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NEW MICROORGANISMS AND PROCESSES FOR MEOR

Final Report

By
Penny L. Sperl
George T. Sperl

December 1993

Performed Under Contract No. DE-AC22-90BC14663

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Bartlesville Project Office
U. S. DEPARTMENT OF ENERGY
Bartlesville, Oklahoma

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NEW MICROORGANISMS/PROCESSES FOR MEOR

I. ABSTRACT

Oil reservoirs naturally contain inorganic and organic materials which may be exploited through simple mineral supplementation to support the growth of denitrifying microorganisms. The growth and metabolic products from the presence of these microorganisms will aid in the release of oil from the rock matrix and improve crude oil quality and oil field operations. These studies have been sucessful in defining new microorganisms and processes for MEOR.

Materials which may serve as nutritional sources for microorganisms are present in the connate or flood waters or may be added to reservoirs during drilling and production operations of oil fields. These materials include sulfate, carbonate, volatile fatty acids, nitrogen-containing corrosion inhibitors, phosphorous-containing scale inhibitors and trace elements. The experiments show that, with simple minimal mineral supplementation to the flood waters, the increased growth of naturally-occurring microorganisms can contribute to the enhancement of oil recovery and are important aspects of many EOR technologies. Sulfate reducing bacteria (SRB), heterotrophic denitrifying bacteria, and denitrifying *Thiobacillus* species were successfully isolated from oil field waters. The SRB and *Thiobacillus* cultures, as a consortium, can utilize the volatile fatty acids and dissolved carbonates found in these waters and formations. These cultures were shown to feed each other sequentially and survive in mixed cultures. In a reservoir environment, these types of organisms are limited due to the development of an additional microflora containing heterotrophic denitrifying bacteria.

New Microorganisms/Processes for MEOR

A consortium of denitrifying microorganisms is useful in new MEOR processes (1) through the production of large volumes of gases (N_2, CO_2, and N_2O), (2) by the production of viscosifying agents, and (3) by the removal and prevention of *de novo* synthesis of hydrogen sulfide. These denitrifying cultures can be easily maintained in an oil reservoir environment. This report shows that denitrifying organisms are also potent agents for the control of SRB growth thereby preventing the development and production of sulfide, and therefore, are important agents for oil field operations. Laboratory experiments with defined mixtures of microorganisms as well as mixed populations showed oil release from sand pack columns and cores.

Methanogens exist in oil reservoirs and are easily cultured from many wells and produced waters. However, their metabolic activities appear to be limited to a very small part of the total microbiological activity.

The following are the results of the project.

1. Denitrifiers. The ability of denitrifiers to utilize sulfide, grow with dissolved organic materials normally found in oil field waters, and to produce viscosifying agents under the anaerobic conditions which are present in an oil field was demonstrated. The study showed the potential for sweetening sour wells by the action of denitrifying organisms.

2. Anaerobic *Thiobacillus* Cultures. It was shown that anaerobic *Thiobacillus* cultures utilize limestone carbonate in rock samples. Improved oil release should result from this dissolution of carbonates in the oil reservoir.

3. Mixed Consortium Experiments. The ability to mix denitrifying bacteria and SRB in the same system was studied.

SRB were established in cores or sand packs or *in vitro* situations and denitrifying bacteria were introduced. Subsequently, the ability of both these types of microorganisms to survive in the presence of each other was tested. Optimally, the SRB became a small minority of the active microbial population, and the greater metabolic diversity of the denitrifiers prevented sulfide production. This competitive interaction should cause the production of significant increased quantities of oil and would also result in improved oil quality.

4. Denitrifying Bacteria and Oil Recovery. The ability of denitrifying bacteria to release additional oil from cores and sand packs in the presence of SRB was demonstrated. The organisms will produce surfactants and/or viscosifying agents *in situ* under anaerobic conditions and therefore lead to more oil production.

II. EXECUTIVE SUMMARY

The data show that development of a mixed denitrifying microbial population in an oil reservoir environment will competitively reduce SRB populations resulting in the removal and prevention of H_2S. At the same time the products resulting from the growth of this replacement population will cause an increase in oil mobilization and oil release by mechanisms involving gas, surfactant and polymer production, and in the case of *Thiobacillus* growth, could cause dissolution of carbonates in the rock matrix. The establishment of this denitrifying population requires only the addition of simple inorganic chemicals without the need for organic nutrients. This new MEOR technology offers industry the potential for a simple, low cost, and effective oil recovery process, while at the same time provides a solution to the microbially generated sulfide problem.

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IV. INTRODUCTION.

The ability to produce more oil from aging and played out oil fields will have a great impact on the future energy dependence of the United States. Microbial Enhanced Oil Recovery (MEOR) offers industry a potentially economically attractive method to recover additional oil.[10] Classically, most MEOR processes involve the injection of carbohydrate-containing substances into wells to stimulate naturally-occurring or introduced microorganisms. These organisms are designed to play various potential roles, including selective plugging, solvent and organic acid formation, surfactant production, gas formation, and possibly oil mobilization through oil modification. The growth of these microorganisms necessarily causes a change in the environment of the formation, and MEOR technologies are designed to encourage the growth of organisms that have favorable effects in the reservoir.[5]

There is great potential, both economically and technically, for microorganisms to help in the release and mobilization of oil. However, we believe that instead of overwhelming the reservoir environment with major nutrient additions, an understanding of the microbiological ecology and a small selective alteration of the reservoir environment would be more effective and have greater potential for increasing oil recovery at low cost.

The anaerobic environment of virgin oil reservoirs is not usually conducive to the growth of microorganisms because of the lack of vital nutrients, although there is some anecdotal evidence that microorganisms may be indigenous to virgin oil reservoirs.[12] If oil degrading aerobic microorganisms were able to grow in oil reservoirs, oil would never be formed except transiently. However, small changes brought about by the penetration of the reservoir during drilling and production can

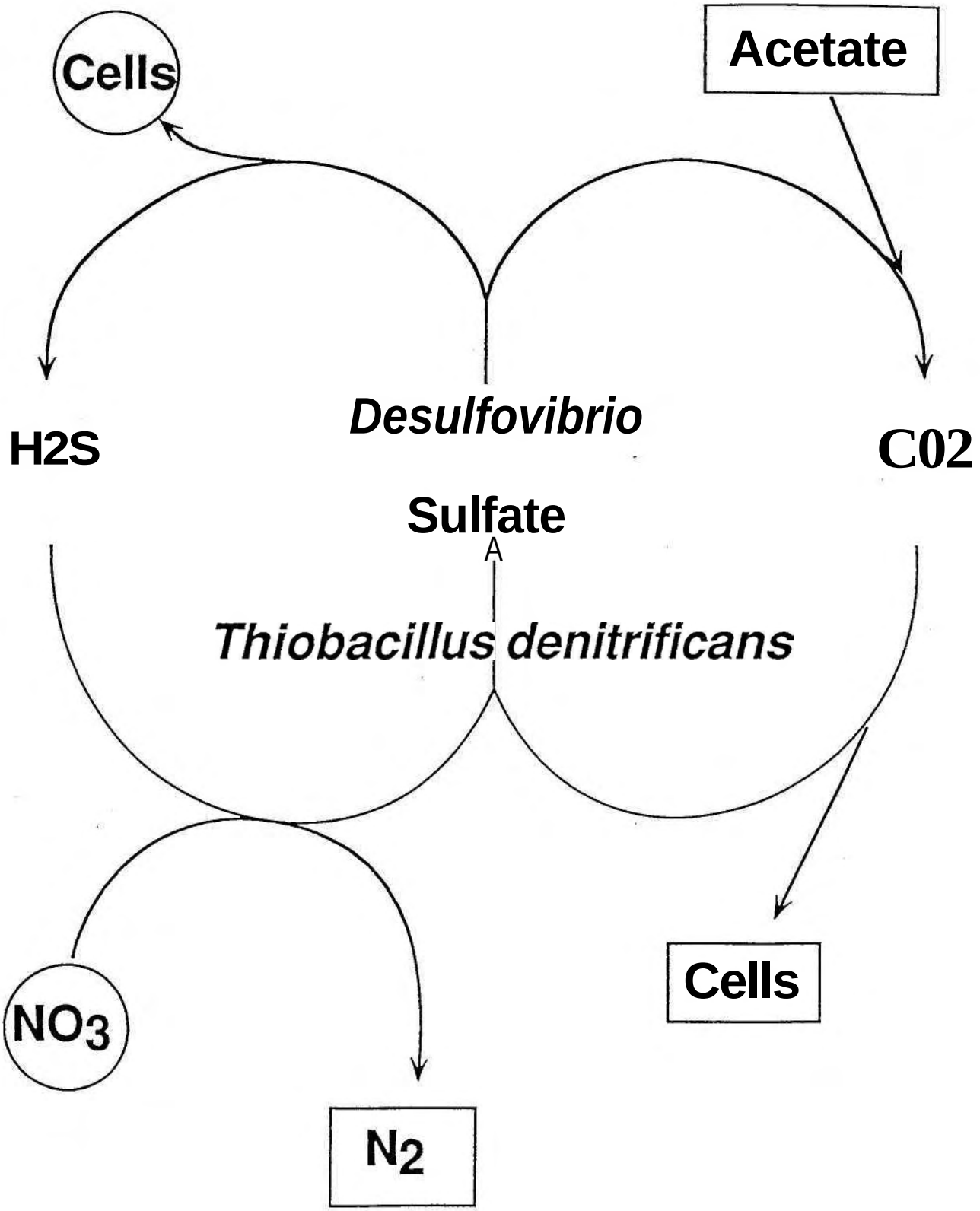
cause rapid growth of microorganisms with a concomitant change in the reservoir ecosystem.[9] Some of these changes can be detrimental, such as souring due to hydrogen sulfide formation. This microbial hydrogen sulfide generation process is currently a severe problem in many major oil fields, including some Alaskan fields. The change in the Alaska fields is probably due primarily to the use of sea water in water flooding operations as well as the addition of chemicals to aid in the surface production and water handling operations. High levels of sulfate come with the addition of sea water, completing the necessary mineral balance required for sulfate reducing bacteria (SRB) to flourish and produce hydrogen sulfide.[8] This leads to souring of the field and results in lower quality crudes and higher operating costs. Thus, this "subtle" change has brought about an enormous change in the reservoir ecology and field operations.

