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SELECTION OF AN EFFECTIVE BIOCIDE AND TOXICITY EVALUATION FOR A SPECIFIC MEOR MICROBIAL FORMULATION

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SELECTION OF AN EFFECTIVE BIOCIDE AND TOXICITY EVALUATION FOR A SPECIFIC MEOR MICROBIAL FORMULATION

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ABSTRACT

The two major environmental impacts associated with microbial enhanced oil recovery (MEOR) are possible contamination of surface and groundwater and contamination of agricultural land. Other potential environmental problems associated with MEOR processes include changes in indigenous microflora in reservoirs caused by injection of nonindigenous microorganisms and nutrient, or the possibility of injected microorganisms mutating and producing undesirable compounds under reservoir conditions.

A specific MEOR microbial formulation, NIPER 1 and 6, was first tested for pathogenicity and mutagenicity of its metabolic products. Pathogenicity testing included both oral ingestion and intraperitoneal injection of the NIPER 1 and 6 formulation using mice. The Ames test was used to determine any mutagenic tendencies of filtered NIPER 1 and 6 metabolic products. Although the MEOR formulation tested negative for both pathogenicity and mutagenicity, biocide tests were conducted to select an effective biocide in the event of a spill or environmental hazard when using this formulation in the field. An aqueous solution of 370 ppm formaldehyde was the most effective biocide for eradicating the microbial formulation. This biocide would be very effective for use in minor spills. However, formaldehyde is considered a toxic chemical and has even been implicated as a carcinogen. For reservoir injection, sodium hypochlorite at an aqueous concentration of 5,000 ppm appears to control growth of the microbial formulation and is less hazardous to the environment.

INTRODUCTION

One of the objectives of the microbial research at NIPER has been to identify possible environmental hazards associated with applying microbial enhanced oil recovery (MEOR) processes in the field. In the past, biocides have been used to control undesirable microbial populations causing production problems such as reservoir plugging and equipment corrosion. Major environmental impacts associated with MEOR include changes in indigenous microflora in reservoirs that may be caused by injection of nonindigenous microorganisms and nutrient and the possibility of injected microorganisms mutating and producing undesirable compounds under reservoir conditions.

This study was designed to test a specific MEOR formulation for pathogenicity and mutagenicity of microbial products and to select a biocide for the formulation in case undesirable microbial contamination should occur in the field. Previous laboratory MEOR studies have involved the use of two microorganisms, *Bacillus licheniformis* and a *Clostridium* species, designated as NIPER 1 and 6, respectively.

Three biocides were selected for toxicity tests with the NIPER 1 and 6 formulation. Sodium hypochlorite, a strong oxidizing agent, and glutaraldehyde have proved to be effective bactericides for sessile bacteria that attach to surfaces within a microbial biofilm and both have been used to dissolve the exopolysaccharide biofilm matrix.[1-3] Alkylating agents such as formaldehyde are nearly as active against spores as against vegetative cells, presumably because they can penetrate easily and do not require water for their action.[4] Formaldehyde has been used extensively in the field.[5-6] Sodium hypochlorite is the least hazardous of the three chemicals for release into the environment.[7] Certain biocides including glutaraldehyde and formaldehyde are extremely toxic over short exposure periods. Bioaccumulation is high, and both are implicated as carcinogens.[6] At this time, regulations for Class II wells do not prohibit injection of such chemicals, but these regulations may eventually be changed.[8]

Tube tests were conducted to determine which biocide has the best potential for eradicating the NIPER 1 and 6 microbial formulation. Two of the biocides were then selected for microbial corefloods to determine their effects on oil recovery, transport, and growth of microorganisms in porous media. Two injection strategies were evaluated for each biocide. The biocides were injected either before or after injection of microbes and nutrient.

Both NIPER 1 and 6 microorganisms have the ability to form endospores. Bacteria capable of forming endospores are used in most field tests, which can take several years to complete. Unlike vegetative cells, the dormant endospores are resistant to heat, drying, radiation, acids, and certain biocides over extended periods of time without access to nutrient. NIPER 1, *Bacillus licheniformis*, has been shown to survive without nutrient for 6 months in porous media, even when other indigenous microbial flora are present.[9] Once the optimal biocide strategy was determined, long-term biocide tests were initiated to provide information on the potential long-term effects of the biocide on the microorganisms and their endospores.

Biocide testing was also performed on a field core from an ongoing MEOR waterflood field project.[10] This test was performed to evaluate the performance of a selected biocide in an actual field core that contained indigenous microorganisms and had a lower permeability than the Berea sandstone used in these tests.

MATERIAL AND METHODS

Core Preparation

Blocks of Berea sandstone were obtained from Cleveland Quarries (Amherst, Ohio) and cut into cylindrical cores of 25 cm in length and 3.7 cm in diameter. Three vertical right cylinders 25 cm in length and 3.7 cm in diameter were also drilled from core samples obtained from the Bartlesville sand formation of

the Mink Unit in Delaware-Childers field in Nowata County, Oklahoma. The field core samples were extracted with either toluene or an azeotrope mixture of chloroform and acetone to remove hydrocarbon. Cores were either encapsulated in epoxy or encased in rubber sleeves and placed inside stainless steel Hassler coreholders with a net confining pressure of 250 psi (1,723.7 kPa). Inlet and outlet valves were attached to the ends of the coreholders. The cores were then evacuated for 2 to 3 hours and flushed with brine. The brine used for the experiments was a 0.5% by weight aqueous solution of sodium chloride. Darcy's law was used to calculate absolute permeability of each core to brine. Two of the three field cores were eliminated from further testing because of horizontal shale and clay streaks and subsequent low-permeability values. Crude oil from the Mink Unit was injected into the Berea sandstone cores and the selected field core until no additional water was produced. Finally, the cores were waterflooded with brine to a simulated residual oil saturation after waterflooding (S_{orwf}). The coreflood apparatus has been previously described.[11] The fluid separators are piston devices used to inject microbial solutions and other fluids into the cores and were designed to prevent corrosive fluids from contacting the pumps. A frontal advance rate of 1 ft/d (to correspond with the Mink Unit field project actual injection rate) was used for all microbial waterfloods.

