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Evaluation of Slime-Producing Bacteria in Oil Field Core Flood Experiments

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Epifluorescence microscopy and carbohydrate determinations indicated that the decrease in permeability of oil reservoir sand to reclaimed sewage water was partially the result of biological plugging. Filtration and biocide addition studies demonstrated that the increase in bacterial densities and slime concentrations in flooded oil field cores appeared to be due to both deposition from the reclaimed water and in situ microbial growth and slime production. Although these biological components increased throughout the cores during flooding, the region where the water entered the core exhibited the highest cell densities and slime concentrations. The approach described in this report should be useful in predicting the potential of a water source to induce biological plugging of oil reservoir sand.

High-quality water is a requirement for the successful application of the reservoir-flooding technique used in the secondary recovery of oil. Projections of future water shortages in southwestern areas of the United States, however, have encouraged exploration for new water sources, some of which will require upgrading for injection purposes. Although efforts are being made to identify and correct characteristics of test water which promote losses during injection, current procedures do not adequately assess the potential of the water to induce biological plugging in reservoir sands. Core permeability testing alone cannot identify the cause of sand plugging.

Most studies have focused on the bacteria present in the test water, rather than on organisms which specifically accumulate in the plugged zone (2, 4, 15). When core floods have been used to simulate a down-hole environment, accurate, quantitative microbiological evaluation has been hampered by the inherent limitations of the culture techniques used for bacterial enumeration and isolation (10, 12, 17).

The microbiological processes that contribute to injection losses are likely to vary from one water flood to another, depending on reservoir and water characteristics. A better understanding of these plugging processes is presently needed for the development of more cost-effective water treatments. The use of more sensitive analytical methods for the evaluation of biological plugging of injection wells should reduce the uncertainty that currently exists in biocide selection, concentration, and dose scheduling for various flood operations.

We used epifluorescence microscopy and carbohydrate measurements to evaluate microbial plugging of reservoir sand cores that had been flooded with reclaimed water. This study also demonstrates a means by which biocides can be tested under conditions that approximate the down-hole environment.

MATERIALS AND METHODS

A core permeability test apparatus (PTA) similar to that described by McCune (13) was used to flood cores (diameter, 5 cm; length, 15 cm) of hand-packed reservoir sand obtained from Aminoil well no. 8 in the lower main zone of the Huntington Beach, Calif., field with reclaimed sewage water that had received upgraded secondary treatment at the Los Angeles County Sanitation District Long Beach Water Renovation Plant (Table 1). The hand-packed cores exhibited porosity values that were slightly higher than the 28 to 29% porosity level estimated for sand in the undisturbed formation. The PTA supplied several cores with test water at the desired temperature from a wastewater line at the above-mentioned plant. When elevated test water temperatures were required, the water delivery tubing and the cores were immersed in a water bath equilibrated to the desired temperature. Constant head pressure and back pressure (Δp) across each core were maintained by a pressure relief valve so that permeability changes in the sand pack could be monitored by the change in the flow rate of water through the core. Flow rates were monitored by determining the volume of effluent collected in a graduated cylinder over a 5-min period.

The porosity of each core was determined by an initial flooding of the cores with 500 pore volumes of tap water to achieve saturation. This method of saturation was found to have no significant effect on core permeability (Fig. 1). The cores were then flooded with test water. Initial permeability values were determined by using Darcy's equation after the measurement of initial flow rates of test water through the cores. The flow rate and volume of test water passing through each core were monitored until the former decreased to 5 to 10% of the initial value or became constant at $>10\%$ of the initial permeability.

When permeability values decreased to the levels described above, the cores were processed for microbiological analysis. Wet sand (0.5 g) was aseptically weighed and transferred to sterile microcontainers (Eberbach Corp.) containing 10 ml of sterile distilled water, and the contents were homogenized in a blender for 40 s to disperse bacterial aggregates. The cell suspension was serially diluted, and a filter-sterilized solution of the fluorochrome acridine orange was added to each dilution at a final concentration of 10 μg/ml to stain the bacteria. A 2-ml volume of the appropriate dilution was filtered through a darkened polycarbonate membrane (pore size, 0.2 μm; diameter, 25 mm). The membrane was transferred to a drop of immersion oil on a microscope

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TABLE 1. Constituents of reclaimed water used in core flood experiments[a]

Constituent	Concn (mg/liter)
Na	172
Ca	55
Mg	14
Cl^-	147
SO_4^{2-}	160
HCO_3^-	150
Total dissolved solids	990
Dissolved O_2	4

[a] Based on data from the Long Beach Water Reclamation Plant and the *Los Angeles County Sanitation District Annual Report*, 1981. pH of the water was 7.3.

