

## Selectivity and depth of microbial plugging in Berea sandstone cores

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### SUMMARY

The depth of plugging by the in situ growth of either injected or indigenous microorganisms was investigated using Berea sandstone cores with pressure taps located along the length of the core. The continuous injection of aerobically prepared sucrose-mineral salts medium with 5% NaCl and 0.1% NaNO<sub>3</sub> resulted in large permeability reductions (70–98%). The plugging was localized at the inlet and outlet faces of the cores, and was attributed to microbial biomass production at the inlet face and biogas accumulation at the outlet face. Batch addition of aerobic medium resulted in more uniform permeability reduction along the core's length, but the magnitude of the permeability reduction was not as large (about 65%). The semi-continuous injection of oxygen-free medium resulted in a slower but a more uniform permeability reduction throughout the core compared to cores which received aerobically prepared medium. The selectivity of the process was investigated in a dual core system where two cores of 240 and 760 mdarcy permeability were connected parallel to each other without crossflow. Initially, about 85% of the total fluid flow passed through the high permeability core. After the addition of *Bacillus* species and medium, the flow pattern changed and about 85% of the total fluid passed through the low permeability core. These results show that the in situ growth of microorganisms can selectively plug high permeability zones and that control of the process may be achieved by alterations in the method of nutrient injection.

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### INTRODUCTION

Hydrocarbon-bearing formations normally consist of strata of sedimentary rock containing a wide range of permeabilities, due to the geologic pro-

cesses by which the formations were formed [17]. In a water-injection process, the amount of fluid conducted within a given stratum is proportional to the permeability of that stratum [5]. Thus, the most permeable strata within a reservoir will conduct most of the injection water, and the least permeable strata will not conduct large quantities of injected water. This results in poor oil recovery efficiency

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during a waterflood or an enhanced oil recovery process [18].

The oil recovery efficiency ( $E_r$ ) is defined as the product of the microscopic oil displacement efficiency ( $E_d$ ) and the volumetric sweep efficiency ( $E_v$ ) [4]:

$$E_r = E_d E_v$$

Volumetric sweep efficiency is the fraction of the reservoir contacted by the recovery fluid and is a measure of the three-dimensional effect of reservoir heterogeneity. Many enhanced oil recovery processes have high microscopic displacement efficiencies under laboratory conditions, but when these processes are applied to actual reservoirs, the volumetric sweep efficiency, rather than the microscopic displacement efficiency, dominates the ultimate success of the process [22].

Selectively plugging the most permeable regions of a reservoir will reduce permeability variation and improve the volumetric sweep efficiency. A variety of techniques have been developed to selectively plug reservoirs, including polymers, clays, cements and waxes [2,6-8,10,22,25]. The difficulty in controlling placement and the instability of some of these agents has limited their use. The use of microorganisms to correct permeability variation has been suggested by several workers. Several studies have shown that bacteria can penetrate sandstone cores under growth or non-growth conditions [11-13,15] and that the injection of nutrients into cores containing microorganisms results in large reduction in permeability [11,14,20].

This study investigates the feasibility of growing injected or indigenous microorganisms in situ to selectively plug high permeability zones using parallel sandstone cores as the experimental system and to determine the factors that affect the depth of plugging within the cores. Selective plugging would reduce the apparent permeability of the core, force more of the injected fluid into the less permeable core, and ultimately increase oil recovery from these sandstone cores.

## MATERIALS AND METHODS

### *Media and conditions of cultivation*

*Bacillus* strain 47 was obtained from the culture collection of the Department of Botany and Microbiology, University of Oklahoma. Strain 47 was grown in medium E with 0.05% yeast extract and 0.1%  $\text{NaNO}_3$  (modified medium E) as described previously [14].

Stationary phase cultures were harvested by centrifugation ( $10000 \times g$ , 10 min,  $4^\circ\text{C}$ ) and were washed by resuspension of the pellet in sterile 2% NaCl and 0.01%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solutions and followed by centrifugation. Cells were resuspended in the above solution at a density of  $1 \cdot 10^5$  cells per ml prior to injection into the core.

