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Effects of biocide treatment and backflow pressure on the permeability of microbially fouled model cores

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SUMMARY

Sintered glass bead cores were plugged until the permeability was reduced to 1% or less of the original permeability by the injection of a slime-producing bacterium isolated from produced water. Scanning electron microscopy of fractured core sections showed that the bacteria were predominantly located in the uppermost sections, around the core inlet. Killing the bacterial cells in the plugging biofilm, using a biocide, had little effect on core permeability. The dead cells were only removed when backflow pressure, simulated by inversion of the test core followed by fluid injection, was applied and maintained at 55–69 kPa. Backflow of plugged cores containing live bacteria produced transient pressure-dependent increases in permeability that were proportional to the backflow pressure applied. We conclude that only sustained backflow procedures reduced permeability: such operations are not effective for oil recovery in field conditions.

INTRODUCTION

Secondary oil recovery by water flooding increases production but it has associated problems. Water injected into oil reservoirs contains a diversity of microorganisms which can block pores within the rock. The problems caused by microorganisms reaching rock strata were considered by Hart et al. [6] and Myers and Samiroden [9] to be two-fold. Firstly, the bacteria penetrate and grow in the rock pores causing blockages or 'plugging', and secondly, their metabolic by-products block the pore spaces. A study by Myers and Sladyj [10] assessed the microbial content of 471 samples of injection water and reported the presence of sulphate-reducing, iron-reducing, slime-forming and filamentous bacteria. The microbial colonization of surfaces in aquatic environments involves polysaccharide production [5] and the formation of confluent biofilms made from polysaccharide-containing materials called glycocalyx [3,4]. Slime production by microbes in injection water has been demonstrated to plug rock pores in both producing oilwells [2] and laboratory model core systems [14]. Several methods have been employed to remove bacterial plugs in rock matrices. Clementz et al. [2] used various combinations of treatments to reverse bacterial plugging, including bleach, acids, back-

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flow of liquids and water injection. Kalish et al. [8] restored some permeability to plugged sandstone in laboratory model cores using acid treatment and backflow. Ruseska et al. [13] used models of oilfield pipelines colonized by bacteria to assess the impact of several biocides at different concentrations on biofilm removal. This work reports a study using model core systems to assess the efficacy of biocide treatment and/or backflow to reverse bacterial plugging by a slime-producing *Pseudomonas* species isolated from oilfield water.

MATERIALS AND METHODS

Cores were made from 90-μm diameter glass beads randomly packed by vibration in Pyrex vials and fused by heating at 680°C for 2 h. All cores were cylindrical and of a uniform size: 5 cm long and 1 cm in diameter. The porosity of the cores was between 36% and 40% and their permeability was between 6.0 darcys and 7.0 darcys. After gas sterilization (Steri-Vac 202, 3 M) the cores were evacuated in a vacuum chamber at 0.001 torr for 40 min, saturated in growth medium and mounted in core holders as described by Shaw et al. [14].

A *Pseudomonas* species [12], isolated from an oilfield water, was used for all experimental work. The bacterium is aerobic, rod-shaped, with a diameter of 0.5 μm and a length of 1.0 μm, and it produces exopolysaccharides. The isolate was grown in 4-liter batch cultures of filter-sterilized sodium citrate medium containing ($g \cdot l^{-1}$ glass-distilled water): $Na_3C_6H_8O_7$, 7.36; $(NH_4)_2SO_4$, 3.30; KH_2PO_4, 7.30; K_2HPO_4, 9.22; $MgSO_4$, 0.12; $FeCl_3$, 0.0041 [12]. The pH of the growth medium was 7.0. The *Pseudomonas* species was incubated at 25°C with orbital shaking at 120 rpm. When the bacterial growth reached the late exponential phase, as determined by absorbance readings, the culture was positioned in a 5-liter reservoir above the core holder. The reservoir was aseptically connected to the core holder using sterile tubing (Tygon) fitted to a drain pipe at the bottom of the reservoir. Culture homogeneity was maintained by a magnetic stirrer. Filtered nitrogen gas was introduced to the culture reservoir by a port in the stopper. The gas supplied a constant pressure of 25 kPa and forced the culture through the core. The culture injection rate was recorded throughout each plugging procedure by the collection of the core effluent in graduated glass vials. Core permeability was calculated by dividing the flow rate at a given time (K) by the initial flow rate (K_i) and expressed as a percentage. The flow rate decreased with time and the plugging procedure was completed when the permeability was 1% or less of the original value. The plugged cores were numbered one to five for separate treatment.