TABLE 2. Relationship between suspended-solid content of injection water and permeability of cores to the water

Test start date (mo/day/year)	Suspended-solids content of water (mg/liter)[a]	Amt (liters) of water causing 50% reduction in permeability
11/25/81	2	50
12/10/81	3	30
12/28/81	2	25
1/12/82	4	15
1/30/82	3	19
5/3/82	2	57
7/7/82	2	32
8/16/82	2	20
9/8/82	3	38

[a] Data from the Los Angeles County Sanitation District, Long Beach Water Reclamation Plant Annual Report, 1981.

slide and overlaid with a glass cover slip. The bacteria on the membrane were enumerated at a magnification of ×1,000 by using a Zeiss epifluorescence microscope (14).

Dilutions of the homogenized cell suspension were also prepared for determination of CFUs and for isolation of bacteria with either plate count agar (medium A) (Difco Laboratories) or a medium consisting of $NaNO_3$ (0.2 g), K_2HPO_4 (0.2 g), $CaCl_2$ (0.03 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), $FeSO_4$ (0.001 g), sodium citrate (0.5 g), sodium succinate (0.2 g), yeast extract (0.5 g), sucrose (5.0 g), agar (15 g), and distilled H_2O (1 liter), with a pH of 7.9 (medium B). Bacterial CFUs were enumerated after 5 days of incubation at a temperature which corresponded to that of the water used for the core floods. Bacteria from dominant colony types were examined microscopically after being stained with India ink.

Carbohydrate determinations for dilutions of homogenized core material were performed by the phenol-sulfuric acid assay (19). The ATP content of the core material was determined by the luciferin-luciferase assay after extraction of the core material with McIlvaine buffer (3). The iron content of the core material was determined by the phenanthroline method after the conversion of Fe^{3+}. Residual chlorine was measured by titration (1).

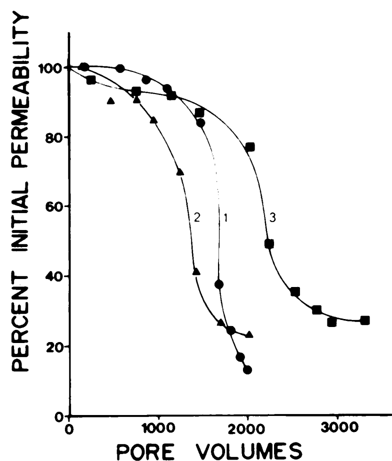

FIG. 1. Permeability profiles for replicate cores flooded with tap water. Porosity values were 34, 32, and 36% for cores 1, 2, and 3, respectively. Initial permeability values were 920, 810, and 1,490 millidarcies for cores 1, 2, and 3, respectively. Δp, 0.68 atm. The suspended-solid content of the water at the time of flood initiation was 2 mg/liter.

RESULTS AND DISCUSSION

Our initial results indicated that replicate cores, flooded simultaneously with reclaimed water, exhibit similar permeability profiles. The mean number of pore volumes of water that passed through the cores (injection volume) before the permeability level decreased to 25% of the initial value had an average standard deviation of 11% of the mean injection volume, determined by evaluation of two replicate cores in each of the seven experiments. In contrast, cores prepared in the same manner as the replicates mentioned above but flooded at different times exhibited permeability profiles in which the average standard deviation was 56% of the mean ($n = 7$). The differences in the profiles could not be attributed to variations in core porosity. The level of suspended solids in the water used for injection varied during the time that the experiments were performed (Table 2). It is likely, therefore, that core plugging was influenced by the fluctuation over time of the level of suspended solids in the reclaimed water. These temporal variations in the level of suspended solids, however, may have been a reflection of changes in the operational efficiency of the water renovation plant.

Inorganic constituents such as iron were uniformly distributed throughout cores plugged by reclaimed water, with amounts ranging from 3.6 mg of Fe^{3+} per g of dry sand at the top to 3.4 mg of Fe^{3+} per g of dry sand at the bottom of the cores. In addition, the iron concentration in sand recovered from plugged cores was not significantly different from that in unflooded sand (mean of 3.2 mg of Fe^{3+} per g of sand). Thus, core plugging did not appear to be due to precipitation and accumulation of iron sulfides.