Effluent samples of some cores were serially diluted in 5% NaCl solution and appropriate dilutions were plated onto agar medium having the same composition as that injected into the core.

### *Core preparation*

Blocks of Berea sandstone (Cleveland Quarries, Amherst, OH) with different permeabilities were cylindrical (5 cm diameter) or square ( $5 \times 5$  cm) cores. Each core was steam cleaned for 2 weeks to remove humic acids [24], dried for 24 h at  $125^\circ\text{C}$ , and then placed in a vacuum desiccator to cool. Cores were coated with epoxy resin, cast in a fiberglass resin and then cut to the specific length. Each core was mounted between two Plexiglass end plates fitted with rubber gaskets to provide a sealed system. Pressure taps were drilled into the fiberglass cast at specified locations along the length of the cores. All connections to the core were made with nylon tubing and Swagelok compression fittings.

Each core was flushed with  $\text{CO}_2$  for 15 min and then vacuum saturated with 5% NaCl + 0.1 M  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  brine. The calcium was added to stabilize the clays in the core and to prevent clay swelling [23]. After vacuum saturation, the core was placed in  $50^\circ\text{C}$  incubator and 100 pore volumes (PV) of the above brine were injected into the core at a constant flow rate, ranging from 1.3 to 1.4 ml

per min using a Gilson HP4 Miniplus peristaltic pump. Next, 10–15 PV of 5% (wt./vol.) NaCl solution were pumped through the core to reduce the amount of calcium to avoid any detrimental interaction with the nutrient medium. Flow rates were measured by volumetric measurements of flow volume versus time. All injected fluids were filtered through 0.22  $\mu\text{m}$  membrane filter to remove particulate material. The fluids injected into some cores were continuously bubbled with  $\text{N}_2$  to limit the amount of  $\text{O}_2$  dissolved in the brine.

Once the initial permeability was determined, sterile modified medium E was injected into the core to stimulate *in situ* microbial growth [14]. In continuous flow experiments, modified medium E, or a 10-fold dilution of this medium, was injected into the core until termination of the experiment. Cells were injected into some cores prior to nutrient injection. The injection of fluids into the cores was not performed aseptically, so the term indigenous refers to those microorganisms which were native to the core and/or those that may have been inadvertently introduced into the core. Some cores received medium with 1 mg/ml of streptomycin to reduce the number of species of microorganisms that grew in the cores. In intermittent flow experiments, a series of treatments was conducted on each core. Modified medium E was injected into the core until a pressure increase was observed. The core was then incubated for 24 or 48 h as indicated. After incubation, 5% NaCl brine was injected into the core (waterflood) to measure the permeability. The core was then flooded with 5% NaCl brine in the reverse direction (backflush) to check the stability of the microbial plug. This process was repeated until permeability did not change between successive treatments.

The weight of suspended particles was determined by filtering 20 ml of the effluent from the core through a pre-weighed, 0.22  $\mu\text{m}$  membrane filter. The filters were dried at 60°C to a constant weight, which was corrected for the weight of NaCl in the samples.

### Permeability and porosity measurements

The permeability of each section of the cores was calculated from Darcy's law [3]:

$$K = \frac{q \mu L}{A \Delta P}$$

where  $q$  is the volumetric flow rate (in cubic centimeters per second),  $\mu$  is the dynamic viscosity of the fluid (in centipoise),  $A$  is the cross-sectional area of the core (in square centimeters),  $L$  is the length of the core (in centimeters), and  $\Delta P$  is the differential pressure across the core (in atmospheres). The permeability ( $K$ ) is given in Darcys.