Microorganisms

A combination of *Bacillus licheniformis* (NIPER 1) and a *Clostridium* species (NIPER 6) is the formulation of choice for some microbial enhanced oil recovery projects. Therefore, the NIPER 1 and 6 formulation was chosen for coreflood biocide tests. *Bacillus licheniformis* is a facultatively anaerobic spore-forming *Bacillus* that produces acids and surfactant when fermenting sucrose. The anaerobic spore-forming *Clostridium* is a member of the butyric acid group that produce acetone, butanol, ethanol, isopropanol, butyric acid, acetic acid, propionic acid, carbon dioxide, and hydrogen when fermenting sucrose.

Two indigenous microorganisms (designated Indigenous 1 and 2) were also isolated from the Mink Unit field core. A small piece of core was chipped from the 3-in-diameter core under anaerobic conditions to expose the inner pore surfaces. The chip was transferred into tryptic soy broth and grown anaerobically at ambient temperature. A streak plate on tryptic soy agar was prepared, and growth of two colony types appeared: an anaerobic spore-forming *Clostridium* species and a facultatively anaerobic *Bacillus*. These microorganisms were easily distinguishable from the NIPER 1 and 6 microorganisms of the same species by microscopic observation of morphology.

Biocides

The following solutions were used in biocide dilutions: a 37% w/w aqueous solution of formaldehyde; a 4 to 6% w/v aqueous solution of sodium hypochlorite; and a 25% w/w aqueous solution of glutaraldehyde.

Nutrient

The molasses used in these experiments was obtained from Pacific Molasses Company in Oklahoma City, and its composition is as follows: total ash, 8.1%; calcium, 0.8%; phosphorous, 0.08%; magnesium, 0.35%; potassium, 2.4%; sulfur, 0.8%; and sodium, 0.2%. The amount of total suspended solids is 74%, of which 3% is total protein, 48% is total sugar (sucrose), and the remaining 23% is fiber. The concentration of molasses used in the experiments was 4% by weight in tap water enhanced with 0.1% ammonium phosphate to facilitate microbial metabolism.

Gas Chromatography

Compositional analyses were performed using a Hewlett Packard 5980A gas chromatograph equipped with a flame ionization detector. A 6-ft (1.83 m) glass column packed with Poropak QS (800-100 mesh) was used for all analyses. A temperature program of 95° to 195° F (35° to 90.6° C) gave the best separation of compounds. Standards used were 0.1 and 0.05% alcohols and fatty acids in an aqueous solution of 0.5% by volume phosphoric acid and 0.25 wt % sodium chloride. Compositional analyses were performed on effluent from long-term biocide cores. The NIPER 1 and 6 metabolic products assay included acetone, butanol, methanol, ethanol, isopropanol, 2-3 butanediol, butyric acid, acetic acid, and propionic acid.

EXPERIMENTAL PROTOCOL

Pathogenicity Tests

Tests for microbial pathogenicity of the MEOR NIPER 1 and 6 formulation were conducted at Oklahoma State University. Mice were used to test for pathogenicity of the microbial formulation. Separate cultures of NIPER 1 and NIPER 6 and a NIPER 1 and 6 mixed culture containing 1×10^8 cfu/mL (colony forming units per milliliter) were each suspended in a solution of PBS (10 mM dibasic potassium phosphate and 0.85% sodium chloride). The solution was given both by oral ingestion and intraperitoneal (IP) injection. For intraperitoneal injection, a 1-mL aliquot was used. For oral ingestion, the mice were deprived of water for 48 hours and then given access to the solution for a period of approximately 20 minutes. Another solution was prepared by dilution of molasses to 4% v/v in PBS and adding a cell concentration (grown anaerobically in 4% molasses) of 1×10^8 cfu/mL. This solution was given orally as described above. Each mouse consumed approximately 9 mL of the cell solution in PBS only and approximately 12 mL of the cell solution containing 4% molasses in PBS. An illustration of the test protocol is presented in figure 1.