Our approach to MEOR recognizes such changes in the reservoir environment. Equivalent large and positive changes in field characteristics will lead to increased oil production and product quality. A model microbial consortium scheme that has the potential to introduce favorable environmental effects in the reservoir is diagrammatically shown in Figure 1.

We consider that the presence of volatile fatty acids (VFA), which are now being identified in many oil field waters, is of key importance in oil field microbiology. The VFA provide a natural carbon source for microbial growth in the oil reservoir. This role of the VFA has previously been neglected in the consideration of reservoir ecology. Our work has centered on the manipulation of the reservoir microbial populations by using the VFA as a constructive carbon source in place of its present detrimental role as a carbon source for the SRB. The VFA are utilized in the presence of nitrate to grow competitive denitrifying type organisms. This interrelationship of carbon,

FIGURE 1

NUTRIENT AND MICROORGANISM CYCLES



sulfur, and nitrogen nutrients in the reservoir waters forms the basis of the new MEOR technology.

The autotroph, *Thiobacillus denitrificans*, and related denitrifying *Thiobacilli* and heterotrophic denitrifying bacteria could have applications for MEOR, especially in oil reservoirs which contain limestone.[14] All denitrifying *Thiobacillus* sp. we have tested will dissolve limestone and use it as their sole source of carbon. If *T. denitrificans* and *Desulfovibrio* can be mixed in a single culture, then the naturally-occurring fatty acids in the field waters will drive sulfur reduction which will serve as the energy source for the denitrifying Thiobacilli. The targeted environments could include applications to straight carbonate (limestone) formations, mixed limestone-sandstone formations, and even some shale-limestone formations (although this would be very dependent on other permeability factors). *T. denitrificans* also could have the effect of the typical MEOR processes, i.e. mobility control and selective plugging by the cellular materials, production of N_2 gas (enough N_2 gas is produced in some cultures to cause the vessels to be highly pressurized), and production of increased CO_2 pressure through the dissolution of carbonate which is in equilibrium with CO_2.

Limestone supplies the CO_2 necessary for the growth of *T. denitrificans*. The dissolution of the carbonate would buffer the growth medium of the culture to a constant pH of 6.5, which is optimum for the growth of *T. denitrificans*. All acid produced during growth from the oxidation of reduced sulfur compounds is neutralized by the dissolution of limestone. The necessary additional nutrients are ammonia and nitrate in the form of ammonium nitrate (supplying both the alternate electron acceptor and a nitrogen source for the organisms), a small amount of phosphate (only just enough to satisfy the nutritional requirements of the microbes otherwise the buffering capacity of

the phosphate may interfere with the dissolution of the limestone), a reduced sulfur source necessary for the energy reactions of the cultures (we used thiosulfate), and trace metals (Zn, Cu, Co, Mn, Fe, Mo, W, Ni, and Se).

A third group of microorganisms that may play an important role in our MEOR studies are the methanogenic microorganisms. We were able to identify methanogens from oil pipelines which may be of use in our system. The microbial consortium cited above is theoretically capable of operating as a two component system, and we feel that methanogens might eventually become a part of the microbial community especially if CO_2 and H_2 are present. The source of reducing power should be the simple volatile fatty acids with the acceptor being CO_2. eH will be an important factor in the balance of the mixed microbial population and if the eH is not strictly regulated the first member of the community to suffer will be the methanogens. However, since these microbes are very fastidious, the possibility that they could play a significant role in MEOR appears to be small.

V. MATERIALS AND METHODS.

A. MICROORGANISMS AND CULTURE METHODS.

Mixed cultures of microorganisms were isolated from produced water from various oil fields, including primarily carbonate reservoirs and sandstone reservoirs. Water from the Kuparuk and Prudhoe Bay oil fields in Alaska were sources of both water for analysis and microorganisms. Strains of *Thiobacillus denitrificans* were obtained from the American Type Culture Collection and additional cultures were isolated from local soils and cultured in medium 1 (Table 1) with trace minerals (Table 4). Other strains were obtained from the Soda Dam thermal area in New Mexico and were cultivated in Medium 1 supplemented with 1 ml Trace Metal Solution SL-4 (Table 5). For the dissolution of limestone, medium 2 was used (Table 2). For the growth of

sulfate reducing bacteria, medium 3 was used (Table 3). Acid-washed Mill Creek sand was used for sand pack experiments and Berea sandstone was used in the core flooding tests. Kuparuk (Alaskan) crude oil (24° API) was used in experiments where crude oil was required.

Table 1
Medium 1

Ingredient	g/l
$Na_2S_2O_3 \cdot 5H_2O$	5.0
NH_4Cl	1.0
KNO_3	2.0
KH_2PO_4	2.0
$NaHCO_3$	2.0
$MgSO_4 \cdot 7H_2O$	0.8
$FeSO_4 \cdot 7H_2O$	0.02
Trace Metal Solution	1 ml
adjust pH to 6.5	

Table 2
Medium 2

Ingredient	g/l
Reduced sulfur source*	5.0
NH_4NO_3	2.5
KH_2PO_4	0.1
Crushed Limestone	2.5
Tap water	1000 ml

* - May be Na_2S, $Na_2S_2O_3$, S°, or $Na_2S_4O_6$.

Table 3
Medium 3

Sulfate-Reducing Bacterial Medium

Chemical	g/l
$MgSO_4 \cdot 7H_2O$	2.0
Na Citrate	5.0
$CaSO_4$	0.5
NH_4Cl	1.0
K_2HPO_4	0.5
Na Acetate	2.5
Na Propionate	0.5
Na Butyrate	0.1
Yeast Extract	0.1
5% solution of $Fe(NH_4)^2(SO_4)$	20 ml

adjust pH = 7.5

The media were reduced in the anaerobic chamber, dispensed in serum bottles, capped and autoclaved. The head space contains 5% CO_2, 10% H_2 and 85% N_2. Samples are injected with a syringe.