The pressure drop across each section of the core was measured using Validyne DP-15 pressure transducer. The signal from the transducer was processed using a Validyne CD-380 signal demodulator and routed to a strip-chart recorder. The transducers were calibrated using pressure gauges. The accuracy of the permeability measurement for each section of the core was checked by comparing the permeability of the entire core calculated from the inlet and outlet pressures to the harmonic average of the permeability of all the sections [3]:

$$\bar{K} = \frac{L_T}{\sum_{j=1}^n \frac{K_j}{L_j}}$$

where  $\bar{K}$  is the harmonic average permeability,  $L_T$  is the overall length of the core,  $L_j$  is the length of section  $j$ , and  $K_j$  is the permeability of section  $j$ .

The effective permeability reduction factor (PRF) was calculated as follows [20]:

$$\text{PRF} = \frac{K}{K_i} \times 100$$

where  $K_i$  is the initial permeability and  $K$  is the permeability after treatment. Lower values for *PRF* imply a greater reduction in the effective permeability.

Porosity and PV of the cores were determined as described previously [15].

## RESULTS

The continuous injection of aerobically prepared, modified medium E, with or without streptomycin, resulted in an exponential increase in the pressure gradient across the core after a lag of 1–5 h (data not shown). When anaerobically prepared medium was used, a lag of 5–20 h occurred before an exponential increase in pressure was observed. Thus, the injection of up to about 2 PV of aerobically prepared medium or up to 16 PV of anaerobically prepared medium to cores did not result in significant permeability reductions (PRF > 90%), unless sufficient time was allowed for microbial growth. After incubation of nutrient-containing cores, visible turbidity and large numbers of bacteria (greater than  $1 \cdot 10^5$  colony-forming units per ml) were observed in the effluent and the permeability of these cores was greatly reduced (PRF < 40%). The above data strongly indicate that the observed permeability reductions were the result of in situ microbial growth and metabolism, rather than chemical or physical changes caused by the injection of the nutrient medium.

Significant permeability reductions, i.e., low PRF values, were observed when aerobically prepared, modified medium E with or without streptomycin was injected into the cores (Table 1). Effluents of cores which received medium without streptomycin had a variety of colony morphologies. These colonies were composed of cells morphologically sim-

ilar to actinomycetes or *Bacillus* species. Effluents of cores which received medium with streptomycin had only one colony type which was composed of a Gram-positive, spore-forming rod. One of these colonies was picked and designated strain BCI-1. BCI-1 grew and produced an exopolymer aerobically and anaerobically in modified medium E with or without 1 mg/ml streptomycin. Smaller PRF values were observed in cores which received medium with streptomycin (cores 1 and 2) compared to core 3, which received medium without streptomycin. This may be due to the large amount of exopolymer that was produced by BCI-1 under these conditions. The addition of 7.5 PV of *Bacillus* strain 47 ( $1 \cdot 10^5$  cells per ml) (core 4), prior to the addition of modified medium E without streptomycin resulted in a more rapid increase in the pressure gradient and a smaller PRF value (< 15%) than that observed for core 3, which did not receive cells.

Analysis of the pressure gradient along the length of cores 1 to 4 showed that large increases in the pressure gradient occurred at the inlet end of the core (between the inlet face and the first pressure tap). There was little or no increase in the pressure gradient in the other sections of the core. However, in core 1, large increases in the pressure gradient were observed at the inlet and outlet ends of the core (Fig. 1) resulting in a rapid decrease in PRF at these two sections of the core (Fig. 2). This phenomenon is called capillary end effect, [9] where gas dissolved in the injected fluid comes out of solution

Table 1

Permeability reductions in cores receiving aerobically prepared medium

The porosity of each core was 18%, the cross-sectional areas of the cores ranged from 20 to 25 cm<sup>2</sup>, and the length of the cores ranged from 12 to 19 cm except core 2 which was 48 cm long. Each core had three pressure taps along the length of the core except core 3 which did not have pressure taps along its length.

