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Bacterial Fouling in a Model Core System

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We have used a sintered glass bead core to simulate the spaces and surfaces of reservoir rock in studies of the bacterial plugging phenomenon that affects waterflood oil recovery operations. The passage of pure or mixed natural populations of bacteria through this solid matrix was initially seen to promote the formation of adherent bacterial microcolonies on available surfaces. Bacteria within these microcolonies produced huge amounts of exopolysaccharides and coalesced to form a confluent plugging biofilm that eventually caused a >99% decrease in core permeability. Aerobic bacteria developed a plugging biofilm on the inlet face of the core, facultative anaerobes plugged throughout the core, and dead bacteria did not effectively plug the narrow (33-μm) spaces of this solid matrix because they neither adhered extensively to surfaces nor produced the extensive exopolysaccharides characteristic of living cells. The presence of particles in the water used in these experiments rapidly decreased the core permeability because they became trapped in the developing biofilm and accelerated the plugging of pore spaces. Once established, cells within the bacterial biofilm could be killed by treatment with a biocide (isothiazalone), but their essentially inert carbohydrate biofilm matrix persisted and continued to plug the pore spaces, whereas treatment with 5% sodium hypochlorite killed the bacteria, dissolved the exopolysaccharide biofilm matrix, and restored permeability to these plugged glass bead cores.

The injection of water into petroleum reservoirs (waterflooding) is the most common form of enhanced oil recovery. Bacteria and nutrients are present in the injected waters, and subsequent proliferation of bacteria in the reservoir can cause severe plugging and loss of water injectivity (2, 4). Sulfate-reducing bacteria, iron-oxidizing bacteria, and many slime-forming and filamentous types are common in oil-field injection waters (1, 17, 18), and we must expect that these aquatic organisms will show the same tendency to colonize surfaces that is shown by all other aquatic bacterial systems studied to date (8, 24). After initial colonization of available surfaces, aquatic organisms tend to form a confluent biofilm within which the sessile bacterial cells are enmeshed in a matrix of acid mucopolysaccharides (7) consisting of the exopolysaccharide glycocalyces (6) of the adherent microorganisms. Previous microbiological studies of the plugging of rock cores (11, 14, 18) have involved the use of dead bacteria, but further studies (16) have suggested that bacterial polysaccharide production plays a major role in the bacterial plugging of sand (10, 16), and our own work has shown that exopolysaccharide production by live bacteria is of pivotal importance in surface colonization (5, 6). For these reasons, we decided to use live bacteria, including mixed natural populations in injection water, and to examine the role of their exopolysaccharide production on the plugging of solid matrices.

The use of cores of natural reservoir rock in bacterial plugging studies is complicated by heterogeneities in chemistry and pore structure, by dissolution or precipitation of mineral matter, and by the presence of variable amounts of clay that may swell and "bridge" across pore structures to reduce permeability. Therefore, we sought to model the spatial aspects of reservoir rock, without its heterogeneity, and decided to use cores made of lightly fused glass beads so that the pore size and available surface area of the cores were both replicable and approximately equal to those of an "open" sandstone. Thus, especially in our early studies in which pure cultures of aquatic bacteria were used, any changes in permeability that occurred in the model cores could be attributed fully to microbiological factors.

MATERIALS AND METHODS

Porous medium. The cores used in this study were made from 90-μm-diameter glass beads which were randomly packed by vibration in Pyrex vials and then consolidated by gradual heating to 700°C for 2.5 h. The vials were broken to release the artificial cores, which were approximately 1 cm in diameter and 5 cm in length. The average throat size for these pore systems was measured at 33 μm by a mercury injection method as described by Wardlaw and Cassan (22). The porosity of the cores was 38 ± 2% and the permeability was 6.5 ± 0.5 darcys.

Apparatus. Figure 1 illustrates the experimental apparatus. A liquid storage reservoir with a capacity of 24 liters contained the test liquid pressurized from a compressed nitrogen tank. All tests were performed at room temperature (23 ± 2°C) with appropriate temperature corrections for viscosity.

Bacterial cells were prevented from settling through the water column by a magnetic stir bar. For every experimental culture volume used, the rate of stirring of the magnetic stir bar was increased until a strong vortex could be seen from the top of the reservoir before the lid was sealed. Cells that may have adhered to the sides of the reservoir were of no direct concern in this study, as they would not interfere with the free-floating cells entering the outlet at the base of the reservoir.

The core holder consisted of machined stainless steel end pieces and a hollow rubber stopper contained in a sleeve. O-rings provided seals against the ends of the rubber stopper where a core was inserted. A pressure gauge connected to the upper end pieces was used to measure the input pres-

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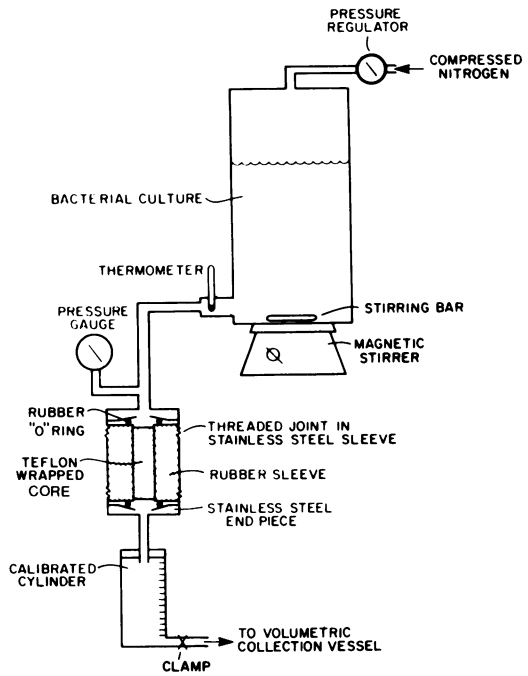

FIG. 1. Schematic diagram of experimental apparatus.

sure. The pressure at the outflow end was atmospheric. A calibrated cylinder and a stop watch were used to monitor the flow rate.