Table 4
Trace Metal Solution

Ingredient	g/1
EDTA	50
H_3BO_3	3.0
$ZnSO_4 \cdot 7H_2O$	22
$CaCl_2$	5.5
$MnCl_2 \cdot 4H_2O$	5.1
$FeSO_4 \cdot 7H_2O$	5.0
$Na_2MoO_4 \cdot 2H_2O$	1.0
$Na_2WO_4 \cdot 2H_2O$	1.0
$Na_2SeO_3 \cdot 5H_2O$	1.0
$CuSO_4 \cdot 5H_2O$	1.6
$CoCl_2 \cdot 6H_2O$	1.6
$NiCl_2 \cdot 6H_2O$	1.0

adjust pH to 6.0 with KOH

Table 5

SL-4 Trace Metals (500X)

Chemical Component	mg/l
EDTA, di Na salt	500
$FeCl_2 \cdot 5H_2O$	100
$MnCl_2 \cdot 4H_2O$	25
$CuCl_2 \cdot 2H_2O$	10
$CoCl_2 \cdot 6H_2O$	20
$NiCl_2 \cdot 6H_2O$	20
$Na_2MoO_4 \cdot 2H_2O$	30
Na_2WO_4	5
Na_2SeO_3	5
H_3BO_3	25

Inocula were obtained from pigging samples from the trans Alaska pipeline and produced water samples from the North Slope in addition to sources from local producing wells and water floods. Preliminary results show abundant growth of both denitrifying *Thiobacillus* cultures and good isolates of sulfate reducing bacteria, presumably *Desulfovibrio sp.* The effect of redox potential on the growth of both pure cultures of *Thiobacillus* and *Desulfovibrio* and the mixed culture enrichments obtained above was studied.

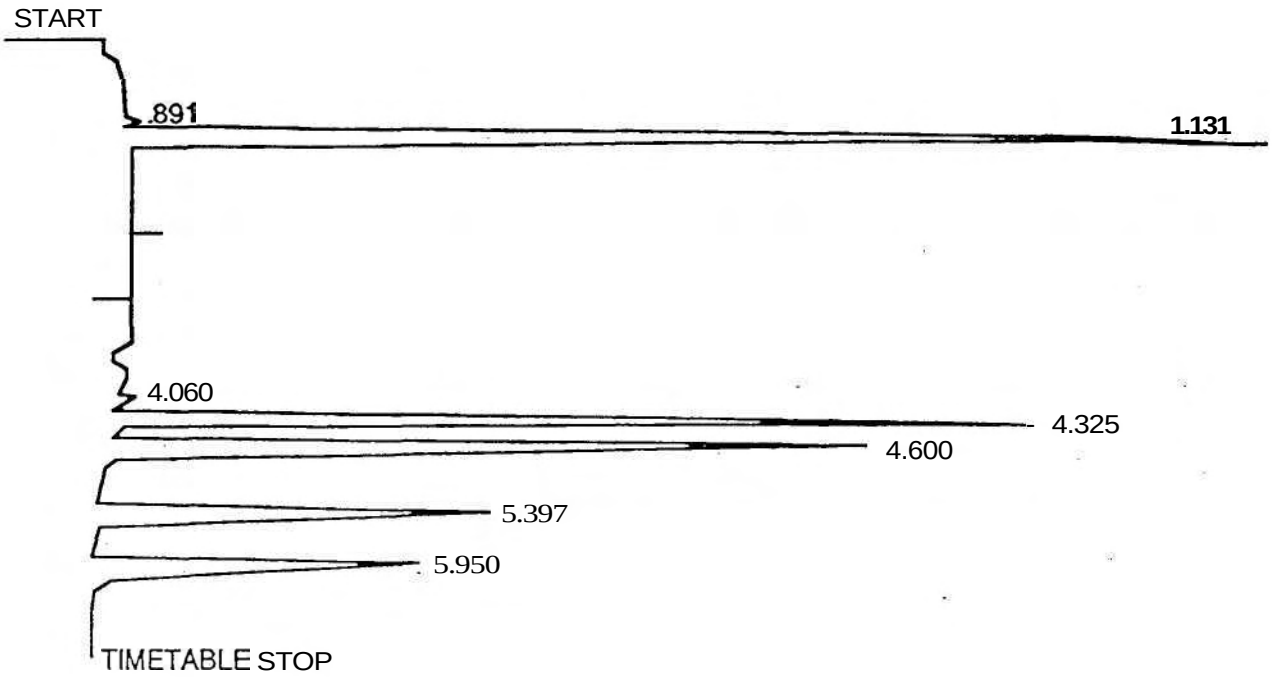
B. GAS ANALYSES.

In order to quantitatively analyze our experiments, we developed analyses for any microbiological gas mixture containing the following: methane, hydrogen sulfide, ammonia, nitrous oxide, nitrogen, hydrogen, oxygen, carbon monoxide, carbon dioxide, nitric oxide and other short chain hydrocarbons. This capability allowed us to both qualitatively determine the products of microbial growth and quantify the gas mixtures necessary to keep the mixed cultures in a state of balance and also allow the analyses of complex mixtures of microorganisms. A typical standard analysis is shown in Figure 2 and an actual cultural gas analysis in Figure 3.

FIGURE 2

CHROMATOGRAPHY OF A STANDARD GAS MIXTURE

RUN 233



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MEOR GAS ANALYSIS

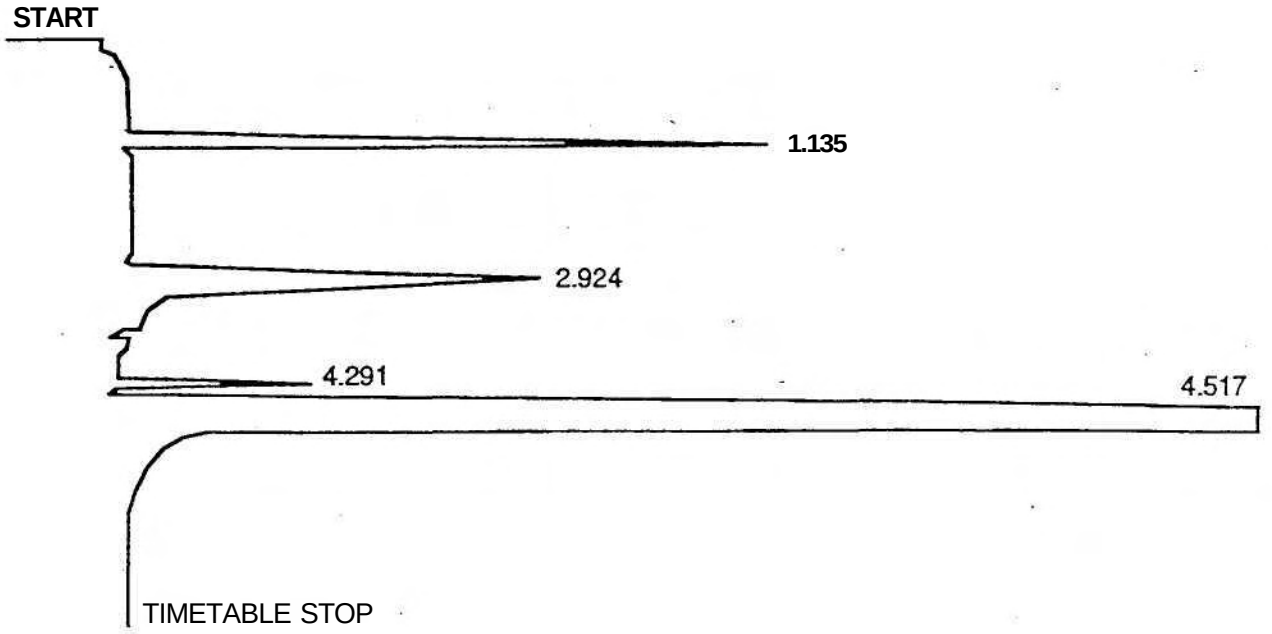
ESTD%-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	VOLUME %	NAME
.891	BB	110201	.368	4995		.000	
1.131	SBB	2633982	.062	702722	1R	4.453	CARBON DIOXIDE
4.060	PB	37748	.064	9823	2	3.289	HYDROGEN
4.325	SPB	2364610	.090	436364	3	5.520	OXYGEN
4.600	SPB	2364610	.090	436364	4	6.339	NITROGEN
5.397	BB	1377436	.113	203965	5	4.209	METHANE
5.950	PB	1891396	166	190081	6	5.251	CARBON MONOXIDE

FIGURE 3

GAS CHROMATOGRAPHY OF A TYPICAL DENITRIFICATION CULTURE

RUN 242



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MEOR GAS ANALYSIS

ESTD%-AREA

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	VOLUME %	NAME
1.135	BB	1231785	.072	285317	1R	2.082	CARBON DIOXIDE
2.924	BV	3357022	.284	196704	9	6.378	H_2S
4.291	PP	388924	.071	91562	3	1.117	OXYGEN
4.517	PB	31881776	.164	3240626	4	85.467	NITROGEN

VI. RESULTS.

A. DENITRIFYING BACTERIA

The inorganic constituents in oil field ecosystems may be manipulated to achieve certain ends. When examining produced waters from various oil fields, it was found that many fields contained sufficient quantities of most mineral and other nutrients for the growth of various classes of microorganisms. Table 6 shows a representative composition of produced water from an Alaskan oil field after flooding with seawater. This water will support the growth of SRB without any addition, although the addition of more nitrogen and phosphate sources greatly enhances growth. We used this as a standard produced water, because it is representative of many oil fields and we have the most detailed analysis of this water. In addition, the Alaskan oil fields represent the largest of recently developed oil fields present in North America and the producers are experiencing severe microbial and H_2S problems.