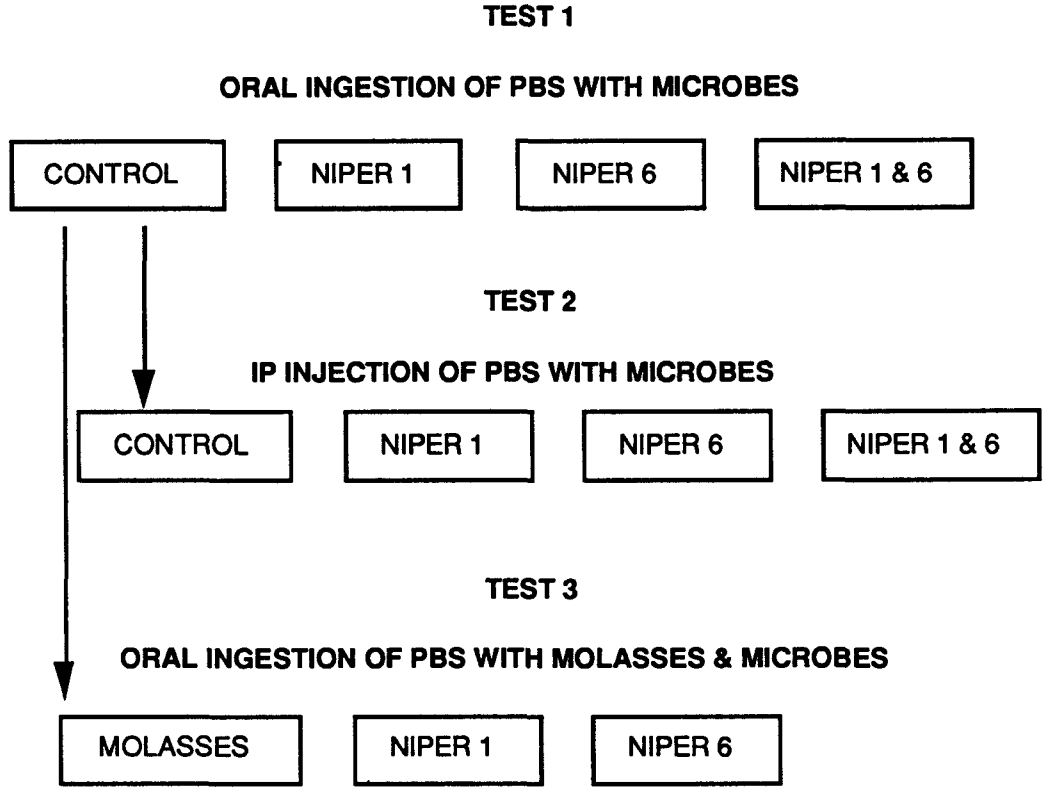


FIGURE 1. - Schematic of pathogenicity testing procedure.

Mutagenicity Tests

The carcinogenic potential of many chemical compounds to which we are exposed in our environment is highly correlated with their ability to induce mutation. A mixed culture of NIPER 1 and 6 grown anaerobically in a solution containing 4% v/v molasses in tap water was filtered with a 0.45-micron syringe filter to remove microbial cells. The microbial product solution was submitted to the Ames test[12] for detecting mutagenic chemicals. The Ames test, which is used as a prescreen for carcinogenic substances, relies on a series of nutritional mutants of *Salmonella typhimurium*.

The assay disk method of the Ames test was used for evaluating mutagenicity of the NIPER 1 and 6 products. In this procedure, a thin layer of agar inoculated with *Salmonella typhimurium* was placed over a base agar plate. The *Salmonella typhimurium* strain requires the amino acid histidine. The medium contained only a very small amount of histidine, which should allow a few cell divisions to occur. Filter paper disks saturated with the NIPER 1 and 6 products, sterile deionized water, and nitrobenzene were placed equidistant on the soft agar. The water and nitrobenzene were used as positive and negative controls for mutagenicity. A duplicate plate was prepared, and both plates were incubated at 37° C for 48 hours.

Biocide Tube and Flask Tests

Initially, tube tests were conducted in which varying concentrations and mixtures of the three biocides were used to determine which biocide had the best potential for eradicating the microbial formulation. Tests were conducted by adding varying concentrations of biocide and mixtures of biocides to 24-hour cultures of NIPER 1 and 6 in tryptic soy broth. Tests were also performed using microbial cultures diluted to 50% concentration with sterile deionized water to simulate dilution by reservoir fluids. Microbial counts in cfu/mL were determined using tryptic soy agar pour plates. Dilutions of the sample were gently mixed into the warm agar and allowed to solidify. All plates were incubated at 30° C aerobically or at ambient temperature anaerobically.

Biocide Coreflood Tests

Two of the biocides were selected for coreflood tests using two different injection strategies. Berea sandstone cores were prepared as described previously and waterflooded to residual oil saturation. Each biocide was added before injection of the microbial formulation and nutrient in one microbial coreflood and added after injection of the microbial formulation and nutrient in a second microbial coreflood. Because the nutrient molasses solution contains microorganisms, control corefloods were performed using nutrient only with each biocide.

The most effective concentration of each biocide was determined from the tube tests. A 0.1-pore volume aliquot of an aqueous solution containing 5,000 ppm sodium hypochlorite and a 0.3-pore volume aliquot of an aqueous solution containing 370 ppm formaldehyde were used in these biocide coreflood tests. A 0.1-pore volume aliquot of microbial formulation was injected in each core followed by injection of a 0.2-pore volume aliquot of nutrient.

For the preflush tests, the biocide was injected, followed immediately by injection of the microbial formulation and nutrient. The cores were shut-in for a 3-day incubation period, then waterflooded. All waterfloods were continued until a total of 2 pore volumes of brine had been injected. For the second injection protocol, the microbial formulation was injected first, followed by injection of nutrient; and the biocide was injected last. Following injection of the biocide, the cores were shut-in for a 3-day incubation period. A third injection strategy was used with the sodium hypochlorite only. After injection of the microbial formulation and nutrient, the core was shut-in for 3 days. After the 3-day incubation period, the sodium hypochlorite was injected; and the core was shut-in for 24 hours with the biocide.

Long-Term Biocide Coreflood Tests

Once the optimal biocide strategy was determined, core tests were conducted to determine long-term effects of the biocide and effects when an indigenous microbial population is present. Three long-

term epoxy Berea sandstone cores were prepared, as described previously, and waterflooded to a simulated condition of S_{orwf}.