Backflow operations were simulated by disconnecting the culture reservoir, aseptically removing the plugged core from the holder, inverting the core and replacing it in the core holder. A second reservoir containing sterile distilled water was connected to the core holder, initially at a pressure of 25 kPa. Backflow pressure was gradually increased until the core permeability was restored. Isothiazolone, a broad-range antimicrobial agent containing 5-chloro-2-methyl-4-isothiazolin-3-one (1.15%, w/v) and 2-methyl-4-isothiazolin-3-one (0.35%, w/v), was used as a solution containing 1.5% (w/v) of active chemicals. This product was used as the 'Kathon WT' formulation supplied by Rohm and Haas, Philadelphia.

As an experimental control, core 1 was not treated with biocide and various backflow pressures were used to remove the bacterial plug from the core. Core 2 was treated with a 10-ml volume of 15 ppm isothiazolone (0.225 ppm active chemical) at an injection pressure of 25 kPa and left to soak in biocide for 4 h. Various backflow pressures were then applied to core 2 until permeability was restored. Core 3 was treated with 230 pore volumes (PV) of 50 ppm isothiazolone (0.75 ppm active chemical) at 25 kPa for 61 h. Backflow pressure was then applied to core 3 until the permeability was restored. Core 4 was treated with 593 PV of 100 ppm isothiazolone (1.5 ppm active chemical) for 4 h at 25 kPa. The biocide reservoir was then disconnected from the core holder and the core was washed with sterile distilled water at an injection pressure of 69 kPa. The washed core was inverted

and a backflow of sterile water was applied to the core at 69 kPa. Core 5 was inverted directly after core plugging was completed and was then treated with 620 PV of 100 ppm isothiazolone (1.5 ppm active chemical) for 4 h at a backflow pressure of 25 kPa. The biocide reservoir was disconnected and further backflow operations were undertaken with sterile distilled water at pressures up to 48 kPa. Core permeability was monitored throughout biocide treatment and backflow operations.

When all of the experimental work was completed, each core was removed from the holder and broken into six sections along the core length using a chisel following shallow scoring with a diamond blade saw. Each section was prepared for direct scanning electron microscopy by glutaraldehyde fixation and gold-palladium coating as previously described [14]. During treatment of each of the cores, samples were aseptically removed from the bacterial culture reservoir and the core effluent and spread onto brain heart infusion (BHI) agar plates. The plates were incubated at 25°C for 48 h and colony counts were recorded.

RESULTS

Core 1 was injected with 2359 PV of a culture containing 8.85×10^7 cfu/ml *Pseudomonas* sp. Over a 48-h injection period the core permeability decreased to 0.2% of its original permeability. Removal of the bacterial plug was attempted without the use of biocides. After core inversion sterile distilled water, at a backflow pressure of 25 kPa, restored permeability to 43% of the original value but this was not sustained (Fig. 1A). With continuous backflow for a further 550 PV the core blocked again and permeability was reduced to 27%. A pressure increase to 35 kPa increased the core permeability to 60%. Full core permeability was restored by the application of 55 kPa backflow pressure. The core retained full permeability at this pressure; however, when the pressure was decreased to 25 kPa, rapid plugging was observed with an associated decrease in permeability to 50% (Fig. 1A). Direct scanning electron microscopy (SEM) of the