Before each injection test, the apparatus and the core were sterilized in a gas sterilizer (Steri-Vac 202, 3M) with ethylene oxide gas. The reservoir was too large to be sterilized by this means, and so 70% ethanol was poured down the sides of the container and drained to the bottom. After the lid was soaked with 70% ethanol, it was closed down over the open end of the reservoir. After 12 to 18 h, the reservoir was rinsed three times with sterile distilled water. To check for contamination, samples of both the 70% ethanol and the sterile rinse water from the reservoir were taken. A standard viable plate count was done, as well as a total direct count by acridine orange epifluorescence microscopy (Zimmermann and Myer-Reil [23] as modified by Geesey et al. [8]). No bacteria were observed, and this method was therefore adopted for routine sterilization of this apparatus.

Before every experiment, to prevent substantial permeability losses due to entrapment of oxygen within the pores of the glass bead cores, the core was put into a vacuum chamber (0.001 torr). The core balanced upright on a sterile glass slide set on the edge of a beaker containing 100 ml of the sterile fluid (e.g., media) used in that particular experiment. After a 40-min evacuation, the sides of the vacuum chamber were lightly tapped so that the core would fall into the beaker of fluid, where it became fully saturated (the vacuum was then stopped). The core was then mounted into the experimental apparatus in this saturated state.

Experiments were conducted with a small pressure drop (ca. 25 kPa) to maintain flow rates such that a single batch of liquid in the reservoir sufficed for an entire experiment. The intial flow rate was approximately 18 ml/min and decreased as the cores became plugged. The flow rate and volume throughput were constantly recorded, and fluid samples from the outlet were collected at given time intervals to monitor bacterial concentrations. Viable cells and total cell numbers were determined by the two methods previously mentioned. The test was continued until there was no change in permeability or until the fluid in the reservoir was consumed.

After each experiment, the core was fractured into six sections along its length for scanning electron microscopy (SEM). Sectioning was done with a diamond blade saw (no. 1125-801; Ruska Instrument Corp.). Preparation of the samples included fixation with 5% glutaraldehyde, washing with 0.067 M cacodylic acid buffer (pH 7.2), series dehydration (30 to 100%) with ethyl alcohol, and critical-point drying from Freon 113 before coating with gold-palladium. This technique enabled us to inspect the plugging characteristics of the bacteria within the core, at the inlet face, and at 0.5, 1.0, 2.0, 3.0, and 4.0 cm and represents a new advance in studies of this kind.

Waters injected and bacteria used. As part of the initial control experiments, studies were first performed with distilled water sterilized by treatment with 30 μg of bleach per liter and nonsterilized distilled water collected directly from the still.

Distilled water was also used in an experiment to simulate produced water, which contains both particulate matter and bacteria. We decided to investigate the effects of mobile particulate matter, independent of bacteria, on decreases in core permeability. Problems surfaced when natural produced waters were used. It was not possible to filter out the bacteria, as a separatory technique, without also filtering out a majority of the mobile fines, resulting in a complete alteration in particulate composition. Attempts to kill the bacteria and remove them by standard autoclave procedures or chemical treatment resulted in either oxidation of or other alterations in the particulate matter.

An artificial system was therefore devised with distilled water and silicon carbide powder (Tech-Met Canada), which is chemically inert. Particle sizes ranged from 5 to 10 μm in diameter. These angular fines were dispersed in distilled water at a concentration of 0.1 g/liter and kept suspended by a magnetic stirrer. The suspension was autoclaved for 30 min and chlorinated (30 μg of bleach per ml) to oxidize the bacteria in the distilled water.

The bacteria used were the following isolates from natural water systems. (i) *Pseudomonas* sp., an isolate from stream water in Marmot Creek, Alberta. This microorganism is gram-negative, rod-shaped, anaerobic and is 0.5 μm in diameter and 1 μm in length. *Pseudomonas* sp. produces exopolysaccharide in the form of a glycocalyx. The culture medium used (Ron Read, Ph.D. thesis, University of Calgary, Calgary, Alberta, Canada, 1983) was of the following composition: $NaC_6H_8O_7$, 2.5×10^{-2} M; $(NH_4)_2SO_4$, 10^{-3} M; KH_2PO_4, 5×10^{-2} M; K_2HPO_4, 5×10^{-2} M; $MgSO_4$, 10^{-3} M; and $FeCl_3$, 5×10^{-5} M (pH 7.0).

Experiments with live cells involved the use of cell concentrations of 10^6 to 10^8 cells per ml; the lower concentrations were achieved by diluting an inoculum grown for 23 h into a reservoir of culture medium. Periodic sampling by standard plate counts and epifluorescence microscopy showed that the concentration of cells in the reservoir did not increase generally more than twofold throughout any experiment (5 to 72 h). This was most probably due to the lack of oxygen in the reservoir, which would retard the growth of aerobic bacteria.

Live cells were used to study permeability losses due to inert particle trapping by bacterial biofilms within the core. This would simulate particles and microbial assemblages in produced waters. Silicon carbide powder was also used in

TABLE 1. Summary of experimental results

Condition of water	Initial suspension concn (cells per ml)	$K_{initial}$ (darcys)	Duration of expt (h)	K_{final} (%)	Throughput vol (pore vol)
1. Nonsterile distilled water	N/A[a]	6.83	18	26.1	7,662
2. Sterile distilled water	0	6.85	18	99.6	13,425
3. Live *Pseudomonas* sp.	1.8×10^8	6.55	43	0.6	714
4. Live *Pseudomonas* sp.	7.2×10^8	6.32	89	0	1,369
5. Diluted *Pseudomonas* sp.	2.0×10^7	5.92	23	0.3	2,482
6. Diluted *Pseudomonas* sp.	5.2×10^6	6.51	72	0.7	8,861
7. Killed *Pseudomonas* sp.	3.18×10^7	6.91	29	61.2	13,425
8. Prefiltered 2% molasses	0	6.50	4	78.0	2,200
9. Prefiltered 2% molasses plus strain BB-2	3.7×10^8	6.50	1.5	0.2	220
10. SiC	0.1 (g/liter)	7.09	50	5.1	3,566
11. SiC and *Pseudomonas* sp.	2.2×10^6	6.70	20	0.1	472
12. Produced water	1.1×10^6	6.44	163	1.0	6,922
13. Biocide treatment	6.2×10^6	6.55	135	0.5–0.8[b]	766 + 1,713[b]
14. Bleach treatment	5.2×10^6	6.88	28	1.6–39[b]	1,564 + 242[b]

[a] N/A, Not applicable.
[b] After treatment.

these experiments. A concentration of 0.1 g/liter (sterilized by autoclaving) was suspended in a culture of *Pseudomonas* sp. (2.2×10^6 cells per ml) growing in liquid.

