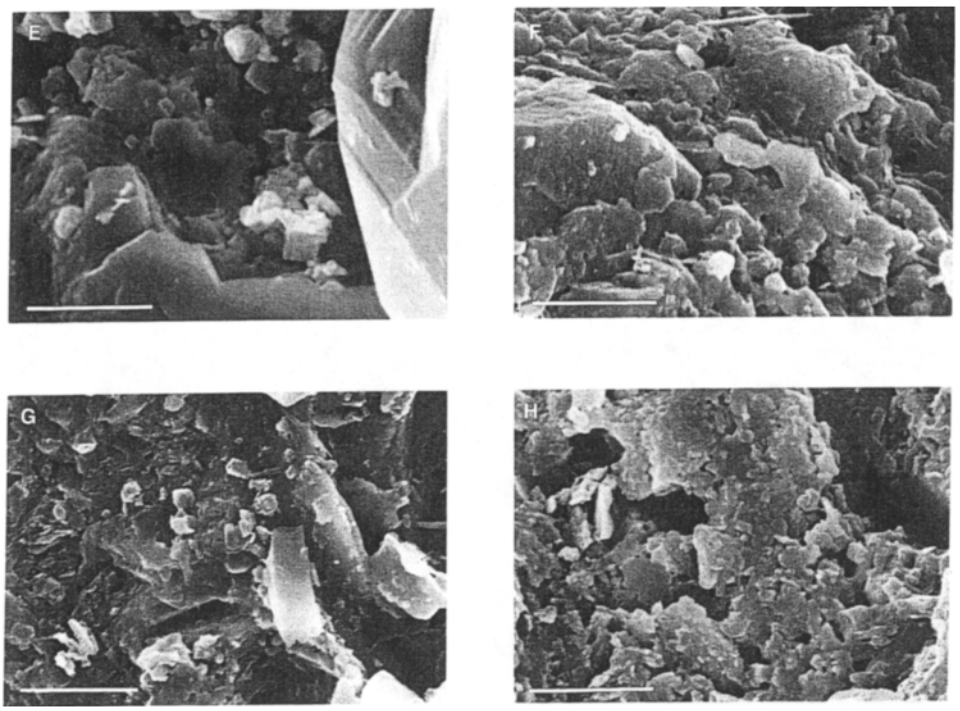

Fig. 7. Scanning electron micrograph of four samples of the sandstone core (E-H) from Run 2. Bacterial polymer is found in each of the high-permeability sand-pack sections, but not in the low-permeability sandstone sections, showing selective plugging of the high-permeability zones. Scale bar represents 5 μm.

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