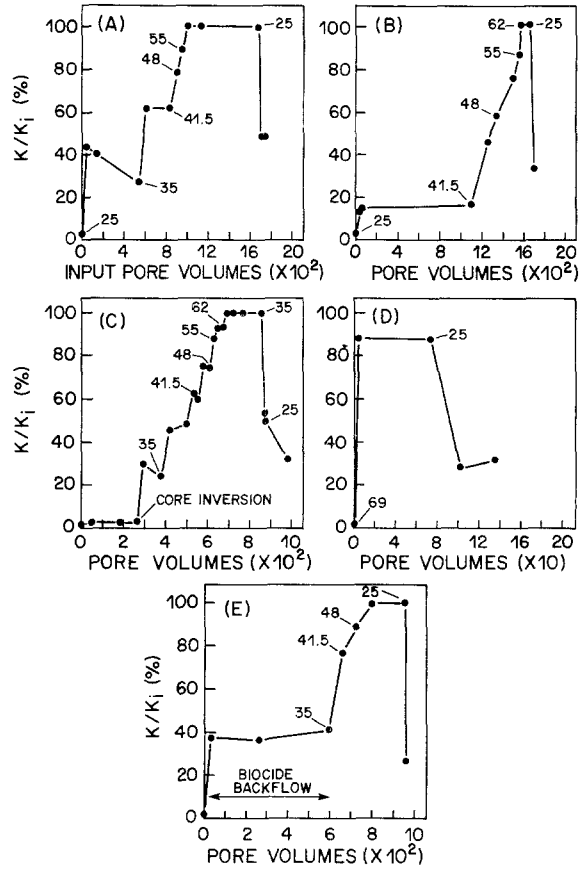

Fig. 1. The restoration of permeability to the five cores using a backflow of distilled water. The changes in pressure of the backflow are indicated in kPa. A, core 1; B, core 2 was soaked in 15 ppm for 4 h before backflow operations commenced; C, core 3 was treated with 230 PV of 50 ppm isothiazolone at 25 kPa for 61 h before core inversion; D, core 4 was treated with 593 PV of isothiazolone for 25 kPa then with sterile distilled water at 69 kPa before core inversion and backflow operations commenced; E, core 5 was inverted after completion of core plugging and treated with 630 PV of 100 ppm isothiazolone for 4 h at a backflow pressure of 25 kPa. Backflow operations were continued with sterile distilled water.

core sections showed that very few bacteria were present throughout the core. The majority of the *Pseudomonas* species were located at the core inlet (Fig. 2) and dehydration-condensed exopolysaccharide slime was observed around the bacteria on these surfaces.

The permeability of core 2 was reduced to 1% of the original value by the injection of 1119 PV of

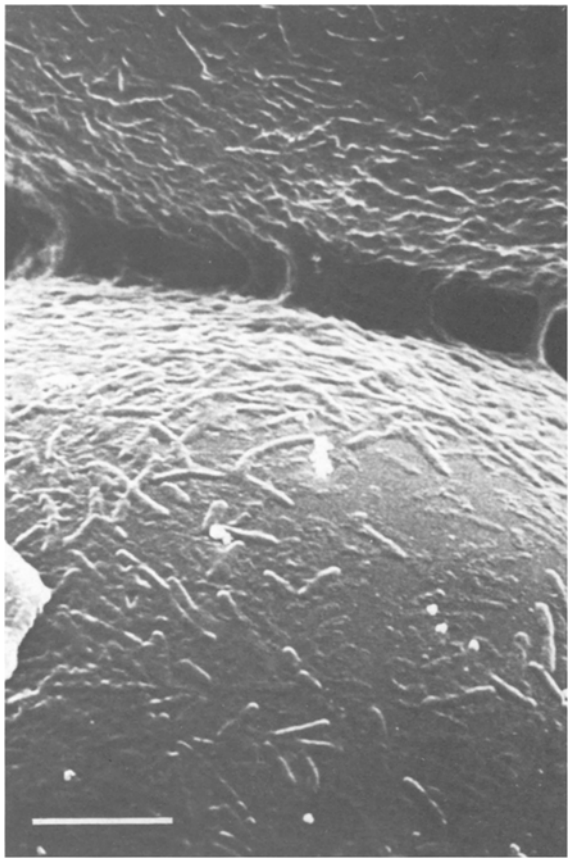

Fig. 2. Direct SEM of the inlet of core 1. The bar represents 5 μm.

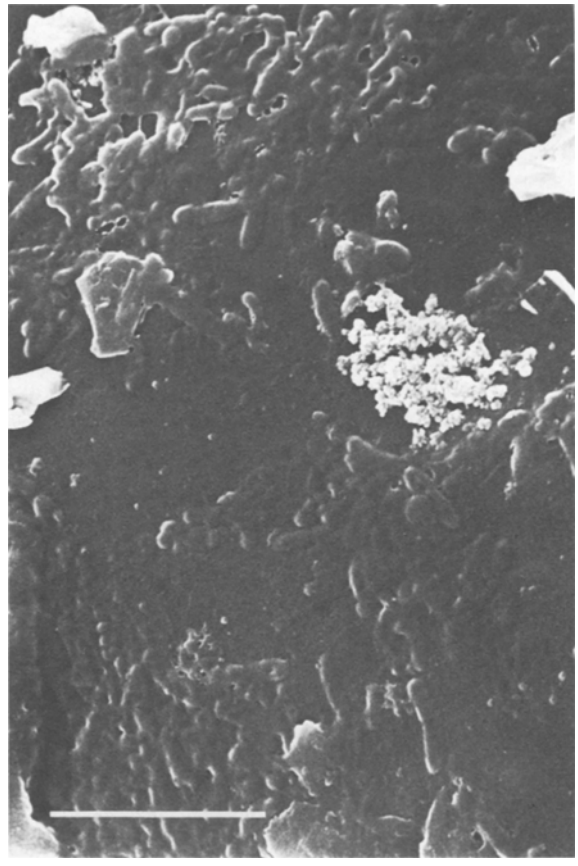

Fig. 3. SEM micrograph of core 2. The bar represents 5 μm.