Live cells were used to study the efficacy of a biocide and to observe the possible restoration of permeability losses. The biocide consisted of 8.5% 5-chloro-2-methyl-4-isothiazoline-3-one, 2.6% 2-methyl-4-isothiazoline-3-one, and 88.8% inert ingredient. A core was partially plugged with bacteria, and then biocide (45 μg/liter) was allowed to flow through the core.

To investigate the ability of bleach to restore losses in permeability, live cells were used once again. A 5% (by weight) sodium hypochlorite solution was passed through a core partially plugged with live *Pseudomonas* sp. cells.

It was necessary to investigate the ability of dead cells to have an effect on permeability losses. For these tests, a 5% formaldehyde solution made up in 0.1 M cacodylic acid buffer (pH 7.2) was found to be very effective in killing bacteria. It was also chosen because of its characteristic of preserving individual bacterial cells.

(ii) BB-2, a gram-negative, rod-shaped, facultatively anaerobic bacterium, 0.5 μm in diameter and 1.5 μm in length. This bacterium was isolated from produced water (water from the formation coproduced with the oil) collected from an oil-field tank at a Petro Canada battery, west of Airdrie, Alberta. BB-2 cells were inoculated into 2% sugar beet molasses medium in an artificial brine within an anaerobic chamber. The artificial brine had a composition similar to that of a coproduced petroleum reservoir brine with sodium chloride as the main constituent. A palladium catalyst which causes any residual oxygen to react with hydrogen, maintaining anaerobic conditions, was incorporated.

BB-2 was used for experiments to study the plugging characteristics of a bacterium isolated from produced water. In an experiment by Jack et al. (12), BB-2 cells were suspended in a prefiltered molasses-brine medium. Prefiltration was done to minimize plugging by non-bacterial particulate matter in the molasses. Also, the culture was not harvested until the bacteria had passed their gas-producing phase to eliminate possible permeability losses related to gas bubbles in pores.

(iii) Heterogeneous microbial population, found as the natural flora in produced water collected from Airdrie, Alberta. This produced water also contained particulate matter and was therefore used to investigate the plugging effects of mixed bacterial populations in the presence of fine suspended particles. This water had a redox potential of −120 mV and a pH of 6.8 and was fairly turbid owing to the contained black nonsulfide particles, oil droplets, and organic material. The bacterial assemblages consisted of aerobic bacteria at a concentration of 10^6 cells per ml and anaerobic sulfate-reducing bacteria at a concentration of 10^5 cells per ml.

RESULTS

When distilled water was passed through the glass bead core, the permeability of this solid matrix was reduced to 26% of its initial value with the passage of 7,662 pore volumes of water in 18 h (Table 1, row 1). Direct SEM examination of pores seen in the inlet face of the glass bead core showed the presence of a large number of bacteria of several different morphological types (Fig. 2a) and these cells could be seen to have produced a very extensive exopolysaccharide matrix that had condensed during preparative dehydration to produce a system of weblike strands representing <1% of their original hydrated volume (20). The bacterial biofilm was continuous and coherent in some areas of the inlet face of this glass bead core, and very large numbers of bacteria were seen in the 0.5 cm closest to the inlet surface, whereas very small numbers of bacterial cells were seen within the core or on the outlet surface. When sterile, chlorinated distilled water was passed through a glass bead core, no perceptible decrease in permeability occurred after the passage of 13,425 pore volumes in 18 h (Table 1, row 2).

When live bacterial cultures containing 1.8×10^8 and 7.2×10^8 cells per ml of an aquatic strain of *Pseudomonas* sp. were passed through a glass bead core, the permeability of this solid matrix was sharply reduced (Table 1, rows 3 and 4). Direct SEM examination of the inlet faces of the cores plugged by these live organisms showed a thick and confluent amorphous film (Fig. 2b) that obscured the details of the glass beads and completely filled the pore spaces. Although the colonizing bacteria were entirely hidden beneath the dehydration-condensed residue of their exopolysaccharide glycocalyces at the plugged inlet face (Fig. 2b),