The bacterial density in reservoir sand, based on direct enumeration by epifluorescence microscopy, was low (<10^7 cells per g of wet sand) before the core was flooded. A high cell density was observed, however, after the cores were flooded with reclaimed water (Table 3). In addition, a bacterial density gradient was observed in which the highest bacterial concentration was obtained at the water inlet region (top) and the lowest concentration was at the water outlet region (bottom) of the 15-cm-long cores. Since the cores were evaluated by direct microscopic examination, which does not detect physiological differences within the microbial population, bacterial density counts were based on both living and dead cells in the sample. Procaryotic cells were the only life forms observed during direct microscopic examination of sand from the flooded cores.

Bacterial recoveries at the core tops measured 1.5×10^8 and 1.3×10^8 CFU per g of wet sand with media A and B,

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TABLE 3. Characteristics of reservoir sand cores flooded with reclaimed sewage water[a]

Treatment	Pore vol	Core section tested	Mean bacterial density ± SD (cells/g of wet sand)[b]	Slime (μg/g of dry sand)
None	500	Top	$(4.5 \pm 0.9) \times 10^9$	400
		Middle	$(2.9 \pm 0.7) \times 10^9$	<50[c]
		Bottom	$(1.6 \pm 0.4) \times 10^9$	<50
Filtration	2,000	Top	$(2.9 \pm 0.5) \times 10^9$	ND[d]
		Middle	$(1.9 \pm 0.4) \times 10^9$	ND
		Bottom	$(1.2 \pm 0.2) \times 10^9$	ND
Hypochlorite	1,500	Top	$(3.0 \pm 0.5) \times 10^9$	240
		Middle	$(2.1 \pm 0.5) \times 10^9$	130
		Bottom	$(1.6 \pm 0.4) \times 10^9$	110
Hypochlorite and filtration	2,700	Top	$(1.4 \pm 0.3) \times 10^9$	80
		Middle	$(1.0 \pm 0.2) \times 10^9$	60
		Bottom	$(8.1 \pm 1.2) \times 10^8$	<50

[a] Δp across cores was 0.68 atm.
[b] $n = 2$.
[c] Limit of detection.
[d] ND, Not determined.

respectively; this indicated that approximately 3% of the cells detected by direct microscopic examination were viable. These viable-cell densities are higher than those determined by other studies (9). Measurements of ATP, which is also an indicator of living biomass (3), corroborated this estimate. If there is 1.2×10^{-10} μg of ATP per bacterial cell (16), the 16.4 ng of ATP per g of wet sand recovered from the top of the cores corresponds to 1.4×10^8 cells, a density consistent with the results from the above-mentioned cultural methods.

Bright-field microscopic examination of India ink-stained preparations of bacteria obtained from the plugged cores revealed extensive capsules or slime layers around individual cells and cell aggregates. Bacterial slime, in many instances, is composed of extracellular polysaccharides (5, 8). Since previous work indicated that carbohydrate determinations from the phenol-sulfuric acid assay provided quantitative estimates of biologically produced slime (7), this method was used to evaluate core plugging due to bacterial slime production. The high carbohydrate concentrations at the water inlet region of the plugged cores (Table 3) confirmed the presence of slime-producing bacteria. These results support the work of others (10, 18) and indicate that in situ plugging occurs mainly at the face of the formation around the well bore.

To determine whether the high density of bacteria in plugged cores was due to entrainment of cells from the water or was the result of in situ bacterial growth, the reclaimed water was filtered (0.2-μm nominal pore size) immediately before injection. This filtration treatment typically removes most suspended solids, including bacterial cells, from solution. When water containing suspended solids was filtered before injection, a 30% decrease in permeability occurred during injection of the first 500 pore volumes of filtered water, but permeability changed very little thereafter during injection of an additional 1,500 pore volumes of the fluid (Fig. 2). In contrast, permeability dropped to less than 5% of the initial value when 500 pore volumes of unfiltered water were injected through replicate cores tested at the same time. These data suggest that deposition of suspended solids accounts for the bulk of the permeability loss in cores flooded with unfiltered reclaimed water.