Table 6
Alaskan Oil Field Produced Water Analysis

pH	8.3
Sulfide	5 ppm
Na	7000 ppm
Ca	94 ppm
Mg	56 ppm
Fe	6 ppm
Cl	11000 ppm
Bicarbonate	2600 ppm
Sulfate	140 ppm
Nitrogen	1 ppm
Phosphorous	11 ppm
Acetate	1000 ppm
Propionate	80 ppm

A study of this oil reservoir environment revealed that a microbial balance might be achieved through the cooperation of two common microbial processes. The first process is the ongoing, natural souring due to hydrogen sulfide production

mentioned earlier. This is defined by the following equation:

$$\text{Sulfate} + \text{Volatile Fatty Acids} \longrightarrow \text{Cells} + CO_2 + H_2S \qquad (1)$$

This reaction operates in some fields in the process of souring. The SRB which are responsible for these reactions are capable of using a wide variety of soluble organic substances as carbon and energy sources [2-4,6,11,14]. The volatile fatty acids, which are quite common in oil reservoirs [7], are good substrates for SRB to drive this process (Table 6).

Most *Thiobacilli* are not normally considered anaerobic bacteria and would not be considered as potential organisms for MEOR processes because of their typically aerobic nature. However, *T. denitrificans* is capable of anaerobic growth if nitrate is supplied as an electron acceptor. The overall growth is defined by the following equation:

$$CO_2 + NO_3^- + \text{reduced S} \longrightarrow \text{Cells} + N_2 + SO_4^{=} + H^+ \qquad (2)$$

Combining equations 1 and 2 yields:

$$\text{Volatile Fatty Acids} + NO_3^- \longrightarrow \text{Cells} + N_2 + \text{fiT} \qquad (3)$$

Equation 3 also describes heterotrophic denitrification, with the addition of acid production. The formation of acid may then dissolve carbonates leading to the formation of dissolved CO_2. In addition, this dissolution of carbonate will buffer the environment at approximately a pH of 6.5 which is optimal for the growth of the autotrophic *T. denitrificans*. Thus, an oil field with some carbonate and a reduced S source (sulfide) may only require the addition of nitrate and minerals for growth of metabolically diverse denitrifiers and subsequent oil release.

Thiosulfate was shown to be the best all around energy

source for *T. denitrificans* and, although sulfur is also a very good energy source, it presents physical problems for the organism which makes it more difficult for them to derive all available energy from this energy source. Sulfide is potentially a very good energy source because of the available chemical energy, but its relatively high toxicity impairs its ease of use as an energy source.

To test these ideas in initial laboratory studies, the sequence below was followed:

1. Isolation and characterization of sulfate reducing bacteria from oil field produced water.
2. Isolation and characterization of denitrifying strains of *Thiobacillus* and their reaction with limestone.
3. Sequential feeding studies.
4. Mixing and oil release experiments.
5. Viscosifying agents and heterotrophic denitrifiers.

Gaseous products produced by denitrifying bacteria under conditions which might occur in an oil reservoir were determined, i.e. reducing conditions [redox potential about -100 mv, sulfide as an energy source, high nitrate concentrations]. Three sulfur sources were tested as sources of energy: thiosulfate, sulfide and elemental sulfur. These cultures were sparged with helium prior to sealing. The produced gases were then collected and subjected to gas chromatographic separations and quantitations. A key indicator of active denitrification is the production of nitrous oxide (N_2O) and nitrogen by the organisms. The *T. denitrificans* strains formed N_2O when using thiosulfate as an energy source. ATCC strains 25259 and 23644 both produced N_2O while using elemental sulfur (S^o), and ATCC strains 29685, 23642 and 25259 produced nitrous oxide with sulfide ($S^=$) as an energy source although growth appeared to be much less than when compared with growth with thiosulfate. In addition, large

amounts of nitrogen were produced by all cultures with each of these sulfur energy sources.

Sulfide is normally inhibitory at concentrations exceeding 80 ppm and must be kept at low concentrations, but may be added daily for good growth. Sulfide resistant strains are readily obtained which can tolerate up to 200 ppm sulfide. We tested the profiles of sulfate reducing bacteria grown in the presence of the same medium as *T. denitrificans*, but with added iron, organic acids (acetate, lactate, propionate, butyrate and formate) and reduced to an eH of about -200 mv. When the cultures are acidified, significant levels of dissolved CO_2 are released from solution along with significant H_2S. Traces of ammonia are also formed in these cultures. It appears from these data that these cultures may be realistically mixed. It also appears that redox potential does have some effect on *T. denitrificans*, although the ranges where they are tolerant to redox potential overlaps well with the redox potential requirements for *Desulfovibrio* and related sulfate reducing bacteria.

We have not been able to show significant methanogenesis in any of our cultures. It could be simply a matter of the media we envision necessary for MEOR cannot support the growth of significant populations of methanogens, or also it could be that the redox potential necessary for the successful cultivation of both denitrifying Thiobacilli and sulfate reducing bacteria is not reducing enough to meet the requirements of typical methanogens.

Limestone supplies the CO_2 necessary for the growth of *T. denitrificans* by the dissolution of carbonate which is in equilibrium with dissolved CO_2. Ordinarily, CO_2 is supplied to Thiobacilli *in vitro* in the form of dissolved carbonate or bicarbonate. The dissolution of carbonate buffers the growth

medium of the culture to a constant pH of 6.5, which is optimum for the growth of *T. denitrificans*. All acid produced during growth from the oxidation of reduced sulfur compounds is thus neutralized by the dissolution of limestone. The necessary added nutrients are ammonia and nitrate in the form of ammonium nitrate (supplying both the alternate electron acceptor and a nitrogen source for the organisms), a small amount of phosphate (only just enought to satisfy the nutritional requirements of the microbes otherwise the buffering capacity of the phosphate may interfere with the dissolution of the limestone), a reduced sulfur source necessary for the energy reactions of the cultures (this may, theoretically, be any of the following: sulfur, thiosulfate, tetrathionate or sulfide), and trace metals (most brines contain high enough concentrations of Zn, Cu, Co, Mn, Fe, Mo, W, Ni, and Se to satisfy the requirements of typical denitrifying microbes for trace elements).

As can be seen, under the best conditions, the only necessary materials which would need to be injected into a well for the proposed new MEOR processes would be ammonium nitrate and low concentrations of phosphate. Under the least ideal conditions this list would include ammonium nitrate, phosphate, a reduced sulfur source, the microbes, and possibly some trace metal nutrients in very low concentrations.

These results demonstrated that the autotroph *Thiobacillus denitrificans*, related denitrifying *Thiobacilli* and heterotrophic denitrifiers should have potential for MEOR, especially in reservoirs which contain carbonate rock. These reservoirs should include straight carbonate (limestone) formations, mixed limestone-sandstone formations and even some shale-limestone formations, although these could be very dependent on permeability and lithology factors. It was shown that *T. denitrificans* and related denitrifying *Thiobacilli are* capable of

dissolving limestone under a variety of conditions (examples are shown in Tables 7-10 below) which could allow the release of oil trapped in the limestone matrix, the limestone-sandstone matrix or, possibly even shale-limestone systems, since shale always contains some limestone ($CaCO_3$ with some $MgCO_3$). By the selective dissolution of limestone, oil trapped in that limestone will be released, thus increasing the ability to recover oil from economically marginal reservoirs.