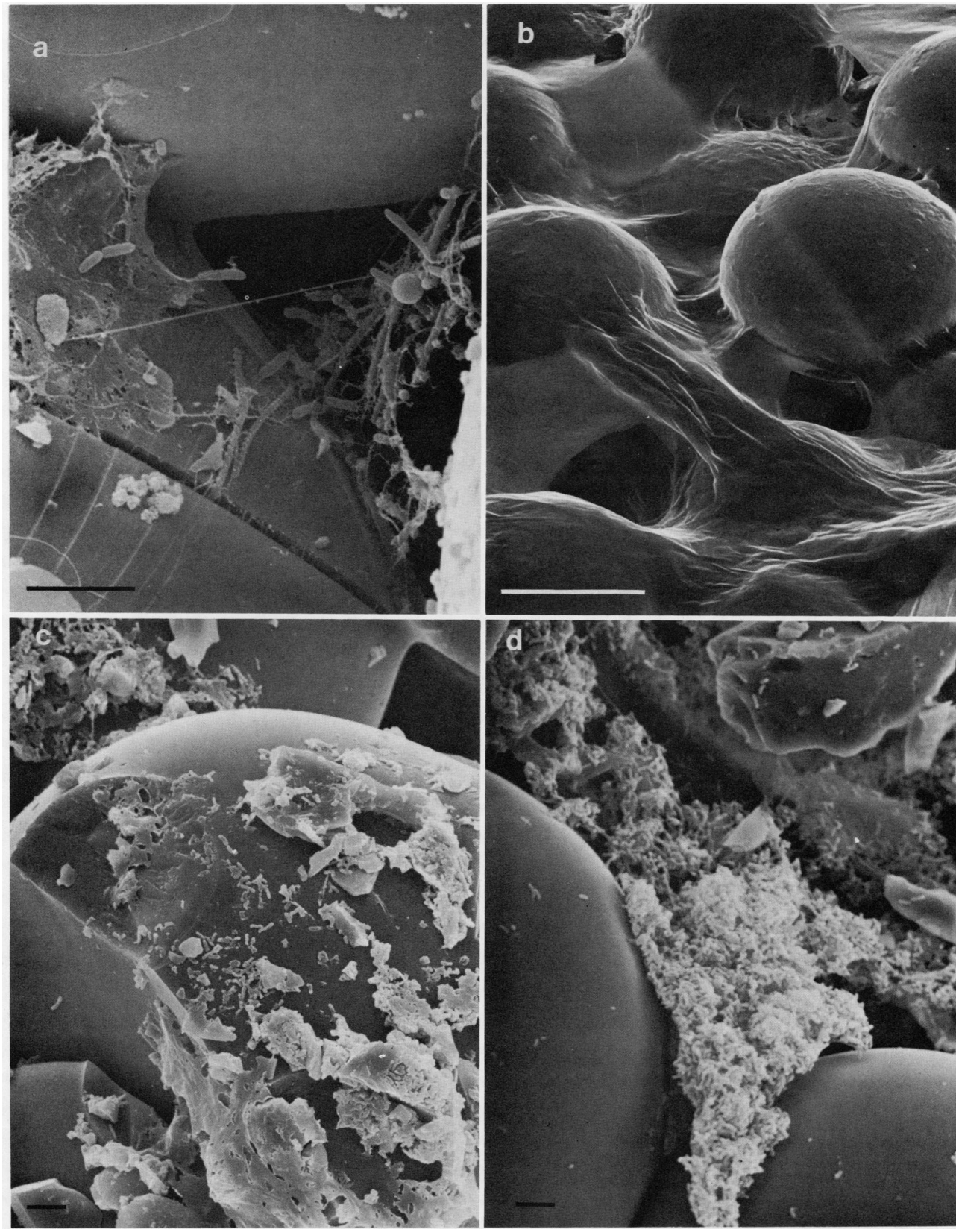

FIG. 2. Scanning electron micrographs. (a) Pore space occupied by numerous microorganisms inherent in nonsterile distilled water. Bar, 5.0 μm. (b) Top surface of a glass bead core completely encased in microbial exopolysaccharide, resulting in partial plugging of fluid flow through the core. Bar, 50 μm. (c) Live *Pseudomonas* sp. colonizing at a depth of 2.0 cm from the inlet face. Colonization is less severe than at the inlet face, but exopolysaccharide is still visible as an effective aid for microbial adherence. Bar, 5.0 μm. (d) Dead cells of *Pseudomonas* sp. Since these cells are incapable of producing exopolysaccharide, they become crowded into the inlet pore spaces, causing an appreciable decrease in permeability. Bar, 5.0 μm.

microcolonies of bacteria could be discerned in the less heavily colonized regions of the 2 cm of the core closest to the inlet face (Fig. 2c), and the last 3 cm of the core showed very few adherent bacteria. This basic aerobic colonization experiment was repeated on several occasions, and SEM of segments of the core consistently showed that the inlet face was plugged by a thick biofilm, whereas bacterial colonization of surfaces was very sparse in the lower areas of the core. When the initial concentrations of cells in the colonizing culture were reduced by dilution with fresh medium, the culture initially containing 2.0×10^7 cells per ml reduced the permeability to 0.3% in 2,482 pore volumes and 23 h (Table 1, row 5; Fig. 3) and that initially containing 5.2×10^6 cells per ml reduced the permeability to 0.7% in 8,861 pore volumes and 72 h (Table 1, row 6; Fig. 3).

When the aquatic bacteria used in these plugging experiments were killed with 5% formaldehyde before passage through the core at concentrations of 3.18×10^7 cells per ml, the permeability was only decreased to 61.2% by the passage of 13,425 pore volumes in 29 h (Table 1, row 7; Fig. 4). The viability of these cells was checked by plating the effluent, which showed no living cells, and by direct light microscopy of the effluent, which showed that the dead cells were numerous and structurally intact. SEM of the inlet surface showed a "cake" of dead cells partially covering the bead surface (Fig. 2d), but these cells were not surrounded by amorphous exopolysaccharide material and so the bacterial layer did not constitute an adherent plugging biofilm.

Plugging experiments were conducted by using an anaerobic oil-field isolate (BB-2) obtained from Tom Jack of Nova/Husky Research (Calgary, Alberta). After filtration, the sterile supplemented brine medium in which this bacterium was grown reduced the permeability to 78% after the passage of 2,200 pore volumes in 4 h (Table 1, row 8), but the passage of 3.7×10^8 cells of isolate BB-2 reduced the permeability to 0.2% in only 200 pore volumes in 1.5 h (Table 1, row 9). The inlet surfaces of these rapidly plugged cores were seen to be occluded by an amorphous mass within which some bacteria were visible (Fig. 5a), and this amorphous plugging material was seen in the spaces throughout the 5-cm depth of the core.