Core No.	Nutrient injection method	Streptomycin added	PV of nutrients	Cells injected (PV)	Initial permeability (mdarcys)	Final PRF (%)
1	continuous	+	17.1		507	2
2	continuous	+	4.7		498	8
3	continuous	—	18.0		313	30
4	continuous	—	6.3	7.4 (strain 47)	273	< 15
5	batch	+	2.8		571	35

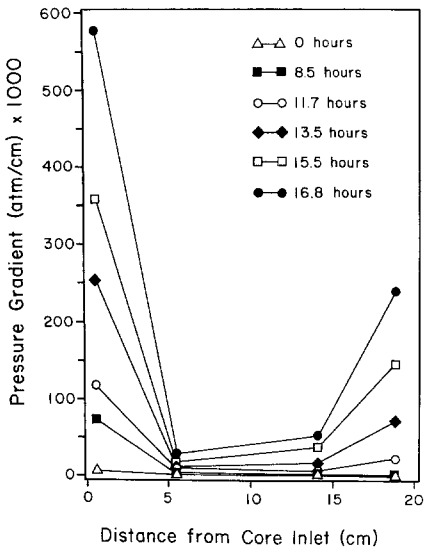


Fig. 1. Pressure gradient along core 1 during continuous injections of aerobically prepared medium with streptomycin. Pressure was measured at the indicated points.

as the pressure drops to atmospheric levels at the outlet end of the core. The dissolution of gas present in the inject fluids may have contributed to the increase in pressure at the outlet end. However, the outlet pressure gradient began to increase at the same time that visible turbidity was observed in the effluent (11.7 h), which was also the time when gas

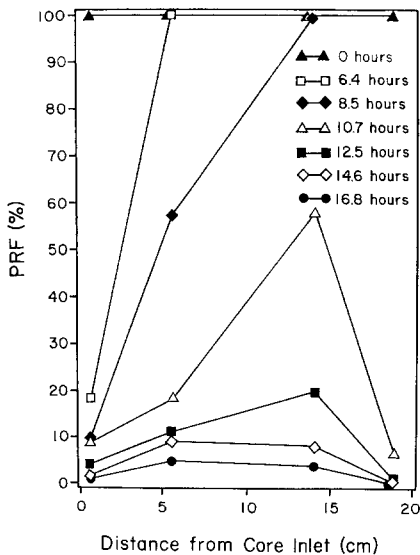


Fig. 2. Permeability reduction factors along core 1 during continuous injection of aerobically prepared medium with streptomycin.

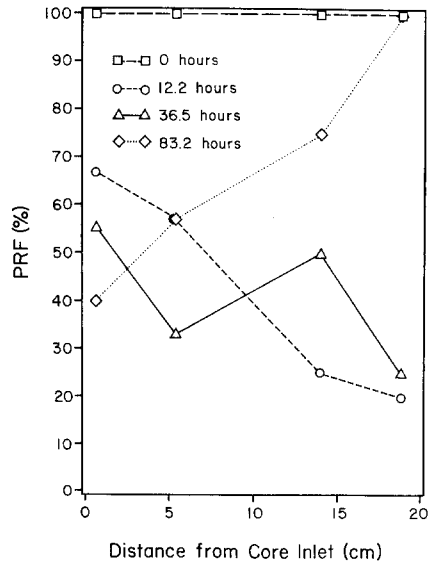


Fig. 3. Effect of batch injection of aerobically prepared medium with streptomycin on the permeability reduction factors along core 5. The core received 2.8 PV of medium, then was incubated without fluid flow except when permeability was measured. Permeability was measured by injecting sterile 5% NaCl solution.

bubbles were observed in the effluent. Thus, it is likely that the gas was microbially produced in the core. This combination of in situ microbial growth and gas production resulted in a uniform reduction in the permeability throughout the length of this core.

When 2.8 PV of aerobically prepared modified medium E with streptomycin were added to core 5 and the core was incubated without fluid flow, higher PRF values, but a more uniform reduction in permeability, were observed in core 5, compared to cores which received continuous nutrient injection (Fig. 3 and Table 1). PRF declined first at the inlet end of the core, followed by a decrease in PRF in the other sections of the core at longer incubation times. The pressure gradient profile showed that some of the permeability reduction could be due to capillary end effects, but increases in the pressure gradient were observed at each pressure tap, suggesting that microbial growth was occurring throughout the core.