Bacterial densities at the inlet end of cores flooded with 2,000 pore volumes of filtered water were lower than those obtained from cores flooded with 500 pore volumes of unfiltered water (Table 3). It is likely, therefore, that bacteria were one of the components of the suspended solids which were deposited in the core and thus contributed to the observed permeability loss. Since the relative abundance of bacteria and the ratio of bacteria to other suspended solids in the reclaimed water were not determined, we could not determine the importance in core plugging of bacterial cells relative to that of the other components of the fraction of suspended solids.

The recovery of high bacterial densities (2.9×10^9 cells per

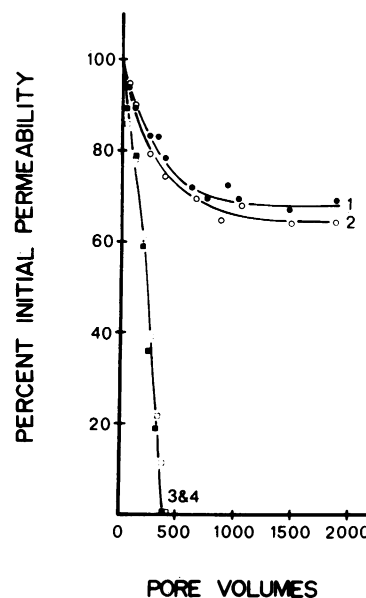

FIG. 2. Permeability profiles for cores flooded with reclaimed water which had been passed through a filter of 0.2-μm nominal pore diameter (cores 1 and 2) or with unfiltered reclaimed water (cores 3 and 4). The porosity value for all cores was 31%. Initial permeability values were 320 millidarcies for cores 1, 3, and 4 and 340 millidarcies for core 2. Δp, 0.68 atm. The suspended-solid content of the water at the time of flood initiation was 4 mg/liter.

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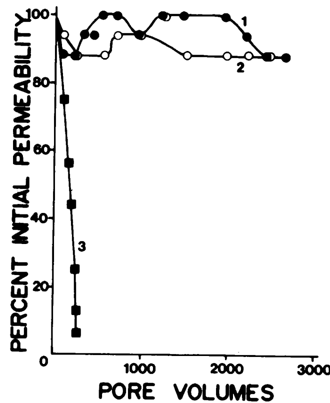

FIG. 3. Permeability profiles for cores flooded with reclaimed water which had been passed through a filter of 0.2-μm nominal pore diameter and treated with sodium hypochlorite (cores 1 and 2) or with unfiltered, untreated reclaimed water (core 3). Porosity values were 32% for cores 1 and 3 and 31% for core 2. Initial permeability values were 260 millidarcies for core 1 and 280 millidarcies for cores 2 and 3. Δp, 0.68 atm. The suspended-solid content of the water at the time of flood initiation was 3 mg/liter.

g of wet sand) from cores injected with filtered reclaimed water indicates that in situ bacterial growth occurred on the surfaces of the sand particles during flooding. These bacteria probably developed from those that were introduced during sand recovery and core preparation. Attempts to flood autoclave-sterilized cores with filter-sterilized reclaimed water to evaluate permeability in the absence of bacteria were unsuccessful. Approximately 10^8 bacteria per g of sand accumulated in the inlet end of sterilized cores flooded with 2,000 pore volumes of reclaimed water that had been sequentially filtered through presterilized cartridge filters (8.0-, 0.45-, and 0.2-μm pore size). Apparently, some bacteria penetrated the filters and established themselves downstream in the sand pack. Thus, it was not possible to demonstrate unequivocally that the reductions in bacterial density and slime levels accompanying the filtration treatment were responsible for the improved permeability of the sand. The use of UV irradiation may be a more realistic alternative for achieving reduced bacterial densities in the injection water. However, the equipment needed to test this approach was not available during this study. Recently, Jenneman et al. (11) showed that dry-heat sterilization was superior to autoclave sterilization of consolidated Berea sandstone cores.