To examine the synergy of bacterial biofilm formation and the incidence of inert particles on the plugging of pores within solid matrices, we compared the permeability reduction by silicon carbide (SiC) particles in the presence and absence of pure cultures of the aquatic *Pseudomonas* sp. These SiC particles, which range in size from 5 to 10 μm, were passed through the core in sterile distilled water at a concentration of 0.1 g/liter and rapidly reduced the permeability to ±15% in 1,000 pore volumes (Fig. 6), but further flow of 3,566 pore volumes only reduced the permeability to 5.1% at 50 h (Table 1, row 10; Fig. 6). SEM of the inlet face of the core (Fig. 5b) showed the formation of an abiotic filter cake within which discrete pores could be seen. When a combination of 0.1 g of SiC per liter and 2.2×10^6 live *Pseudomonas* cells were passed through the core, the permeability was reduced to 0.1% in just 472 pore volumes and 20 h (Table 1, row 11; Fig. 6) and the inlet surface was seen to be plugged by a coherent amorphous biofilm within which SiC particles were partly buried (Fig. 5c). The spaces within the core did not contain SiC particles when examined by SEM, and these inert particles seemed to have been completely trapped by the developing bacterial biofilm.

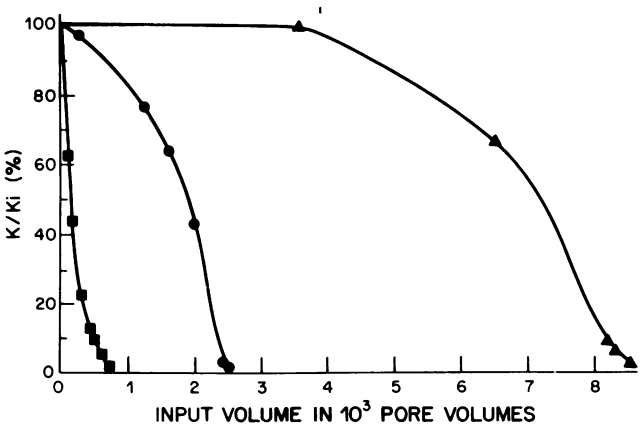

FIG. 3. Different rates of plugging of the glass bead cores as a result of differences in concentration of live cultures of *Pseudomonas* sp. Symbols: ▲, 5.2×10^6 cells per ml; ●, 2.0×10^7 cells per ml; ■, 1.8×10^8 cells per ml.

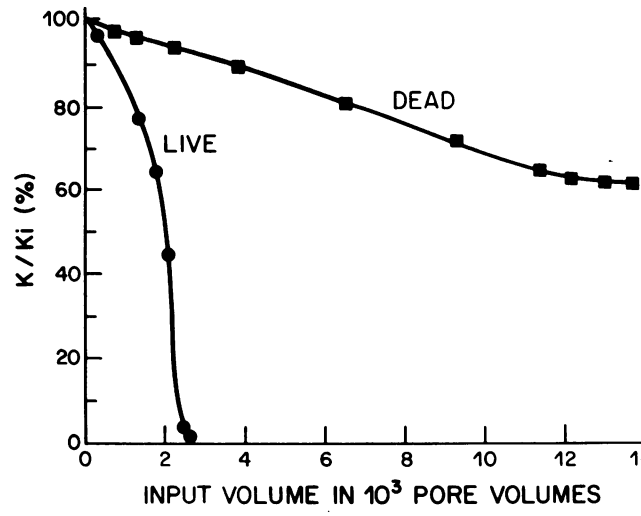

FIG. 4. Marked differences in the permeability loss due to live and dead cells of *Pseudomonas* sp.

Investigations of the plugging of the glass bead core by natural populations of oil-field bacteria showed that this water reduced the permeability to 1.0% in 6,922 pore volumes and 163 h (Table 1, row 12), and SEM showed (Fig. 5d) a wide variety of particles trapped in an amorphous matrix at the inlet surface of the core. As previously stated, all attempts to remove the inert particles from this water without removing the bacteria failed completely, as did all attempts at killing the bacteria without changing the inert components, and so the biotic and abiotic plugging factors could not be assessed independently.

In an examination of the effects of various treatments on partially plugged cores, we doubled the pressure at which the plugging medium flowed (25 to 50 kPa) and noted a transient increase in permeability (20 to 46%) that decayed to the previous value with the passage of 2,000 pore volumes in 4 h (Fig. 7). A biocide was also used to treat a partially plugged core, and only a very small increase in permeability (0.5 to 0.8%) was noted (Table 1, row 13; Fig. 8), even though the bacteria in the flowing-water phase were completely killed, as indicated by plate counts. The inlet surface of the biocide-treated core was seen to be heavily fouled by a thick bacterial biofilm, and we conclude that this biocide kills the bacteria in the system but fails to remove the