a culture containing *Pseudomonas* sp. (viable cell count 4.61×10^7 cfu/ml) over 52 h. The core was then soaked in 10 ml of biocide for 4 h and the permeability remained at 1% (Fig. 1B). After the core was removed and inverted, the injection of sterile distilled water as a backflow at 25 kPa increased permeability to 15%. It was necessary to increase the backflow pressure to 48 kPa to give 60% core permeability. Backflow pressure of 62 kPa restored full permeability, but this was drastically reduced to 33% when the pressure was lowered to 25 kPa. In comparison with the untreated core (Fig. 1A), at each backflow pressure investigated the core permeability was less after biocide treatment. SEM of core section showed slime-encased rod-shaped cells on the glass beads of the inlet surfaces (Fig. 3). No bacteria were detected by SEM in any of the deeper core sections. Sampling of core effluent onto BHI agar demonstrated that no live bacteria were washed from core 2 after biocide treatment.

Having observed that the biocide treatment of core 2 at 15 ppm was unsuccessful in assisting plug removal, the effects of higher concentrations were examined. The permeability of core 3 was reduced to 0.5% in 48 h using 961 PV of a culture containing *Pseudomonas* species at a viable count of 1.03×10^8 cfu/ml. The core was washed with 50 ppm isothiazolone but an injection of 250 PV of the biocide failed to improve the permeability (Fig. 1C). Core inversion and backflow water injection at 25 kPa raised permeability to over 30%. A backflow pres-

sure of 62 kPa restored full permeability. However, the core plugged rapidly when the injection backflow was decreased, initially to 35 kPa then to 25 kPa. SEM of core 3 revealed the presence of the *Pseudomonas* sp. in the upper core sections (Fig. 4); the remaining sections did not contain any bacteria. No live cells were observed when the core effluent was sampled onto BHI agar.

Core 4 was injected with 2127 PV of a culture of *Pseudomonas* sp. (2.02×10^7 cfu/ml) and the permeability was reduced to 0.8% of the original value in 42 h. The experimental procedure was modified to investigate the accumulative effects of biocide treatment and injection pressure on bacterial plugging prior to the backflow operation. After biocide treatment at 100 ppm an injection pressure of 69 kPa was applied to the core but the permeability was not restored. However, when the core was inverted and a backflow pressure of 69 kPa applied, permeability was restored to 88% (Fig. 1D). This demonstrated the importance of the direction of the applied pressure on successful bacterial plug removal. When the backflow pressure was reduced to 25 kPa the permeability decreased to 32%. No colonies were detected when samples of the core effluent were plated onto BHI agar, demonstrating that no live bacteria were washed from the core after biocide treatment.

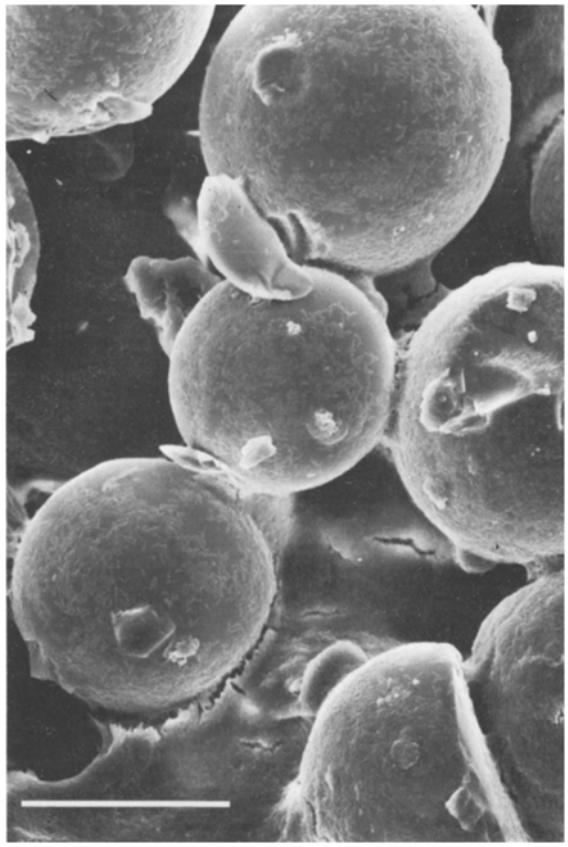

Fig. 4. SEM micrograph of core 3. The bar represents 50 μm.