The first core, designated MEOR 34, was injected with 0.1 pore volume of microbial formulation, followed by injection of 0.2 pore volume of nutrient, and shut-in for a 3-day incubation period. Following the incubation period, the core was waterflooded. After the waterflood, a 0.1-pore volume aliquot of an aqueous solution containing 5,000 ppm sodium hypochlorite was injected, and the core was shut-in for a period of 3 months. The second core, MEOR 35, was injected with 0.1 pore volume of microbial formulation, followed by 0.2 pore volume of nutrient, and was shut-in for a 3-day incubation period. After 3 days, the core was injected with sodium hypochlorite (5,000 ppm) and shut-in for a period of 3 months. The third core, MEOR 36, was injected with 0.1 pore volume of microbial formulation, followed by injection of 0.2 pore volume of nutrient, and allowed to incubate for a period of 2 weeks. After 2 weeks, another 0.2 pore volume of nutrient was injected; and the core was shut-in for a 3-day incubation period. After the 3 days, 0.1 pore volume of sodium hypochlorite (5,000 ppm) was injected, and the core was shut-in for a period of 3 months.

After the 3-month shut-in period, each core was injected with sterile, filtered molasses to stimulate any microbes or spores present. Plate counts of colony forming units per milliliter in core effluent were taken at each injection step and after a final 3-day shut-in period. Compositional analyses for NIPER 1 and 6 products were also performed on a final effluent sample using gas chromatography.

Field Core Biocide Test

Biocide tests were performed on an actual field core. The core was evacuated, then saturated with brine. Absolute permeability to brine using Darcy's law was 150 md, compared to measurements of 800 md to 1 darcy for the Berea sandstone cores. The core was injected with oil from the Mink Unit until no more brine was flushed out, then the core was injected with brine and waterflooded to a simulated S_{orwf}. A 0.1-pore volume aliquot of indigenous 1 and 2 formulation was injected in the core, followed by injection of 0.2 pore volume of nutrient. The core was shut-in for a 3-day incubation period, after which 0.2 pore volume of NIPER 1 and 6 formulation was injected and followed by an injection of 0.2 pore volume of nutrient. Once again, the core was shut-in for a 3-day incubation period.

After the second shut-in period, a 0.3-pore volume aliquot of brine containing 370 ppm formaldehyde was injected. The core was shut-in with the biocide for 7 days. On the 7th day, the core was injected with sterile, filtered molasses to stimulate any viable microorganisms or spores. After another 3-day shut-in, the core was waterflooded (2 pore volumes of brine was injected). Microbial plate counts were taken at each injection step.

RESULTS AND DISCUSSION

Pathogenicity Tests

The mice subjected to the NIPER 1 and 6 formulation both orally and by intraperitoneal injection were observed for a period of 2 months. Pathogens of the genus *Clostridium* and *Bacillus* produce toxins that have incubation periods of short duration. During the period of observation, all control and test mice appeared to be healthy.

Mutagenicity Tests

Scattered colonies appeared on the surface of both plates. A positive result would be indicated by a relatively high concentration of colonies surrounding the disk. No increase in colony formation was observed surrounding the paper disks with filtered NIPER 1 and 6 formulation. The NIPER 1 and 6 metabolic products were non-mutagenic as defined by the Ames test.

Biocide Flask and Tube Tests

Results of initial biocide tests are presented in table 1. Initially, low concentrations of glutaraldehyde and formaldehyde were used. Because none of the biocides were effective, the concentrations were increased in a second test. A concentration of 4,500 ppm sodium hypochlorite appeared to control microbial growth but did not completely eradicate the NIPER 1 and 6 formulation. However, the concentration was not increased because it would not be feasible to exceed this concentration in a reservoir. The formaldehyde at a concentration of 370 ppm appeared to be the most effective.

Biocide combinations in low concentrations have proved to be effective against some microbial populations.[13] Results of tests with biocide combinations are presented in table 2. The biocide combinations selected for testing appeared to be less effective than the formaldehyde and sodium hypochlorite used separately.

Previous tests had indicated that formaldehyde was not effective at 75 ppm but was bactericidal at 370 ppm. Intermediate dilutions of 150 and 250 ppm formaldehyde were tested for bactericidal effectiveness. Test results are presented in table 3. At first, the 250 ppm dilution appeared to be effective, but at 120 hours, aerobic counts reappeared, indicating possible survival and germination of spores.

TABLE 1. - Initial biocide tests

Microorganisms	Initial anaerobic microbial counts, cfu/mL[2]	24 hour[1] anaerobic microbial counts, cfu/mL	120 hour[1] anaerobic microbial counts, cfu/mL
Test No. 1 - 24 hour anaerobic cultures in TSB[1]			
Control - No Biocide			
NIPER 1	1.6×10^9	7.6×10^8	5.2×10^8
NIPER 6	3.6×10^9	7.2×10^8	3.7×10^8
NIPER 1 & 6	4.3×10^8	3.0×10^8	1.6×10^8
Glutaraldehyde - 50 ppm			
NIPER 1	1.6×10^9	6.2×10^8	6.0×10^8
NIPER 6	4.2×10^9	4.9×10^8	3.0×10^8
NIPER 1 & 6	2.4×10^8	3.8×10^8	1.8×10^8
Formaldehyde - 75 ppm			
NIPER 1	3.6×10^9	5.1×10^8	6.2×10^8
NIPER 6	3.2×10^9	5.1×10^8	5.7×10^8
NIPER 1 & 6	7.1×10^8	5.1×10^8	2.0×10^8
Sodium Hypochlorite - 4,500 ppm			
NIPER 1	3.2×10^9	1.0×10^6	2.6×10^3
NIPER 6	8.9×10^8	2.5×10^8	1.3×10^8
NIPER 1 & 6	2.50×10^9	(3)	(3)