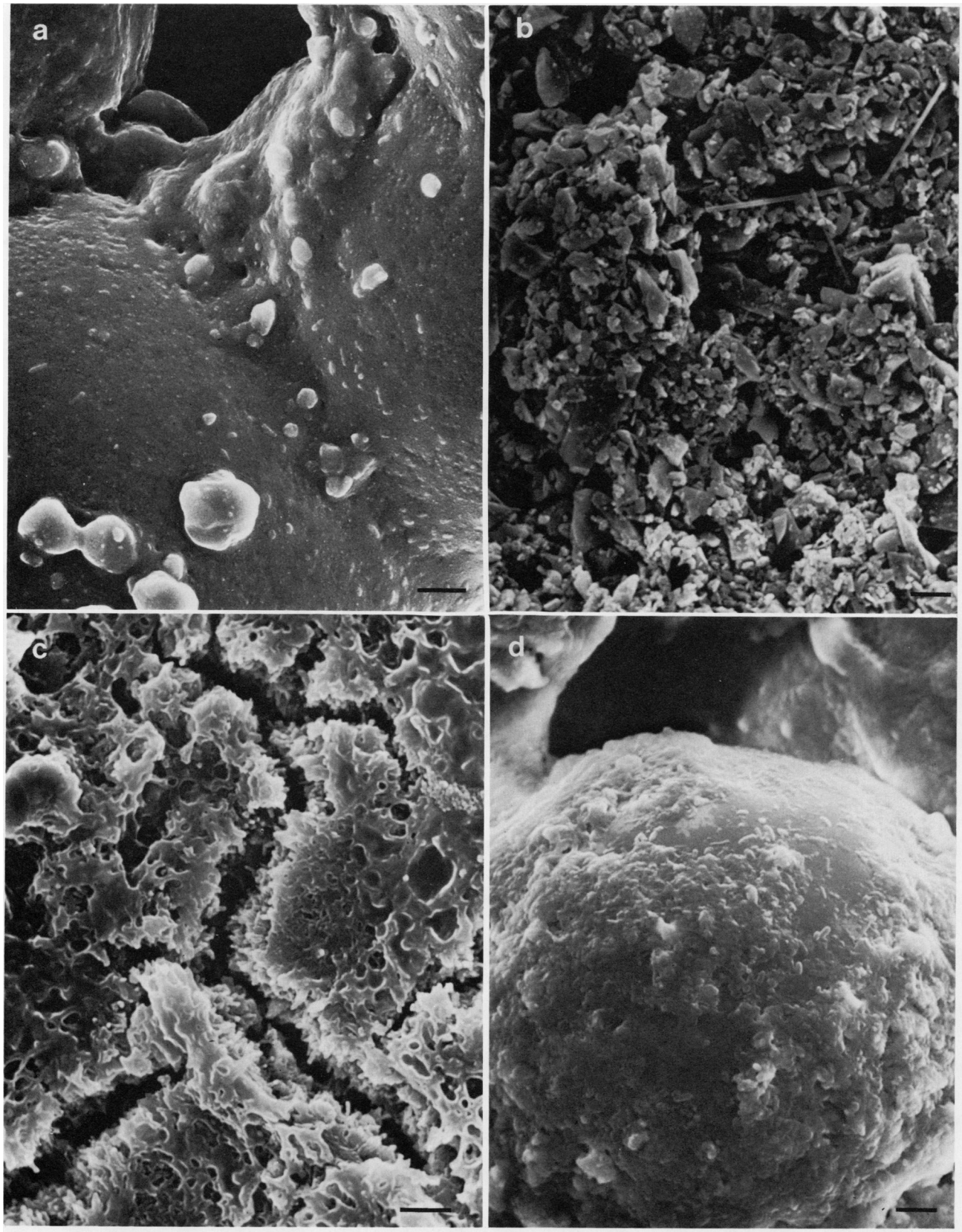

FIG. 5. Scanning electron micrographs. (a) Oil-field isolate BB-2 embedded in a layer of amorphous exopolysaccharide. This isolate is very capable of plugging the pore spaces to reduce the core permeability considerably. Bar, 5.0 μm. (b) SiC beads accumulated at the inlet face of the core produce their own pore network while decreasing the permeability of the glass bead core. Bar, 5.0 μm. (c) Inlet face blocked by a dense mat of *Pseudomonas* sp. and SiC beads, completely enclosed by the exopolysaccharide, resulting in a very effective decrease in core permeability. Bar, 5.0 μm. (d) Live produced-water microorganisms encased in their own exopolysaccharide. The ability of organisms found in oil-field waters to actively plug pores and adhere to surfaces is demonstrated. Bar, 5.0 μm.

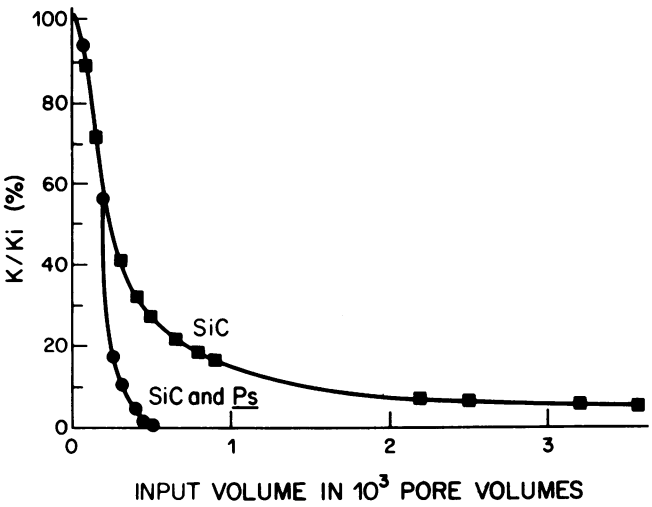

FIG. 6. Pore-plugging capabilities of inert particles alone (SiC) and inert particles in conjunction with bacteria (SiC plus *Pseudomonas* sp. [*Ps*]).

plugging biofilm. When a strong oxidizing agent (5% [by weight] sodium hypochlorite) was passed through a partially fouled core, the permeability was increased from 1.6 to 39% and the core did not return to the 1% permeability level until 242 additional pore volumes (2 h 40 min) had passed through the core (Table 1, row 14; Fig. 9). Direct examination of the inlet face of the bleach-treated core showed that the plugging bacterial biofilm had been largely removed by this oxidative treatment.

DISCUSSION

Aquatic bacteria have shown a strong tendency to adhere to available surfaces in natural (8) and simulated (13) stream systems, and this tendency is especially pronounced when groundwater flows through soil (21) or through the pores of a solid matrix like that of a sand filter (16). After initial, reversible adhesion to these surfaces (15), the bacteria adhere irreversibly by means of their exopolysaccharide surface structures (6) and begin to form adherent microcolonies within which the cells are enclosed and protected by their enveloping exopolysaccharide glycocalyces (6). The

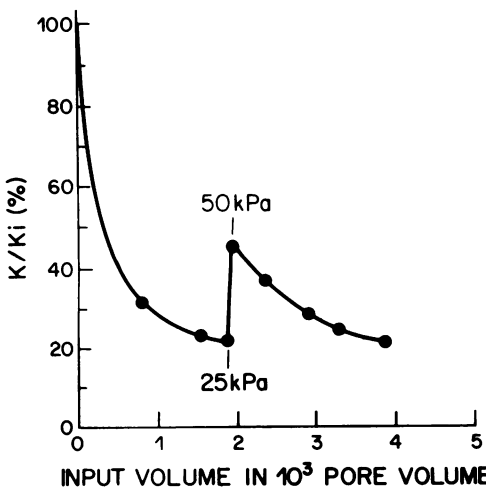

FIG. 7. Differences in the rate of pore plugging when various differential pressures are used.