Core 5 was plugged with 2828 PV of a culture containing *Pseudomonas* sp. (6.9×10^7 cfu/ml). Over a 48-h injection period the core permeability was reduced to 0.2% of the original value. The core was inverted and treated with 100 ppm isothiazolone at 25 kPa. The biocide backflow treatment was continued for 620 PV and permeability increased from $<1\%$ to 41% (Fig. 1E). Continued backflow pressure at 25 kPa failed to increase core permeability above 41%. Water injection at backflow pressure of 35–48 kPa completely cleared the bacterial plug and restored full permeability. Again, a decrease in backflow pressure to 25 kPa caused a corresponding loss in permeability. Sampling onto BHI agar demonstrated that no viable *Pseudomonas* species survived the isothiazolone application.

DISCUSSION

Aquatic microorganisms adhere to solid surfaces and colonize them by forming biofilms composed of cells and exopolysaccharides [5]. Bacterial production of polysaccharides and slimes in oil-bearing rocks causes core plugging [2,14], and the biofilms are strengthened by the capture and entrapment of inert particles present in well injection waters [8]. The cores used in this study were made of sintered glass beads that model the pore and throat size of oil reservoir rock, but without having the variety of constituents of solid matrices that may cause plugging problems. Therefore, the permeability changes in the cores could only be attributed to the injection of microorganisms into them followed by biocide treatment and high-pressure backflow operations.

A similar trend was observed for the five cores

tested; that is, the injection of bacteria caused a gradual reduction in core permeability to less than 1% of the original value. Permeability was only fully restored and maintained when backflow operations, simulated by core inversion, were applied at elevated pressures. Rapid reduction in backflow pressure from 55 to 25 kPa in core 1, plugged with live cells, and between 48 and 69 to 25 kPa in the biocide-treated cores caused a loss in core permeability. Some cells, protected in biofilms, may have survived biocide treatment and proliferated when the backflow pressure was reduced to 25 kPa. However, the rate of plug reformation was so rapid, completed within the addition of between 5 and 15 PV, as to exclude the possibility that microbial growth caused the permeability loss. Alternatively, we suggest that the rapid permeability losses observed when the backflow pressure was decreased were caused by the bacterial biofilm being displaced, but not dislodged, by the backflow pressure. As a consequence of reduced backflow pressure to 25 kPa the biofilm acted as a valve and closed again, lowering core permeability. Direct SEM confirmed that no bacterial cells remained in the pores after increased backflow pressure, but some cells remained on the glass bead surfaces.

Sections of cores containing either live cells or biocide-treated cells were shown by SEM to contain bacteria on the uppermost portions of the core, closest to the flow inlet. The predominance of cells by the inlet was the result of either backflow pressure removing the cells from the lower core sections or the pseudomonad preferentially forming skinplugs. Work in our laboratories has demonstrated that aerobic bacteria produce skinplugs in model core systems [14]. Jang et al. [7] showed a relationship between cell numbers injected onto cores and skinplug formation. Bacterial plugs formed only when suspensions containing cell counts of at least 5×10^7 cfu/ml *Pseudomonas putida* were used. Lower cell concentrations did not form plugs.

In this study the application of 15 ppm biocide was sufficient to kill all of the bacteria that were dislodged from the cores and reached the core effluent. Although the biocide killed the bacteria it did not remove the biofilms between the glass beads, and the cores remained plugged. All of the five cores, plugged with either live or biocide-killed cells, required approximately the same backflow pressure to restore permeability. The use of biocide assisted plug removal only when it was injected as a backflow. Other researchers have used backflow operations to partially restore core permeability, using solutions of 5% NaCl [11], or in conjunction with either acid [8] or bleach treatment of cores [2]. Increased injection pressure into model cores was not successful in clearing plugging for prolonged periods [14] and resulted in the formation of biofilms that could withstand higher pressure [1]. This study has shown that injection pressure at 69 kPa failed to restore permeability (core 4).

This work has demonstrated that the important factor in efficient plug removal was the application of sustained liquid backflow at pressures of 48–69 kPa. We conclude that the isothiazolone treatment was not efficient at removing bacterial plugs that were already established but may be used to keep rock matrices free from microbial contamination after other more successful plug removal procedures, such as acid or bleach treatment [2], have been completed.

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