Next, the effect of in situ anaerobic microbial growth and metabolism on the permeability of Be-

Table 2

Permeability reductions in cores receiving anaerobically prepared medium

The porosity ranged from 21 to 22%, the cross-sectional areas ranged between 21 and 23 cm<sup>2</sup>, and the core lengths ranged from 10.2 to 13.4 cm. Each core had one pressure tap located in the middle of the core. A 'treatment' is the injection of anaerobically prepared modified medium E without streptomycin until an increase in pressure was observed, incubation at 50°C for 24 h except for core 9 which was incubated for 48 h, injection of 5% NaCl solution until a constant pressure reading was obtained (waterflood), then injection of 5% NaCl solution in the opposite direction (backflush) to determine the degree of facial plugging. All fluids injected into the cores were continuously bubbled with O<sub>2</sub>-free N<sub>2</sub>.

Core No.	Number of treatments	Nutrients injected (PV)	Initial permeability (mdarcys)	Final inlet PRF (%)	Final outlet PRF (%)	Average final PRF (%)
6	10	44.6	867	10	2	4
7	10	36.7	246	5	5	5
8	11	86.7	728	8	1	4
9	10	39.2	489	20	9	14

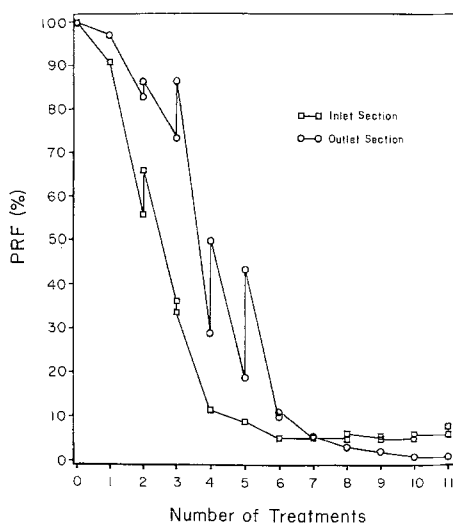


Fig. 4. Permeability reduction factors along core 8 during the batch injections of anaerobically prepared medium. PRF values were measured during the waterflood and backflush of each treatment. Vertical lines indicate the amount of permeability restored by backflushing.

rea sandstone was studied (Table 2). Treatments were continued until the PRF value did not change (<5%) between successive treatments. The PRF values were as low as those obtained when aerobically prepared medium was used, but a larger volume of nutrients was injected into the cores compared to those which received aerobically prepared

medium, indicating that microbial growth and metabolism occurred at a slower rate in these cores under anaerobic conditions. Low PRF values were obtained for both the inlet and outlet sections of the cores, indicating that microbial plugging occurred uniformly throughout the length of the cores. Cores 6 and 7 were incubated in a vertical posi-

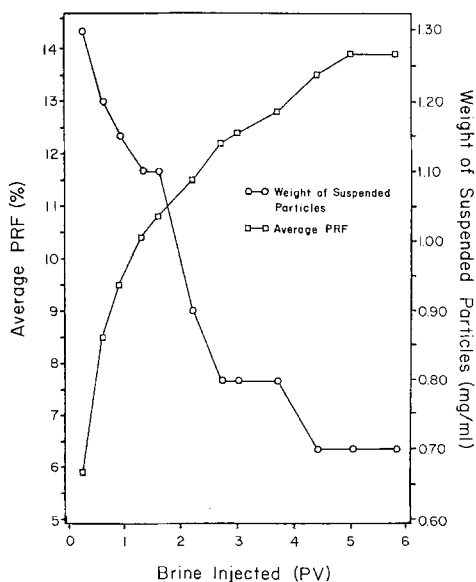


Fig. 5. The change in PRF and concentration of suspended solids in the effluent of core 8 during the waterflood which followed the incubation period.

tion with the outlet end at the top and had similar PRF values for both sections as did cores 8 and 9 which were incubated horizontally, which suggests that capillary end effects did not contribute to the permeability reduction in the outlet section. Similar PRF values were obtained when the incubation time was increased from 24 (cores 6 to 8) to 48 h (core 9).