An alternative approach to the elimination of in situ growth of bacteria in the cores was attempted by the application of the biocide sodium hypochlorite. Cores which received filtered reclaimed water that had been continuously dosed with sodium hypochlorite (minimum dose, 237 ppm [237 μl/liter] of residual chlorine [as measured in the water that had exited the core]) maintained 90 to 100% of their initial permeability after being flooded with 3,000 pore volumes of this type of water (Fig. 3). Under these conditions, bacterial density and slime (carbohydrate) levels at the top of the cores were reduced by 69 and 79%, respectively, when compared with levels from plugged cores flooded with untreated reclaimed water (Table 3). In fact, bacteria and slime levels were substantially reduced throughout the cores when the water was filtered and treated with the biocide. Although bacterial densities were reduced by the treatment, it was still not possible to completely eliminate bacteria from

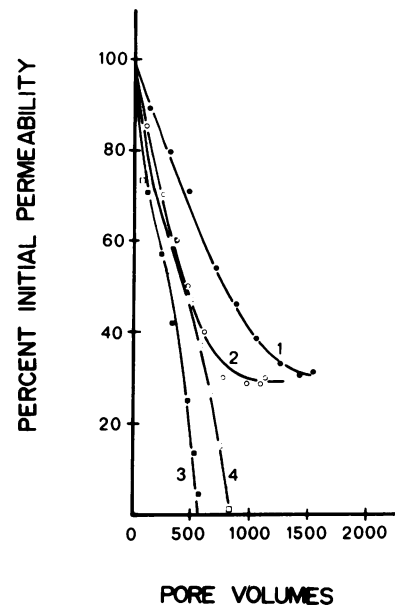

FIG. 4. Permeability profiles for cores flooded with hypochlorite-treated reclaimed water (cores 1 and 2) or with untreated reclaimed water (cores 3 and 4). Porosity values were 32% for cores 1 and 4 and 31% for cores 2 and 3. Initial permeability values were 420, 350, 530, and 420 millidarcies for cores 1, 2, 3, and 4, respectively. Δp, 0.68 atm. The suspended-solid content of the water at the time of flood initiation was 2 mg/liter.

the flooded cores or even to reduce their number to that observed before flooding. The data indicate, nevertheless, that whenever a reduction in bacterial and slime concentrations occurred, there was a concomitant improvement in permeability of 90 to 100% of the initial value. The results also indicate that the cores were able to accommodate an accumulation of bacteria above that present in unflooded sand (i.e., 1.4×10^9 cells per g of sand according to data obtained from cores flooded with filtered, biocide-treated water) without permeability being noticeably affected. If bacterial density increased to approximately 3.0×10^9 cells per g of sand, however, permeability was decreased.

The continuous addition of hypochlorite to unfiltered reclaimed water during core injection also resulted in an improvement of permeability greater than that obtained with untreated water but less than that achieved by the combined filtration and biocide treatments. When hypochlorite was added continuously to water to obtain residual chlorine levels similar to those achieved previously, core permeability decreased during injection of the first 1,000 pore volumes of reclaimed water but remained constant thereafter at approximately 30% of the initial permeability (Fig. 4). Efforts to achieve a higher final permeability by increasing the hypochlorite concentration to 400 ppm or more resulted in the formation of a precipitate at the water inlet end of the core and an immediate and complete loss of permeability (data not shown). Chemical analysis of the precipitate by atomic emission spectroscopy indicated that it was composed primarily of calcium and phosphorus and, therefore, was probably a hydrous calcium phosphate. This salt was not detected in significant amounts in plugged cores flooded with the lower concentrations of hypochlorite. These results demonstrate that a reduced but constant injection was achieved when a modest dose of hypochlorite alone was used to treat the reclaimed water.

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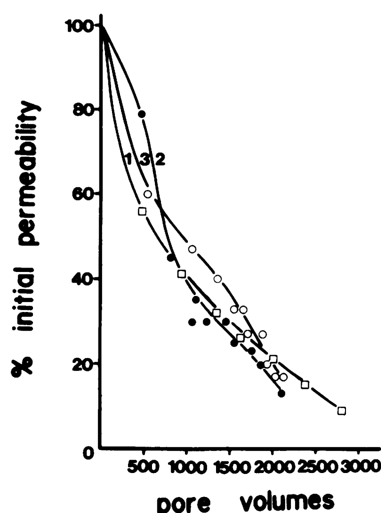

FIG. 5. Permeability profiles for cores flooded with untreated reclaimed water maintained at 20°C (core 1) or with the same type of water heated to 52°C (cores 2 and 3). Porosity values were 36, 34, and 32% for cores 1, 2, and 3 respectively. Initial permeability values were 475, 148, and 111 millidarcies for cores 1, 2, and 3, respectively. Δp, 1.7 atm. The suspended-solid content of the water at the time of flood initiation was 2 mg/liter.