All denitrifying *Thiobacillus* sp. that were tested will dissolve limestone, using it as their sole source of carbon. The examples cited in the tables used *T. denitrificans* ATCC 25259 and ATCC 23642, as well as some wild isolates from our laboratory experiments and enrichment studies, including "moderately" thermophilic isolates (40-45° C). Although there are no reported true thermophilic strains of denitrifying *Thiobacilli*, proprietary strains from produced waters and other natural oil field related sites and geothermal areas that grew at higher temperatures were isolated.

According to *The Procaryotes*, *T. thioparus* may also denitrify. We included this organism in our studies, but it did not appear to have any superior capabilities and was not as good a denitrifier as *T. denitrificans*.

Table 7

Dissolution of crushed (≥ 100 mesh) limestone with thiosulfate as energy source by denitrifying cultures.

Strain	% Dissolved
ATCC 25259	88.3
ATCC 23642	82.9
Buck Tail	85.0
Meadow Creek	86.1
1 N HC1	87.4

It appears from these data that there is about 12-17% acid insoluble material in this limestone sample.

Table 8

Dissolution of solid limestone with thiosulfate as energy source by denitrifying cultures.

Strain	mg/l Dissolved
ATCC 25259	740
ATCC 23642	760
Buck Tail	723
Meadow Creek	635

Table 9

Dissolution of solid limestone with sulfur as energy source by denitrifying cultures.

Strain	mg/l Dissolved
ATCC 25259	380
ATCC 23642	320
Buck Tail	370
Meadow Creek	330

Table 10

Dissolution of solid limestone with sulfide as energy source by denitrifying cultures.

Strain	mg/l Dissolved
ATCC 25259	60
ATCC 23642	160
Buck Tail	30
Meadow Creek	35

The amount of limestone dissolved creates the potential for new channels to be opened for water flow, which would have significant effects for oil recovery. In addition to the obvious effect of limestone dissolution, *T. denitrificans* would also have

the effect of the typical MEOR microorganisms, i.e. selective plugging by microbes of water washed channels, production of considerable N_2 gas for well pressurization (enough N_2 gas is produced in some cultures, that if the vessel is sealed tightly then the gas pressure can actually cause a glass tube or bottle to rupture) and production of increased CO_2 pressure through the dissolution of carbonate which is in equilibrium with CO_2. Also, this produced CO_2 may dissolve into the oil increasing oil mobility. With optimization, about 1 g of limestone can be dissolved by *T. denitrificans* per liter of culture in the laboratory. Thus, a waterflood which injects 15,000 liters of water per day could dissolve about 15 kg of limestone per day or 2 to 3 tons of carbonate could be dissolved in a year in a typical well if the microorganisms are continuously and actively growing. This is not likely to happen, but the numbers do indicate the potential of using *T. denitrificans* in MEOR processes.

Heterotrophic denitrifying bacteria can be readily isolated from oil field produced waters as long as the temperature is less than 65°C. Above 65°C, less nitrogen is formed from nitrate and more ammonia is formed, with a concomitant switch in the microbiological population from Pseudomonad type microorganisms to organisms resembling *Clostridium* and *Bacteroides*. However, both *T. denitrificans* and other denitrifying bacteria are present in significant numbers.

B. ISOLATION AND CHARACTERIZATION OF SULFATE REDUCING BACTERIA FROM OIL FIELD PRODUCED WATER.

In addition to the culture work done with *T. denitrificans* and other denitrifiers, we also isolated and obtained strains of sulfate reducing bacteria from oilfield produced waters (Table 11).

Table 11

Oil Field Produced Water Analysis[1]

Sample Location	TDS,%	Sulfide	Sulfate	Acetate	Propionate	pH
Alaska #1	2.2	12	140	1000	80	8.1
Alaska #2	2.4	22	95	1100	79	8.1
California #1	12.3	0	5	60	0	7.6
California #2	7.6	21	250	200	12	7.7
California #3	4.8	23	65	710	44	8.4
Texas #1	3.6	17	120	920	37	8.2
Texas #2	11.5	23	30	80	2	8.5
Oklahoma #1	18.2	0	50	0	0	7.9
Oklahoma #2	15.1	11	23	10	0	8.0

[1] Sulfide, sulfate, acetate and propionate are expressed as ppm.

Sulfide quantities were determined using an ion specific electrode. Sulfate levels were determined using a wet chemical method. Amounts of acetate and propionate were determined by a gas chromatographic method. Total dissolved solids were determined by residue after evaporation. We also analyzed for P and N which were not expected nor detectible in most of the samples.

We injected samples from these produced waters into medium which supports the growth of sulfate reducing bacteria. This medium contained 3.5% salt and contained acetate or a mixture of acetate/lactate as the carbon and energy source. We determined a rough titer of SRB numbers by serial dilution techniques. The results are shown in Table 12.

Table 12

Presence of SRB in Produced Waters

Water Sample	SRB	SRB Titer/ml
Alaska #1	+	10^5
Alaska #2	+	10^5
California #1	-	<1
California #2	+	10^5
California #3	+	10^3
Texas #1	+	10^3
Texas #2	+	10^3
Oklahoma #1	-	<1
Oklahoma #2	+	10^4

It can be noted that the produced waters which had no sulfide also had no (or small amounts) acetate or propionate and had the lowest pH measured and high TDS levels. This relationship of the volatile fatty acid content and sulfide presence and other water characteristics should be investigated and compared with data from other samples of produced field waters. Should a correlation between VFA content and SRB (sulfide) content occur, it may offer industry a method to predict which oil fields would become sour through microbial SRB action. These studies emphasize for the first time the importance of the VFA content of flood and connate waters.

Desulfovibrio desulfuricans (ATCC 7757) and *Desulfotomaculum nigrificans* (ATCC 19858) were obtained from the American Type Culture Collection and have been cultured at 40°C. These organisms were cultured on a minimal medium containing acetate, propionate and butyrate as carbon sources. These fatty acids are the typical ones found dissolved in waters from most oil fields and the concentrations used (100 to 750 ppm) are also typical (Table 11). We have also isolated strains from material obtained from the Trans-Alaska pipeline and other pipeline fouling material.

Sulfate reducing bacteria were very common in these samples and could use a wide variety of organic substances including the common volatile fatty acids normally found in produced waters. These organisms were active from 30-75* C and could use acetate and propionate individually or together. When both acetate and propionate were present, the propionate was used preferentially over the acetate. The key to these organisms is that if *T. denitrificans* and *Desulfovibrio* can be mixed in a single culture then the naturally-occurring fatty acids which drive sulfur reduction will serve as the energy source for the denitrifying Thiobacilli.

Thus, the only necessary nutrients which will need to be added will be ammonium nitrate and some phosphate. All other nutrients will be supplied by naturally-occurring chemicals in the formation and brines. It should be noted that these organisms exist mixed in nature, but it is difficult to know whether or not they can be artificially mixed in a non-typical environment and both be active at the same time. There is a possibility that eH may be an important factor.

The addition of nitrate to the produced water systems (Table 11) caused the level of sulfate reducing bacteria in the system to drop to <10/ml and the heterotrophic denitrifying bacteria increased to about 10^8/ml (Table 12-13). These data show that high concentrations of the carbon sources (acetate-propionate) are required for both SRB and denitrifying organisms. In the presence of the nitrate and carbon sources the denitrifiers grow and reduce the level of SRB. These results show the importance of the presence of the carbon source on both sulfide generation and its removal. Sulfide dropped to 0 and there was no apparent new sulfate reduction. We were also able to recover about 500 *T. denitrificans*/ml.

Table 13
Inhibition of SRB by Denitrifying Bacteria (DB)
in the Presence of Nitrate

Sample	Acetate	Sulfide	N_2	N_2O	SRB/ml	DB/ml
Alaska #1	0	<5	+	-	<10	$>10^7$
Alaska #2	0	<5	+	+	<10	$>10^7$
California #1	55	0			<10	0
California #2	0	<5	+	-	<10	$>10^7$
California #3	0	<5	+	-	<10	$>10^7$
Texas #1	0	<5	+	+	<10	$>10^7$
Texas #2	70	15	-	-	<10	0
Oklahoma #1	0	0	-	-	<10	0
Oklahoma #2	10	15	-	-	<10	0

C. METHANOGENIC BACTERIA.

The third group of microorganisms which may play an important role in these MEOR studies are the methanogenic microorganisms. Methanogens are a part of the microbial community in most oil fields,[11] but they are always a minor proportion of the population. The source of reducing power should be the simple volatile fatty acids, but these have not been identified in oil fields with the acceptor being CO_2. Instead we have found that the major methanogens are those which use methanol as a source for methane and that they comprise less than .1% of the microbial population. For these reasons we feel that this aspect of the project is not as favorable as the positive response with denitrifying population effects.