Microorganisms	96 hour[1] anaerobic microbial counts, cfu/mL	144 hour[4] anaerobic microbial counts, cfu/mL
Test No. 2 - 24 hour cultures in 50% TSB and 50% sterile water		
Control - No Biocide		
NIPER 1	1.8×10^6	1.9×10^4
NIPER 6	6.4×10^8	2.2×10^7
NIPER 1 & 6	9.4×10^8	2.4×10^7
Glutaraldehyde - 250 ppm		
NIPER 1	1.2×10^6	6.3×10^4
NIPER 6	6.2×10^8	1.6×10^6
NIPER 1 & 6	8.7×10^8	1.5×10^6
Formaldehyde - 370 ppm		
NIPER 1	1.2×10^2	0.0×10^0
NIPER 6	0.0×10^0	0.0×10^0
NIPER 1 & 6	0.0×10^0	0.0×10^0

TABLE 1. - Initial biocide tests -- continued

Microorganisms	96 hour[1] anaerobic microbial counts, cfu/mL	144 hour[4] anaerobic microbial counts, cfu/mL
Test No. 2 - 24 hour cultures in 50% tryptic soy broth and 50% sterile water		
Sodium Hypochlorite - 4,500 ppm		
NIPER 1	6.9×10^2	6.0×10^0
NIPER 6	TNTC[5]	2.3×10^4
NIPER 1 & 6	TNTC[5]	6.4×10^3

[1]Designates time from injection of biocide.
[2]cfu/mL - colony forming units per milliliter.
[3]Counts not available due to broken tube.
[4]A second injection of biocide was performed at 312 hours. 144 hours denotes time after second injection.
[5]TNTC-Too numerous to count (at 1/10 dilution).

TABLE 2. - Tube test evaluation of biocide combinations

Microorganisms	Initial anaerobic microbial counts, cfu/mL[2]	24 hour[1] anaerobic microbial counts, cfu/mL
Control - No Biocide		
NIPER 1	6.7×10^6	7.0×10^4
NIPER 6	2.2×10^8	2.3×10^8
NIPER 1 & 6	2.8×10^7	1.9×10^7
370 ppm formaldehyde and 2,500 ppm sodium hypochlorite		
NIPER 1	7.9×10^7	5.9×10^5
NIPER 6	1.5×10^8	9.0×10^6
NIPER 1 & 6	1.4×10^7	1.1×10^6
Control - no biocide		
NIPER 1	[3]0	[3]0
NIPER 6	1.7×10^8	6.3×10^8
NIPER 1 & 6	1.8×10^8	1.5×10^8
185 ppm glutaraldehyde and 2,500 ppm sodium hypochlorite		
NIPER 1	4.0×10^5	8.9×10^3
NIPER 6	1.8×10^8	2.4×10^6
NIPER 1 & 6	1.8×10^8	1.5×10^8

[1]24 hours from time biocide was added.
[2]cfu/mL - colony forming units per milliliter.
[3]Culture did not grow, so control was invalid.

TABLE 3. - Formaldehyde dilution test

Flask Test

24 hour anaerobic culture of NIPER 1 and 6 in tryptic soy broth

Time, hours	Control		Formaldehyde - 150 ppm	
	Aerobic cfu/mL[1]	Anaerobic cfu/mL	Aerobic cfu/mL	Anaerobic cfu/mL
[2]0	1.2×10^9	1.0×10^9	1.6×10^9	1.5×10^9
[3]24	1.8×10^9	1.3×10^9	9.2×10^8	4.6×10^8
48	1.2×10^9	1.2×10^9	5.7×10^8	6.7×10^8
72	1.4×10^9	2.0×10^9	1.3×10^9	1.1×10^9
96	1.3×10^9	1.9×10^9	7.6×10^8	6.3×10^8

Tube tests

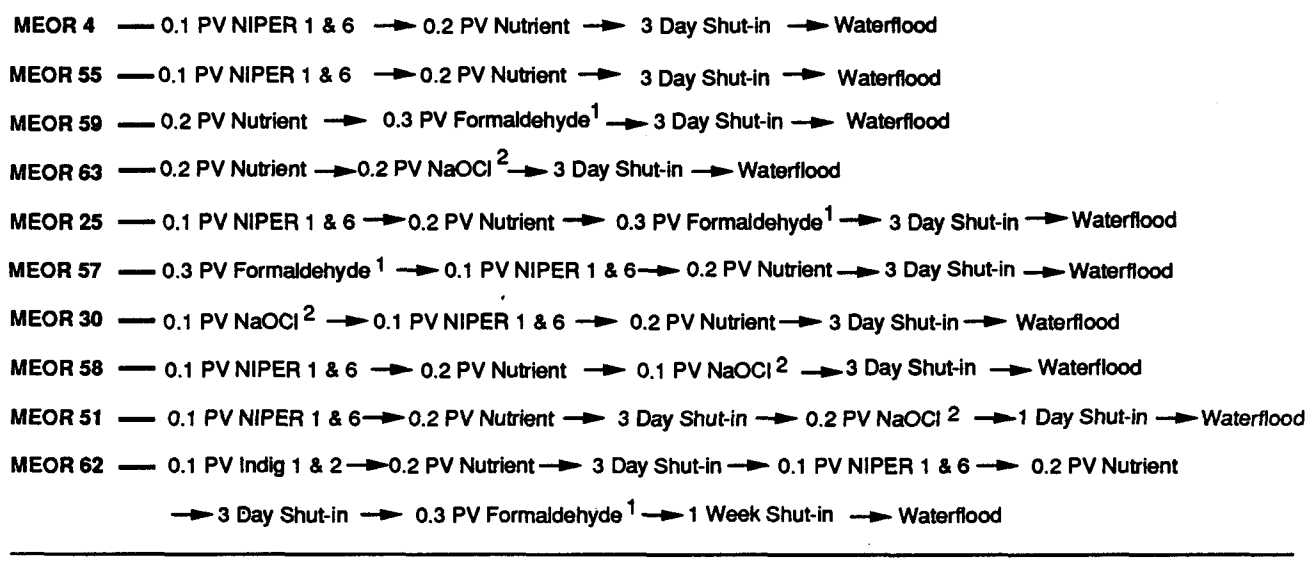
24 hour anaerobic culture of NIPER 1 and 6 diluted 50% with sterile water