The PRF values obtained during the waterflood and backflush portions of the treatment process for core 8 are shown in Fig. 4. Similar trends were observed for cores 6, 7 and 9 (data not shown). Initially, plugging was greater in the inlet section of the core. As the treatments continued, permeability was reduced in both sections of the core. Backflushing restored much of the permeability (indicated by the vertical lines in Fig. 4) in the outlet section of the core during treatments 2 to 5, indicating that the permeability reductions were not stable in this section. As treatments continued, backflushing had little effect on permeability in either section of the core.

The PRF usually increased during the first 4 to 5 PV of 5% NaCl solution injected into the cores after the incubation period (Fig. 5). This was probably due to the removal of suspended solids from the core, since the effluent contained 0.7–1.3 mg per ml (dry wt.) of suspended solids. Electron micrographs of the effluent material collected on membrane filters showed that the presence of bacterial cells, clays and other small particulates (data not shown). The PRF value became constant after 4–5 PV of brine were injected. The PRF values reported above were measured at this time.

The selectivity of the microbial plugging process (i.e., plugging of the high permeability zone as opposed to the low permeability zone) was tested using two cores of different permeabilities connected in parallel without crossflow of fluids from one core into the other. Initially, 76% of the total fluid passed through core 10 (higher permeability) and 24% through core 11 (Fig. 6). For the first 24 h of flow time, 17 PV of *Bacillus* strain 47 suspended in 5% NaCl solution were injected into the core. At the end of cell injection, the flow pattern had changed and all of the flow was diverted into the

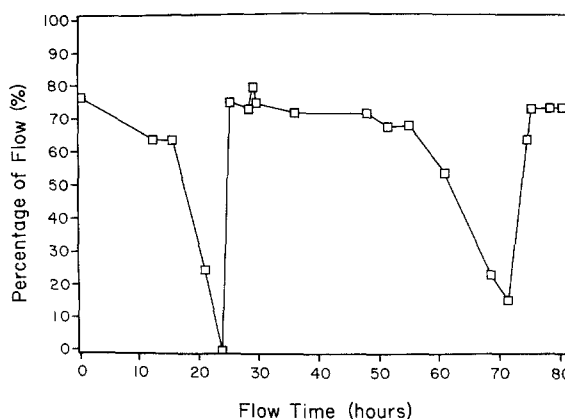


Fig. 6. The selectivity of the microbial plugging process in parallel core system using *Bacillus* strain 47 and aerobically prepared medium. Core 10 (initial permeability of 760 mdarcys) and core 11 (initial permeability of 240 mdarcys) were connected in manifold to a common liquid reservoir. The abscissa represents the flow time of core 10 and does not account for the time when the system was incubated without fluid flow. Data for core 11 are not shown. During the first 24 h of flow time,  $1 \cdot 10^4$  cells of *Bacillus* strain 47 suspended in sterile 5% NaCl were injected into the system. The system was incubated for 13 h without flow. From 24 to 29 h, each core was backflushed with sterile 5% NaCl solution. From 29 to 49 hours of flow time, a 10-fold diluted solution of modified medium E without streptomycin was injected. From 47 to 71 hours of flow time, modified medium E without streptomycin was injected. The system was incubated for 50 h without fluid flow. Then, sterile 5% NaCl solution was injected.

low permeability core (core 11). Backflushing with 5% NaCl solution for 5 h restored the initial flow pattern, suggesting the facial plugging of core 10 had occurred. For the next 19 h of flow time, a 10-fold dilution of modified medium E was injected into the system and little change in the flow pattern was observed. Then, undiluted modified medium E was injected. The fluid flow pattern began to change and, after 22 h of injecting undiluted medium, about 90% of the total flow was diverted into core 11. After about 65 h of flow time, the effluent was turbid, viscous and contained a large number of cells. After 71 h of flow time, the system was incubated without fluid flow. This restored the flow pattern to that initially observed at the start of the experiment.