D. MIXING AND OIL RELEASE EXPERIMENTS

1. Sequential Feeding Studies.

Under normal laboratory conditions, both the SRB and the denitrifying *Thiobacilli* grow at roughly the same rates. Therefore, the organisms might be capable of coexisting and

supporting each other. If *T. denitrificans* and *Desulfovibrio* can be mixed in a single culture, then the naturally-occurring fatty acids which drive sulfate reduction will serve as the energy source for the denitrifying *Thiobacilli*.

We again grew various species of *Desulfovibrio* in media containing acetate and propionate (25:1) on a molar basis, which is the typical ratio of these volatile fatty acids found in oil field waters). This medium was then filtered through a .22 μM filter and sodium bicarbonate and ammonium nitrate were added. The medium was then inoculated with *T. denitrificans* which grew well as long as the sulfide content was not too high (<80 ppm). *T. denitrificans* did not grow in the medium if no reduced sulfur was present in the medium. Clearly the products of the metabolism of the sulfate reducing bacteria can supply necessary nutrients for the denitrifying bacteria, especially the reduced sulfur source.

However, once the systems were not controlled for pure cultures, heterotrophic denitrifiers became dominant and used the acetate, propionate and nitrate for growth. They did not prevent the growth of sulfate reducing bacteria or the *T. denitrificans*, but they became more than 90% of the total microbial population. Simply put, the addition of nitrate to oil field waters prevents the growth of sulfate reducing bacteria because denitrifiers are more efficient and grow more rapidly. They also metabolically remove sulfide. The results demonstrate the important advantage in that sulfate reducing bacteria are completely inhibited, but not immediately killed, by the introduction of nitrate into their environment.

After these data were collected a series of cultures were prepared to determine if heterotrophic denitrifying bacteria were present and to determine their effect on the SRB population and

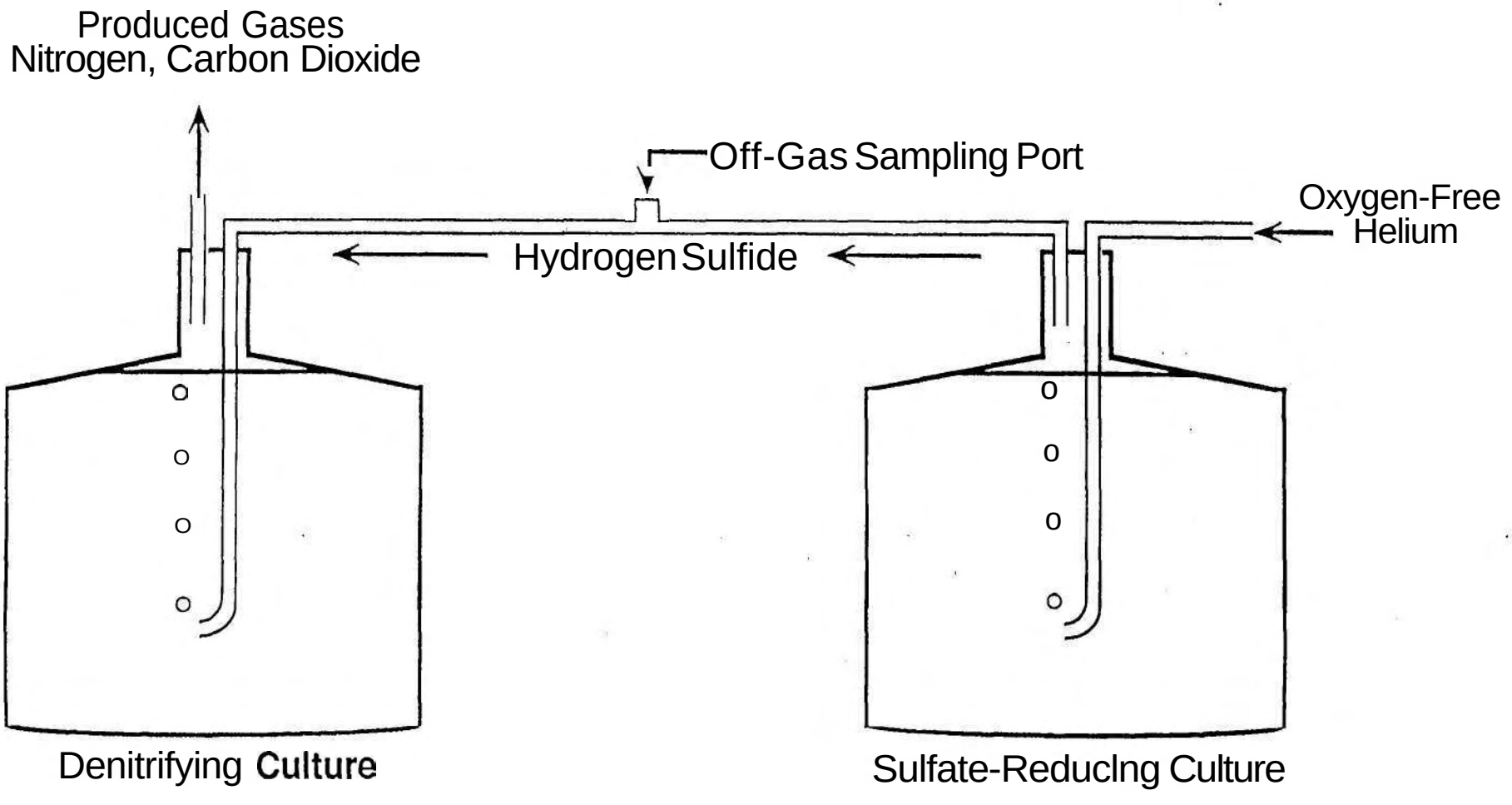
sulfide concentration. Fifty ml of each water sample was put into a 100ml serum bottle and sparged with He for 15 minutes to drive off nitrogen and oxygen gas. The bottle was then capped with a butyl rubber stopper and a deaerated solution containing $NH_4H_2PO_4$ and NH_4NO_3 was introduced with a 10 ml syringe. The syringe was left in the stopper to collect produced gas. The produced gas was chromatographically separated and quantified by gas chromatographs. If denitrifying bacteria were present they would consume the acetate and produce N_2O and N_2. In addition, they should also inhibit the formation of sulfide by SRB and reduce the number of viable SRB remaining in the system. The addition of nitrate to this system indeed did cause the level of sulfate reducing bacteria in the system to drop to <100/ml and the heterotrophic denitrifying bacteria increased to about 10^8/ml. Sulfide dropped dramatically and there was no apparent new sulfate reduction.

Thus, the injection of nitrate into these waters helps remove sulfide. It was also found that high salt (i.e. TDS>5-7%) inhibits the growth of these denitrifying bacteria. There are halotolerant (up to 8% salt) denitrifiers and very halophilic (up to saturation) denitrifiers reported in the literature. However, these microbes may not be present in all oil field produced waters. Clearly the denitrifiers not only remove existing sulfide but also prevent *de novo* sulfide production. This action extends to the inhibition of SRB growth and possibly also acts to kill viable SRB cells. These results offer a potential treatment for the control of microbial induced corrosion.

The next stage in these experiments was to link the cultures more directly. This was done via a two stage culture as diagrammed in figure 4. This system was designed to supply the necessary reduced sulfur sources as sulfide from the growth of the sulfate reducing bacteria to the denitrifying bacteria in the

FIGURE 4

SEQUENTIAL CULTURE DIAGRAM



second vessel. The first vessel contained all of the necessary nutrients for sulfate reducing bacteria and after 12 days good growth of the sulfate reducing bacteria was evident. Helium was passed through the first vessel to scrub the sulfide as hydrogen sulfide out of the culture and introduce it into the second vessel where the medium was complete for the growth of the denitrifying bacteria, except that it lacked a reduced sulfur source. Growth commenced 3 days after the first vessel began to turn black, but was not very vigorous. We found it necessary to attach an off gas trap from this culture through 1 N NaOH to trap sulfide which completely passed through the system.