Bacterial densities and slime levels were also reduced in cores flooded with hypochlorite-treated unfiltered water, compared with those receiving untreated water (Table 3). Oxidizing biocides such as sodium hypochlorite not only prevent the growth of microorganisms, but also hydrolyze the extracellular polysaccharides produced by the cells for attachment to surfaces (5). Control of bacterial slime development in the cores during water injection was, therefore, probably due to the abililty of this biocide to impede both attachment and growth of bacteria on the surfaces of the sand particles.

The reason for the initial rapid decrease in permeability of the filtered, biocide-treated cores is not presently understood. Although we do not have mineral composition data for the sand used in this study, the sand exhibited properties similar to those of sand obtained from a different well that contained only trace amounts of clay (such as montmorillonite) and had a cation exchange capacity of 0.015 meq/g (V. T. Lieu, unpublished report). Thus, initial loss of permeability was probably not due to clay swelling. Nevertheless, there appears to be some incompatibility between the sand and reclaimed water that does not exist with tap water.

Although the temperature of reclaimed water generally ranges from 18 to 22°C upon discharge from the treatment plant, its temperature upon injection into the formation is likely to be considerably higher because of mixing with hot recycled water. The water temperature in the injection well, and hence in the formation around the well bore in the upper main zone of the Huntington Beach field, is approximately 52°C. Since this and other studies indicated that formation plugging occurs primarily at the site of water injection, a core flood experiment was conducted at 52°C to simulate the temperature at the formation face.

Core flooding performed at 52°C produced permeability profiles similar to those produced at 20°C (Fig. 5). However, bacterial densities at the tops of cores flooded with water at 52°C were significantly higher than those in the same region of cores flooded with water at 20°C, as determined by direct microscopic enumeration (Table 4). Slime concentrations at the tops of the cores were also higher at the elevated temperature (Table 4). In contrast, bacterial densities, as measured by CFUs on either medium A or B, were noticeably lower at the higher temperature. These data suggest that the elevated water temperature selected for a population of bacteria that did not grow on the culture media used in this investigation. Since the results of this and other (6, 20) studies suggest that microbial growth, attachment, and extracellular polysaccharide production are influenced by temperature, core permeability tests should be conducted at injection water temperatures for accurate evaluation of biological plugging of formation sands.

The capacity of all cores used in our temperature study to accept additional pore volumes of untreated water and to accumulate higher concentrations of bacteria and slime than were seen in previous experiments was probably due to the increased Δp (1.7 atm [1 atm = 101.29 kPa]) across the cores. Although this modification was intended to increase the sensitivity to the PTA to small changes in core permeability, it also demonstrated that pressure may influence biological plugging processes. Unfortunately, the PTA used in this study was not capable of operating at the high down-hole pressures (50 to 350 atm) where plugging occurs. However, the Δp (1.7 atm) across the 15 cm of sand in the cores used in this study is approximately the same as that which occurs across a comparable distance of formation sand subjected to down-hole water flood (unpublished data).

Other environmental parameters such as O_2 and SO_4^{2-} concentrations and E_h may also influence bacterial growth

TABLE 4. Effect of water temperature on bacterial densities and slime concentrations in cores[a]

Sample location in core	Mean bacterial density ± SD at:				Slime concn (μg of carbohydrates/g of dry sand)	
	20°C		52°C			
	Direct count (10^9)[b]	Plate count (10^8)[c]	Direct count (10^9)[b]	Plate count (10^6)[c]	20°C	52°C
Top	2.1 ± 0.5	1.6[d] 1.4[e]	5.3 ± 1.1	3.5[d] 4.6[e]	300	510
Middle	2.9 ± 0.5	ND[f]	3.7 ± 0.7	ND	<50[g]	<50
Bottom	2.1 ± 0.4	ND	1.5 ± 0.3	ND	<50	<50

[a] Δp, 1.07 atm. Samples taken after injection of 2,200 pore volumes of water.
[b] Cells per gram of wet sand. n = 2.
[c] CFU per gram of wet sand.
[d] On medium A.
[e] On medium B.
[f] ND, Not determined.
[g] Limit of detection.

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and slime production around the well bore. Although these factors were not included in our investigation, they can be controlled by the PTA and should be considered before final recommendations on the acceptability of a water source for injection are made.

In conclusion, the test procedures described here provide a means of evaluating the potential of a water source to stimulate biological plugging of sand from oil formations which are being considered for water flooding. These methods also provide a means of evaluating the effectiveness of various water treatments for controlling biologically related permeability problems before these treatments are tested in the field.

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