Time, Hours	Control		Formaldehyde, 150 ppm		Formaldehyde, 250 ppm	
	Aerobic, cfu/mL	Anaerobic, cfu/mL	Aerobic, cfu/mL	Anaerobic, cfu/mL	Aerobic, cfu/mL	Anaerobic, cfu/mL
[2]0	2.8×10^8	2.3×10^8	2.6×10^8	2.0×10^8	2.7×10^8	2.8×10^8
[3]24	3.2×10^7	4.0×10^8	9.1×10^7	2.5×10^7	1.3×10^7	1.6×10^7
48	9.5×10^8	7.6×10^7	6.5×10^6	1.9×10^7	0.0×10^0	0.0×10^0
120	7.0×10^6	8.8×10^6	1.0×10^4	2.3×10^4	2.2×10^2	0.0×10^0
144	6.9×10^6	8.2×10^6	6.3×10^3	1.5×10^2	3.3×10^2	0.0×10^0
24 hour anaerobic culture of indigenous 1 and 2 diluted 50% with sterile water						
[2]0	9.9×10^6	1.6×10^7	2.5×10^6	5.0×10^5	4.4×10^6	1.9×10^7
[3]24	1.3×10^6	1.6×10^6	0.0×10^0	0.0×10^0	0.0×10^0	0.0×10^0
48	2.5×10^6	5.7×10^6	0.0×10^0	5.0×10^3	0.0×10^0	3.8×10^3
120	2.5×10^6	1.0×10^5	0.0×10^0	3.4×10^4	0.0×10^0	0.0×10^0
144	1.3×10^6	1.5×10^6	0.0×10^0	5.0×10^2	0.0×10^0	0.0×10^0

[1] cfu/mL - colony forming units per milliliter.
[2] Before addition of biocide.
[3]Designated time after addition of biocide.

Biocide Corefloods

The injection strategy for each biocide coreflood is presented in figure 2. Data for oil recovery efficiency and preflood microbial counts performed on core effluent are presented in table 4. Many of the microbial counts appear to be high, but the molasses microbes were observed in the effluent. In previous corefloods, when using nonsterile molasses as the nutrient for microbial systems, contaminating bacteria



[1]370 ppm formaldehyde.
[2]5,000 ppm sodium hypochlorite.

FIGURE 2. - Injection protocol for short-term biocide corefloods.

from the molasses would interfere with the injected *Clostridium* or *Bacillus* species.[9] Neither biocide appeared to be effective against the molasses microorganisms.

The percent recovery efficiency appeared to show good correlation with effectiveness of the biocides because even though the molasses microbes survived, they were not effective in mobilizing oil. The injection strategy for MEOR 58, in which sodium hypochlorite was injected immediately following the microbial solution and nutrient, did not appear to be as effective. However, when a larger aliquot of sodium hypochlorite was injected after the 3-day shut-in period (MEOR 51) and was shut-in for 24 hours, the recovery efficiency was decreased by a factor of 10. The biocide was less effective when injected with the microbial formulation. Sodium hypochlorite may not be as effective in killing the microbes when they are in the exponential phase of growth.

Both formaldehyde and sodium hypochlorite appeared to be most effective when used as a preflush before microbial injection. However, the use of biocides in conjunction with the MEOR process would normally be because of contamination after a microbial injection and treatment. Both biocides appeared to be effective in controlling the microbial formulation in the short-term corefloods.

Field Core Biocide Test

Core MEOR 62 was used for the biocide field core test, and results of the coreflood are presented in tables 4 and 5. A concentration of 370 ppm aqueous solution of formaldehyde was the biocide selected for this test. Because Indigenous 1 and 2 microbes were injected in the core initially, biocide tests were performed on the indigenous microbes, and results are presented in table 3. A concentration of 250 ppm formaldehyde was effective in eradicating the indigenous microbes. Plate counts were taken of core effluent at each injection step, and the internal pressure of the core was monitored. Although anaerobic counts of the Indigenous 1 and 2 microbes were negative, both microbes were observed in the effluent after the 3-day shut-in of NIPER 1 and 6. Either the dilution was too high or the microbes had not transported to the end of the core. Microbial counts did not decrease significantly during incubation with the biocide. However, internal pressure declined from 70 to 0 psi indicating probable loss of the gas-producing *Clostridium* species, NIPER 6. The effluent was streaked aerobically, and only molasses microbes were apparent -- NIPER 1 was not observed. Indigenous 1 and 2 microbes were also not observed in the preflood effluent. A recovery efficiency (E_r) of only 2.7% was obtained, while the controls MEOR 4 and MEOR 55 (no biocide) had E_r values of 34.9 and 39.0%, respectively. These results indicate that the biocide was effectively controlling growth of the NIPER 1 and 6 formulation.