The first vessel supported the growth of $5x10^8$ sulfate reducing bacteria per ml and the second vessel contained about $2x10^8$ denitrifying *Thiobacilli* per ml culture. Thus, the products of the metabolism of sulfate reducing bacteria can supply necessary nutrients for the denitrifying bacteria, especially the reduced sulfur source. Since these two cultures could be directly linked in sequence to each other we began the next stage of mixing in sand packs.

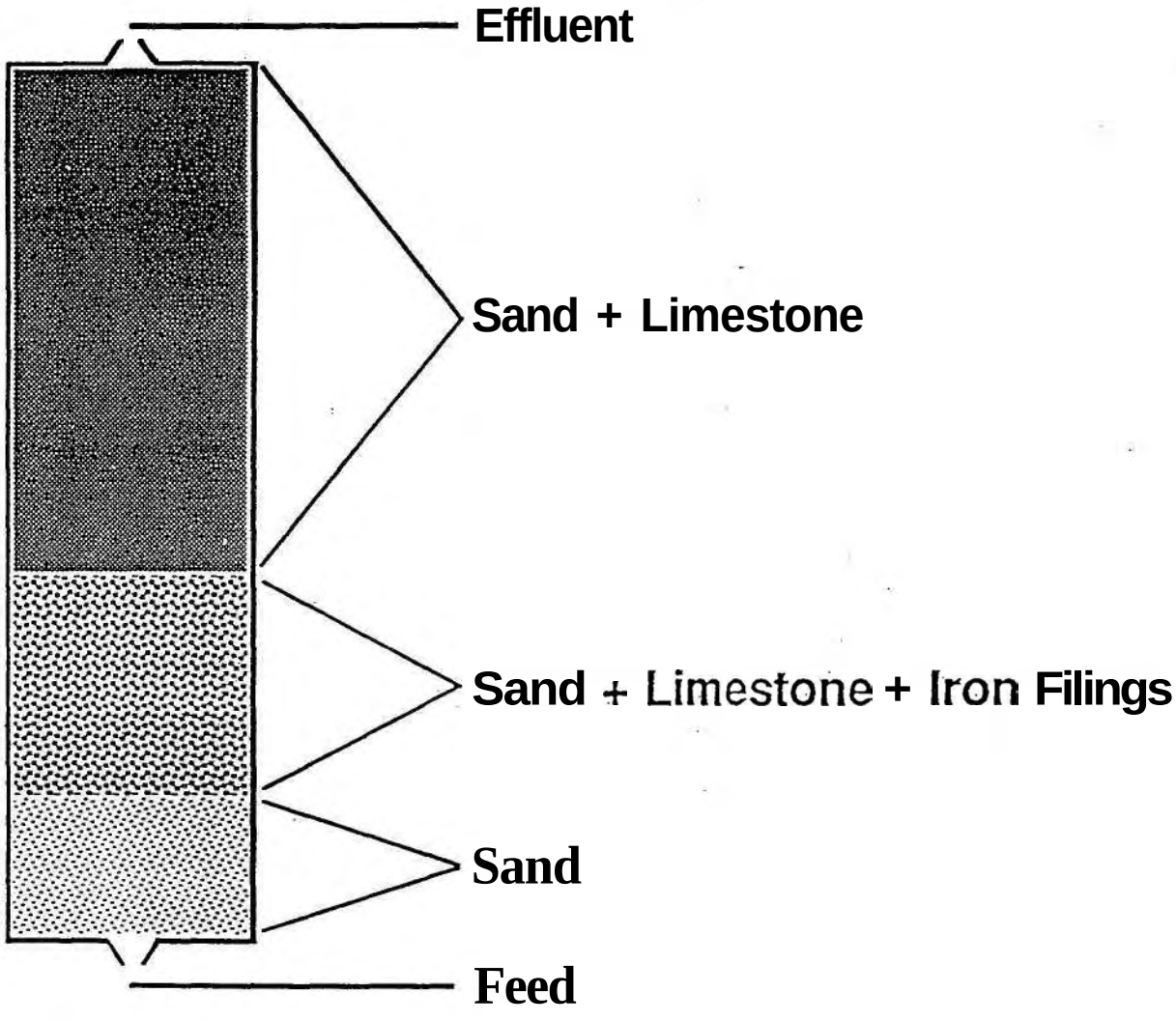
2. Oil Release by Denitrifying Cultures.

a. Sand Pack Experiments-Microbial Growth Interactions.

To determine the effect and extent of microbial interactive growth on the oil releasing capabilities by employing denitrifying cultures and conditions, a series of sand pack growth and oil releasing studies were conducted. A sand pack containing acid washed Mill Creek sand and 0.1% w/w crushed limestone was constructed. This was an 80 cm column and was fed from the bottom using an hydrostatic head. The feed was the nutrient solution representing field water compositions at a redox <-300 mv and 100 mesh iron filings were included in a layer to detect the formation of FeS. See Figure 5 for a diagrammatic

FIGURE 5

DIAGRAMMATIC REPRESENTATION OF A SAND PACK COLUMN



representation of this experiment. After two weeks the column became blackened at the layer of the iron filings and gas was produced in the sand matrix above the iron layer. This gas was shown to be nitrogen and some carbon dioxide, consistent with what would have been expected from the process of denitrification. After the experiment was concluded we found high levels of sulfate reducing bacteria in the iron filing layer of the sand pack. In addition, the effluent water contained no acetate or propionate. *T. denitrificans* was found in the effluent at approximately 10^9/ml and sulfate reducing bacteria were also found in the effluent at about 10^4/ml. These experiments showed that these two microorganisms can survive well together and that one organism was sequentially feeding the other.

b. Sand Pack Experiments - Oil Release.

Sand packs also were used to simulate cores for oil release studies. A 2.5 x 70 cm column was packed with acid washed sand and 0.1% (w/w) crushed limestone. Mill Creek sand was saturated with oil over a period of one week exposure and was then washed very slowly with a reverse gravity flow of water until very little oil was released over a period of 24 hr. This washing took approximately two weeks. This sand was then drained of free water. The system was flooded with a liquid which contained an artificial produced water as well as different carbon and energy sources in the presence and absence of ammonium nitrate. The system was inoculated with a mixed consortium of organisms which contained denitrifying bacteria. Air was removed by sparging with helium. The column was fed from the bottom using a hydrostatic head. The feed was the nutrient solution representing field water composition at a redox <-300 mv. Released oil was measured on the surface of the liquid in the graduate cylinder as the oil was released (Table 14).

Table 14
Sand Pack Oil Release Studies
in the Presence of Denitrifying Organisms.

Additions to Water	Oil Released ml	% Increase over Control
None (control)	.75	0
Nitrate	.8	6
1000 ppm acetate	.75	0
100 ppm propionate	.85	13
Acetate + nitrate	1.64	119
Propionate + nitrate	1.55	107
Acetate + Propionate + nitrate	1.45	93
Molasses (.2%)	1.25	67
Molasses + nitrate	1.38	84

These data in Table 14 are very revealing. The addition of a non-fermentable carbon source (such as acetate), by itself does not increase oil release. This would be expected because such carbon sources are already present in many oil fields. The data show that the addition of nitrate in the absence of a carbon source also does not release oil. The introduction of a fermentable carbon source (sugars), such as found in molasses, (which contains some nitrogenous compounds), causes an increased oil release. This is the standard MEOR technology which has been shown to have some applicability. However, the additional additive of nitrate with the fermentable carbon substances will produce additional oil. More importantly, the data show that the addition of nitrate in the presence of non-fermentable carbon sources (acetate and propionate) will cause a significantly greater increase in oil release. These data demonstrate that the new MEOR system, which employs the utilization of pre-existing carbon sources in the reservoir waters in the presence of nitrate, has the potential to cause additional oil recovery.

3. Core Flooding Experiments.

a. Flooding in Absence of Nitrate.