## DISCUSSION

The ability of microorganisms to selectively plug the high permeability zones was demonstrated using a parallel core system. The results indicate that microbial cells and the required nutrients were selectively transported into the high permeability core, since this core initially received most of the fluid flow. The in situ growth of microorganisms in this core apparently reduced the permeability of this core and caused the flow to be redirected into the low permeability core. Bacterial growth in the high permeability core was confirmed by the large amounts of biomass present in the effluent of this core. The restoration of the permeability in the high permeability core after 3 days of incubation at 50°C may be due to cell lysis. The substantial plugging of the high permeability core that occurred when cells alone were injected is probably due to facial plugging, since backflushing restored the original flow pattern. Facial plugging can be expected when a large number of pore volumes of cells are injected, especially when a relatively large rod-shaped cell such as *Bacillus* species is injected [16,2]. Dilute medium was injected into the system without any change in the flow pattern, which suggests that a controlled decline in permeability could be achieved by adjusting the concentration of the nutrient medium.

Contrasting permeability layers in a reservoir are often in capillary contact with each other [17]. Thus, crossflow between the two layers will occur. If the high permeability zone is only plugged at the inlet face, little stratification correction will occur, since crossflow will allow the injected fluids to migrate into the high permeability zone. The continuous injection of nutrients under aerobic conditions results in large permeability reductions. However, the plugging seems to be almost exclusively located at the inlet face of the core. Similar conclusions were made by Shaw et al. [21]. The addition of cells prior to nutrient injection decreased the lag time before permeability reductions were observed, and seemed to increase the degree of plugging. The batch addition of aerobically prepared nutrients resulted in a more uniform permeability reduction

throughout the core (Fig. 3), but large permeability reductions were still observed at the inlet section of the core. This should allow for significant flow diversion into low permeability regions, even in cases where crossflow occurs. We have preliminary experimental data to show that large flow diversions occur in Berea sandstone slabs in capillary contact with each other.

When anaerobically prepared medium was injected into cores, a much lower rate of permeability reduction was observed compared to cores receiving aerobically prepared medium. About 10–20 PV of anaerobic medium could be injected before permeability reductions were observed. Thus, the injection of nutrients under anaerobic conditions should allow for dispersal of the nutrients in the reservoir before inlet plugging reduces the injectivity. Use of anaerobically prepared medium and allowing sufficient time for microbial growth to occur between nutrient injections resulted in uniform reductions in the permeability of both sections of the core.

The large number of PV of anaerobically prepared nutrients used in these experiments was, to a great extent, the result of the experimental design. Since nutrients were injected into the cores until a pressure increase was observed, i.e., microbial growth began, much of the nutrients initially injected into the core was not metabolized. Also, treatments were continued until the PRF did not change. Thus, a greater number of treatments were used than would be the case in an actual field trial.

Electron micrographs of a high-permeability, fused-glass bead core system showed that the production of exopolymers by bacteria was an important factor leading to the observed permeability reductions [21]. Electron micrographs of the plugged inlet sections of our cores also showed large amounts of biomass, both cellular and extracellular, and clays and other particulate matter were also found to be associated with the biomass. Myers and Samiroden [19] suggested that exopolymers produced by bacteria can increase the degree of plugging by retaining the small particulate material present in the injection water. Our data are consistent with this hypothesis.



Other work (Torbati et al., unpublished results) has shown that in situ microbial growth in Berea sandstone preferentially plugs the larger pore throats, since these pores conduct most of the injected fluid. This diverts flow from these pores into smaller pore channels which have higher oil saturations. We believe that this improved microscopic sweep efficiency can explain the increased oil recoveries observed in some cores after the in situ microbial growth [20].

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