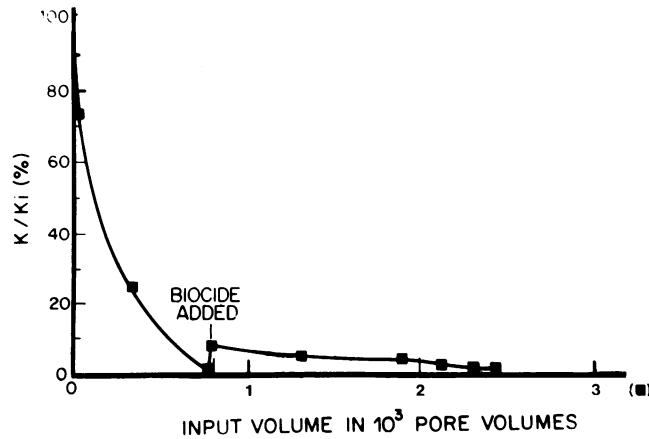

FIG. 8. Effect of biocide treatment on restoring permeability losses due to pore plugging by *Pseudomonas* sp.

bacterial glycocalyx is a very highly hydrated matrix of largely anionic polymers (20) that binds charged organic and inorganic nutrients by ion exchange (6) and thus provides a source of nutrients for the bacterial transport mechanisms even in environments in which nutrients are in very low concentration (6). Large bacterial populations (ca. 5×10^8 cells per cm^2) are often found on surfaces in low-nutrient, pristine alpine streams (8), and it is therefore not surprising that nonsterile distilled water could support the growth of a sufficient number of bacteria to form a biofilm and partially plug the ca. 33-μm pores of the glass bead core. The production of very large amounts of exopolysaccharide glycocalyx material by organisms within the biofilm eventually buries the individual bacterial cells, and SEM, which reveals only surfaces, shows the biofilm as an amorphous mass plugging the inlet surface and pores of the core (Fig. 2b). This type of biofilm formation is seen in the fouling of cores by aerobic bacteria, in that SEM shows that the first 0.5 to 2.0 cm of the 5.0-cm core are very heavily plugged by

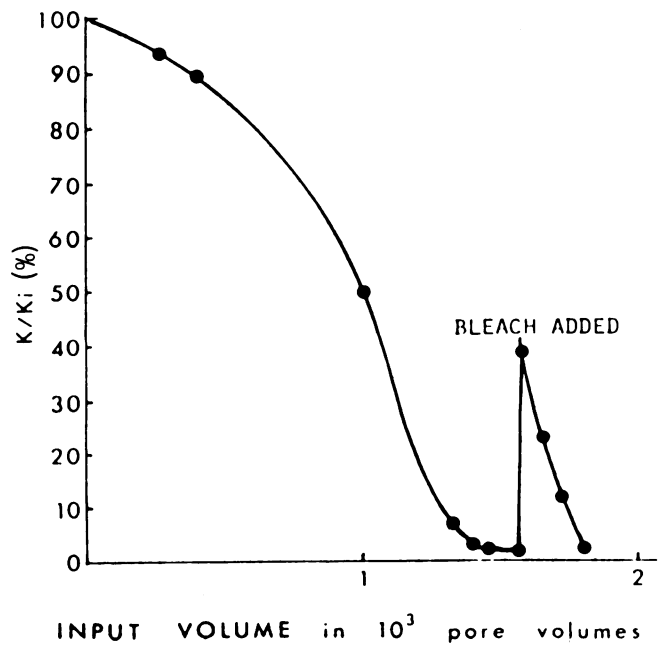

FIG. 9. Ability of sodium hypochlorite to restore core permeability losses due to microbial activity within the core.

bacteria in their amorphous matrix, whereas the pores of the remaining 2.0 to 5.0 cm contain only small numbers of single unencapsulated cells. We postulate that this "skin plugging" may result from a depletion of oxygen or nutrients, or both, in the deeper layers of cores colonized by aerobes, and the even fouling of pores throughout the 5.0-cm depth of cores colonized by the anaerobic strain BB-2 argues for the former hypothesis. We would expect that the rate of plugging of a core would be influenced by the number of live bacteria impinging on the solid matrix and by the amount of nutrient available for eventual biofilm formation. The slow rate of plugging by the few organisms in nutrient-poor distilled water supports this suggestion. Dead bacterial cells would be expected to plug the core by acting as small, inert particles (ca. 1 μm), just as SiC crystals act as larger inert particles (5 to 10 μm), and both of these particles are seen to form a filter cake on the inlet surface and to reduce permeability to a certain point (9), 61.2 and 5.1%, respectively, past which no further losses of permeability occur because the particulate filter cake has its own pores and inherent permeability. However, the trapping of inert particles (e.g., SiC) within the developing biofilm produced by live bacteria accelerates plugging to a remarkable degree, and the fastest plugging rates seen were those caused by media containing both live bacteria and inert particles.

Once established, the bacterial biofilm plugging the pores of the solid matrix of a core is difficult to dislodge. Changes of pressure produce a transitory increase in permeability, but this increase is soon lost, and Characklis et al. (3) have clearly shown that such increases simply cause the formation of a more coherent bacterial biofilm that is able to withstand the increased shear forces. Killing the bacteria within an extensive biofilm does not immediately remove this plugging structure from the pore of the solid matrix, and therefore the use of high (45 μg/ml) concentrations of an effective isothiazalone biocide (19) kills the biofilm bacteria but does not immediately affect the permeability. The use of an oxidizing agent that both kills biofilm bacteria and dissolves their exopolysaccharide glycocalyces would be expected to yield significant increases in the permeability of plugged cores, and we note that 5% bleach (sodium hypochlorite) increased the permeability of a plugged core from 1.6 to 39% and that this increase in permeability was sustained for at least an additional 242 pore volumes when the flow of the colonizing medium was resumed. The usefulness of this oxidizing agent is attested by the success of bleach treatments to increase the water injectivity in a number of injection wells in the Los Angeles basin (4).