TABLE 4. - Biocide corefloods

Coreflood designation	Preflood pressure, psi	Preflood counts, cfu/mL[1]		Oil Recovery efficiency, %
		Aerobic	Anaerobic	
MEOR 4[2]	58	2.4×10^6	1.0×10^6	34.9
MEOR 55[2]	51	2.0×10^5	2.0×10^4	39.0
MEOR 59[2]	18	8.8×10^6	5.7×10^5	4.3
MEOR 63[2]	13	8.8×10^6	3.8×10^6	7.6
MEOR 25[2]	5	2.9×10^5	3.1×10^5	6.0
MEOR 57[2]	20	4.0×10^5	8.0×10^2	7.3
MEOR 30[2]	2	5.7×10^4	8.1×10^2	0.3
MEOR 58[2]	38	6.6×10^5	3.2×10^4	29.0
MEOR 51[2]	10	5.7×10^6	1.4×10^8	2.9
MEOR 62[3]	0	3.2×10^5	5.0×10^5	2.7

[1] cfu/mL - colony forming units per milliliter.
[2] Unfired Berea sandstone.
[3] Field core.

TABLE 5. - Biocide field core MEOR 62

	Microbial plate counts, cfu/mL[1]		Internal pressure, psi
	Aerobic	Anaerobic	
Initial counts - control	2.8×10^4	5.3×10^3	
Indigenous microbes - 3-day shut-in	1.9×10^6	(2)	60
NIPER 1 & 6 - 3-day shut-in	2.3×10^7	4.4×10^7	70
After biocide - 1 week shut-in	1.1×10^6	1.3×10^6	10
Sterile nutrient - 3-day shut-in	3.2×10^5	5.0×10^5	0

[1]Colony forming units/ mL.
[2]No colony forming units at 1/10,000 dilution.

Long-Term Biocide Core Tests

Results of the long-term biocide core tests and a schematic of injection strategies are presented in figure 3. Tabular results of gas chromatographic analyses for NIPER 1 and 6 products are presented in table 6. The low product concentrations indicate that NIPER 1 and 6 did not survive in core MEOR 34. The waterflood may have removed most of the microbial cells before the sodium hypochlorite was injected. The biocide did not appear to be effective in cores MEOR 35 and MEOR 36. High microbial counts and gas chromatographic results indicate survival of the NIPER 1 and 6 microorganisms in core MEOR 35. High internal pressure and gas chromatographic results suggest that the microbial plates used for counts on core MEOR 36 were questionable because microbes were present in relatively high concentrations. Plate counting errors can occur if the agar is too warm when it is poured into the microbial dilution.

CONCLUSIONS

1. All microbial formulations used in MEOR field processes should be tested for pathogenicity and mutagenicity. NIPER 1 and 6 microorganisms do not appear to be pathogenic to mice, and NIPER 1 and 6 microbial products do not appear to be mutagenic.

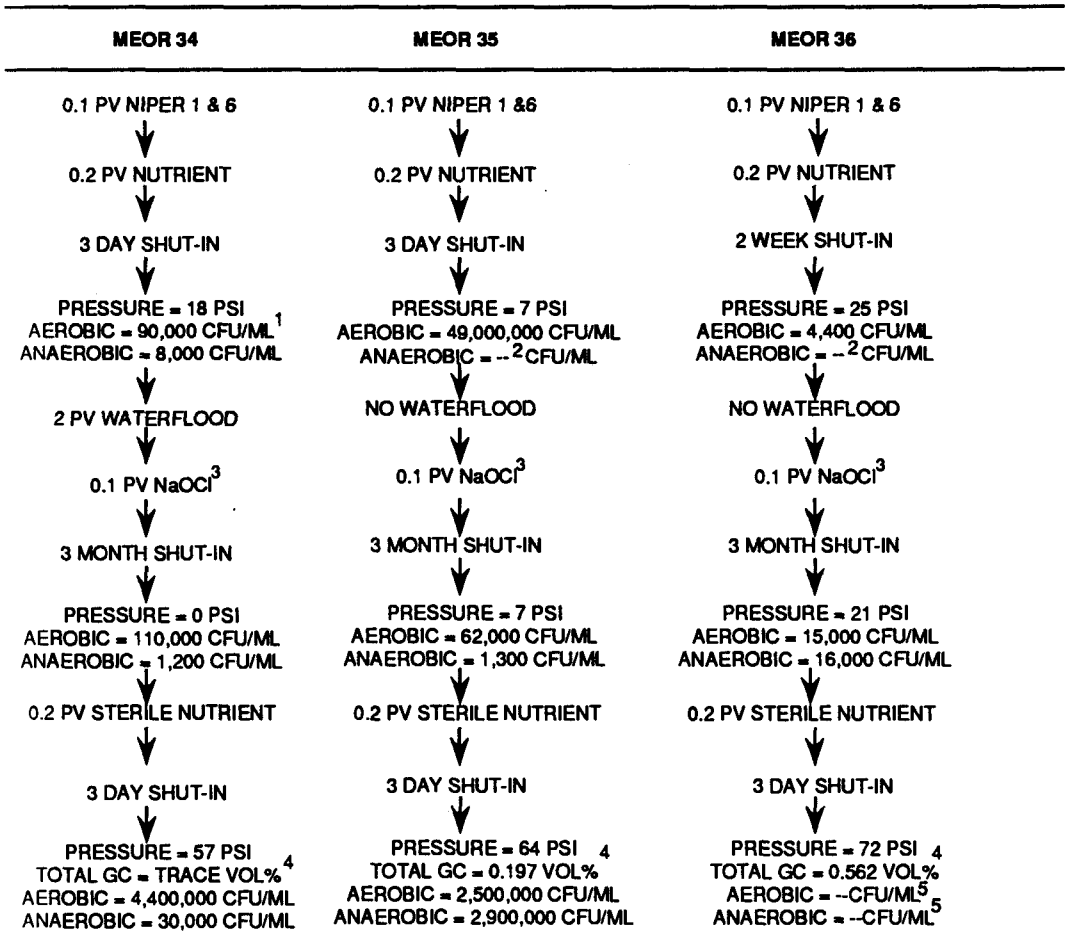
2. Formaldehyde is the most effective of the three biocides tested for short-term use in eradicating the NIPER 1 and 6 microbial formulation.