The glass bead core has proven to be a useful model of reservoir rock in that it isolates biological from abiotic plugging factors and allows us to use SEM to see not only the inlet face of a core but also the pores in fractured sections that pass through the core at various depths. The conclusions that we reach in this study are ecologically predictable, namely that bacteria in fluid menstrua adhere avidly to surfaces to form a biofilm and that the biofilm restricts flow and traps nutrients for use by the biofilm population. By demonstrating this biofilm mode of growth, the glass bead model core may contribute to our general understanding of bacterial growth in solid matrices such as soil and reservoir rock.

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LITERATURE CITED

1. **Carlson, V., E. O. Bennett, and J. A. Rowe.** 1961. Microbial flora in a number of oilfield water-injection systems. Soc. Pet. Eng. J. **1**:71–80.
2. **Cerini, W. F., W. R. Battles, and P. H. Jones.** 1946. Some factors influencing the plugging characteristics of an oil-well injection water, p. 52–63. *In* Petroleum Technology Publication no. 2028. American Institute of Mining Engineers.
3. **Characklis, W. G., J. D. Bryers, M. G. Trulear, and N. Zelver.** 1979. Biofouling film development and its effects on energy losses. A laboratory study. Condensor Biofouling Control Symposium, 25–29 March 1979. Electric Power Research Institute, Atlanta, Ga.
4. **Clementz, D. M., D. E. Patterson, R. J. Aseltine, and R. E. Young.** 1982. Stimulation of water injection wells in the Los Angeles Basin by using sodium hypochlorite and mineral acids. J. Pet. Technol. **34**:2087–2096.
5. **Costerton, J. W., J. M. Ingram, and K.-J. Cheng.** 1974. Structure and function of the cell envelope of gram-negative bacteria. Bacteriol. Rev. **38**:87–110.
6. **Costerton, J. W., R. T. Irvin, and K.-J. Cheng.** 1981. The bacterial glycocalyx in nature and disease. Annu. Rev. Microbiol. **35**:299–324.
7. **Fletcher, M., and G. D. Floodgate.** 1973. An electron microscopic demonstration of an acid polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J. Gen. Microbiol. **74**:325–334.
8. **Geesey, G. G., R. Mutch, J. W. Costerton, and R. B. Green.** 1978. Sessile bacteria: an important component of the microbial population in small mountain streams. Limnol. Oceanogr. **23**:1214–1223.
9. **Gruesbeck, C., and R. E. Collins.** 1979. Entrainment and deposition of fine particles in porous media. SPE paper no. 8430. Society of Petroleum Engineers-American Institute of Mining Engineers 54th Annual Fall Meeting, Las Vegas, Nev. Society of Petroleum Engineers.
10. **Gupta, R. P., and D. Swartzendruber.** 1962. Flow-associated reduction in the hydraulic conductivity of quartz sand. Soil Sci. Soc. Am. Proc. **26**:6–10.
11. **Hart, R. T., T. Fekete, and D. L. Flock.** 1960. The plugging effect of bacteria in a sandstone system. Can. Min. Metall. Bull. **53**:495–501.
12. **Jack, T. R., B. G. Thompson, E. diBlasio, and B. Bramhill.** 1982. The plugging problems associated with the injection of anaerobic bacteria and their growth substrate into oil reservoirs for enhanced oil recovery, p. 152. Proceedings of the American Chemical Society 183rd Annual Meeting, Las Vegas, Nev. American Chemical Society, Washington, D.C.
13. **Jones, H. C., I. L. Roth, and W. M. Sanders III.** 1969. Electron microscopic study of a slime layer. J. Bacteriol. **99**:316–325.
14. **Kalish, P. J., J. E. Steward, W. F. Rogers, and E. O. Bennett.** 1964. The effect of bacteria on sandstone permeability. J. Pet. Technol. **16**:805–814.
15. **Marshall, K. C., R. Stout, and R. Mitchell.** 1971. Selective sorption of bacteria from sea water. Can. J. Microbiol. **17**:1413–1416.
16. **Mitchell, R., and Z. Nevo.** 1964. Effect of bacterial polysaccharide accumulation on infiltration of water through sand. Appl. Microbiol. **12**:219–223.
17. **Myers, G. E., and B. M. Slabyj.** 1962. The microbiological quality of injection water used in Alberta oilfields. Prod. Mon. **26**:12–14.
18. **Ralish, J. T., and D. L. Flock.** 1965. A study of formation plugging with bacteria. J. Pet. Technol. **17**:201–205.
19. **Ruseska, I., J. Robbins, J. W. Costerton, and E. S. Lashen.** 1982. Biocide testing against corrosion-causing oilfield bacteria helps control plugging. Oil Gas J. **March**:253–264.
20. **Sutherland, I. W.** 1980. Polysaccharides in the adhesion of marine and freshwater bacteria, p. 329–338. *In* R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent

(ed.), Microbial adhesion to surfaces. Ellis Horwood, Ltd., Chichester, England.
21. **Wallis, P. M., and T. I. Ladd.** 1982. Organic biogeochemistry of groundwater at a mountain coal mine. Geomicrobiol. J. **3**:49–78.
22. **Wardlaw, N. C., and J. P. Cassan.** 1979. Oil recovery efficiency and the rock-pore properties of some sandstone reservoirs. Bull. Can. Pet. Geol. **27**:117–138.
23. **Zimmermann, R., and L. Myer-Reil.** 1974. A new method for fluorescence staining of bacterial populations on membrane filters. Kiel. Meeresforsch. **30**:24–27.
24. **ZoBell, C. E.** 1943. The effect of solid surfaces upon bacterial activity. J. Bacteriol. **46**:39–56.