3. Sodium hypochlorite is effective in controlling the growth of NIPER 1 and 6 in the short term, but loses its effectiveness over a longer 3-month period.

4. Biocide mixtures of sodium hypochlorite-glutaraldehyde and sodium hypochlorite-formaldehyde were not effective in controlling the NIPER 1 and 6 formulation.

5. Gluteraldehyde was not bactericidal for the NIPER 1 and 6 formulation at a concentration of 250 ppm.

6. Neither formaldehyde nor sodium hypochlorite was bactericidal for the microbial flora contained in the unsterile molasses nutrient.



[1] Colony forming units per mL.

[2] No colony forming units at 1/10,000 dilution.

[3] 5,000 ppm sodium hypochlorite was used in all tests.

[4] Gas chomatography analyses for NIPER 1 & 6 products.

[5] No colony forming units at 1/100 dilution, which does not agree with GC results and pressure. Agar may have been too warm.

FIGURE 3. - Injection protocol and results of long-term biocide core tests.

TABLE 6. - Gas chromatographic analyses of long-term core effluents

Coreflood	Compound	Volume, %
MEOR34	Methanol	[1]0.005
	Ethanol	0.005
	Acetone	0.005
	Isopropanol	0.000
	Acetic acid	0.005
	2,3 Butanediol	0.000
	Propionic acid	0.000
	Butanol	0.005
	Butyric acid	0.005
	TOTAL	0.030
MEOR 35	Methanol	0.005
	Ethanol	0.029
	Acetone	0.005
	Isopropanol	0.000
	Acetic acid	0.028
	2,3 Butanediol	0.058
	Propionic acid	0.005
	Butanol	0.000
	Butyric acid	0.067
	TOTAL	0.197
MEOR 36	Methanol	0.005
	Ethanol	0.099
	Acetone	0.005
	Isopropanol	0.005
	Acetic acid	0.037
	2,3 Butanediol	0.130
	Propionic acid	0.009
	Butanol	0.005
	Butyric acid	0.267
	TOTAL	0.562

[1]A value of 0.005 is the limit of detection and indicates a trace amount.

RECOMMENDATIONS

As a result of this research, the following tests are recommended:

1. Biocide core tests should be performed on 4-ft-long cores using effluent sampling ports along the length of the core to evaluate microbial populations at various intervals along the core. Figure 4 presents injection and sampling techniques recommended for use with 4-ft-long biocide corefloods. Both formaldehyde and sodium hypochlorite should be used in these corefloods.

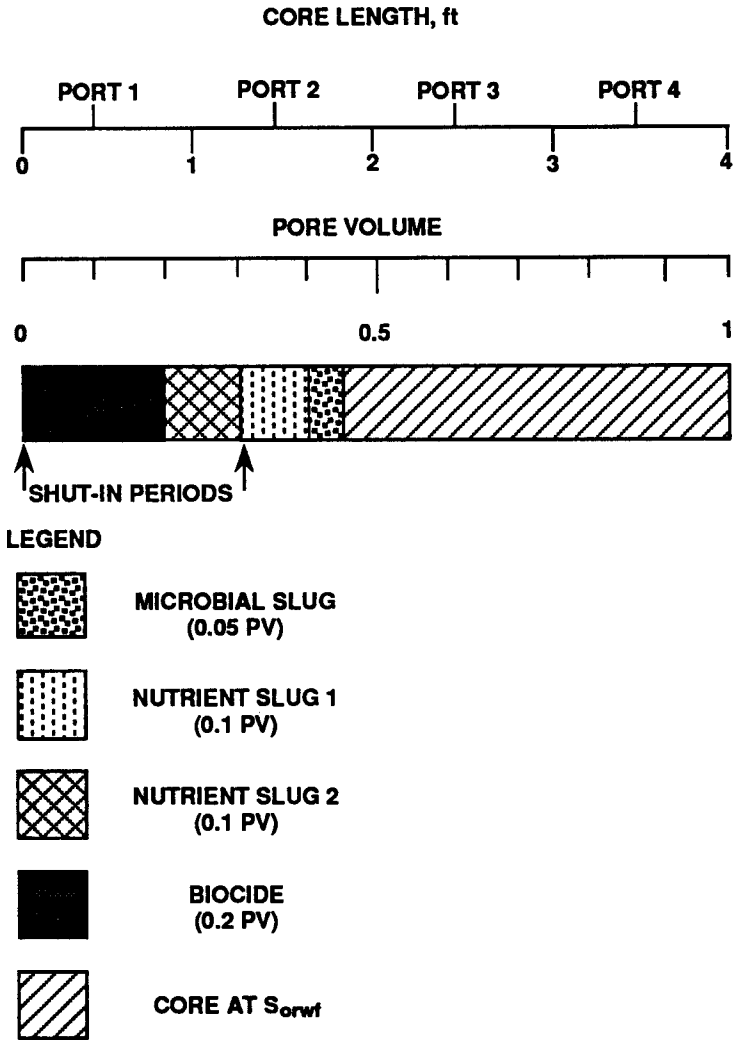


FIGURE 4. - Example of a possible injection strategy for a 4-foot-long biocide coreflood.

More tests should be performed with sodium hypochlorite because it is less toxic than formaldehyde and more environmentally acceptable for reservoir injection.

2. A coreflood should also be performed with sterile molasses to evaluate the effect of the biocides on only the NIPER 1 and 6 formulation.

3. A series of tests should be performed with variation in pore volumes of biocide injected and also with multiple biocide slugs to determine the most effective injection strategy.

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