A Berea sandstone core (6", 400 mD) was prepared and flooded. The water contained sufficient mineral nutrients to support about 10^6 sulfate reducing bacteria/ml at 45°C. We made an artificial mixture of salts to approximate this analysis for use in our core flooding and bottle experiments. A rate of flooding of 1ml of liquid/hour was targeted as a steady state. The driving force was nitrogen gas pressure. The water was constantly sparged with nitrogen to keep it anaerobic. During the course of this flood the sulfate reducing bacteria increased to about 10^8/ml, the sulfate concentration went down to 0, sulfide increased to 85-120 ppm, the propionate was totally used up and the acetate reduced to <800 ppm. In order to keep the 1ml/hr flow rate, the pressure on the system was increased up to about 250 psi. This system represented a typical flood that is experiencing SRB and sulfide problems.

b. Flooding in the Presence of Nitrate.

A similar Berea sandstone core (6", 400 mD) was prepared and flooded with nitrate present. The system utilized the same feed as above, but with bicarbonate added, since we felt that the Berea sandstone may not have enough carbonate to satisfy the denitrifying bacteria. The results were essentially the same as for the sand pack, except it was noticed, for the first time, that heterotrophic denitrifying bacteria were present in high concentrations. In experiments utilizing pure cultures of *Desulfovibrio desulfuricans* and *T. denitrificans*, the heterotrophic denitrifiers did not have a chance to compete.

In the Berea sandstone cores, and probably in the sand packs, the heterotrophic organisms became dominant, although they did not obviate the growth of either the sulfate reducing bacteria or the denitrifying *Thiobacilli*. Quite simply, once the

systems were not controlled for pure cultures, the heterotrophic denitrifiers became dominant and utilized the acetate and propionate and nitrate for growth. They did not completely prevent the growth sulfate reducing bacteria or the *T. denitrificans*, but they became more than 90% of the total microbial population. Thus, the addition of nitrate to oil field waters reduces the growth of sulfate reducing bacteria because denitrifiers are more efficient and grow more rapidly. However, in most oil fields the concentration of total volatile fatty acids dissolved in the water is below 500 ppm and if sulfide is present then *T. denitrificans* could become a dominant organism as long as nitrate is present.

The big unexpected advantage of the results of this experiment is that sulfate reducing bacteria could be completely inhibited by the introduction of nitrate into their environment (see Table 13). Thus, we have a method for the prevention of additional souring in an oil field and the sweetening of the field by the removal of the sulfide. Both the heterotrophic denitrifiers and *T. denitrificans* are capable of oxidizing sulfide, even in the precipitated form of FeS.

c. Oil Recovery Tests.

A flood water representing a produced field water was prepared and contained sufficient nutrients to support about 10^6 sulfate reducing bacteria/ml at 45°C. A new sandstone core (200-400 mD, 6 inch core) was flooded with this solution for about ten days and then flooded with oil obtained from the same field. The oil was filtered through a 1 μM filter to remove particulate matter which might clog the core. A rate of flooding of 1ml of liquid/hour was targeted as a steady state. The driving force was nitrogen pressure. The water was constantly sparged with nitrogen to keep it anaerobic. After about 12 days a steady rate of oil production was noted through the core and the test flood

began. This core was flooded for 60 days, constantly measuring the amount of oil produced. The data are graphically presented in Figure 6. A second core was treated in an identical manner, except after oil loading the core was inoculated with 10 ml of a mixed culture of sulfate reducing bacteria, *T. denitrificans* and heterotrophic denitrifying bacteria. Then the system was flooded with sterile water supplemented with 0.1 g/l monobasic ammonium phosphate. During the course of this flood the sulfate reducing bacteria increased to about 10^8/ml, the sulfate concentration went down to 0, sulfide increased to 85-120 ppm, the propionate was totally used up and the acetate reduced to <200 ppm. In order to keep the 1ml/hr flow rate the pressure on the system was increased to about 250 psi. As can be seen, about 15% more oil was recovered when compared to the control flood (Figure 6).

An additional core was treated identically up to the point of water flooding. At this point, the flood was treated similarly, but 2g/l ammonium nitrate was additionally added. Under these conditions, the level of sulfate reducing bacteria in the system decreased and the heterotrophic denitrifying bacteria increased to about 10^8/ml. The results of this flood are shown in Figure 7. As can be seen, additional oil was recovered (approximately 30%). Sulfide content dropped to 0 and there was no apparent sulfate reduction. We were also able to recover about 500 *T. denitrificans*/ml. We did not observe any in the previous flood.

E. VISCOSIFYING AGENTS AND HETEROTROPHIC DENITRIFIERS.

We have shown that the heterotrophic denitrifying bacteria in produced water of oil fields are uniformly able to produce cultures of high viscosity when starved for ammonia. Many denitrifying bacteria are not capable of operating both dissimilatory and assimilatory nitrate reducing processes simultaneously. Therefore, the addition of the Na or K salt of

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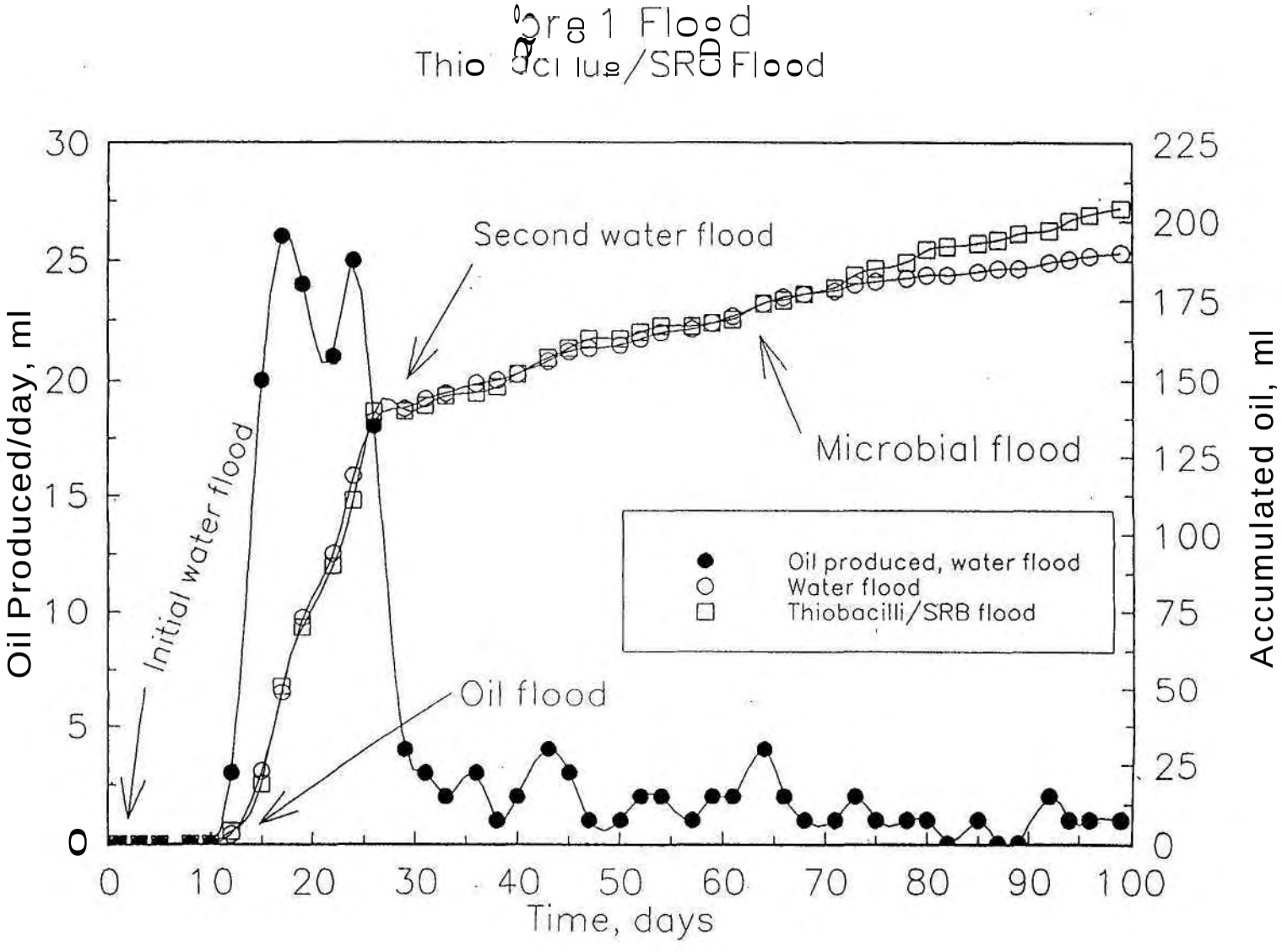
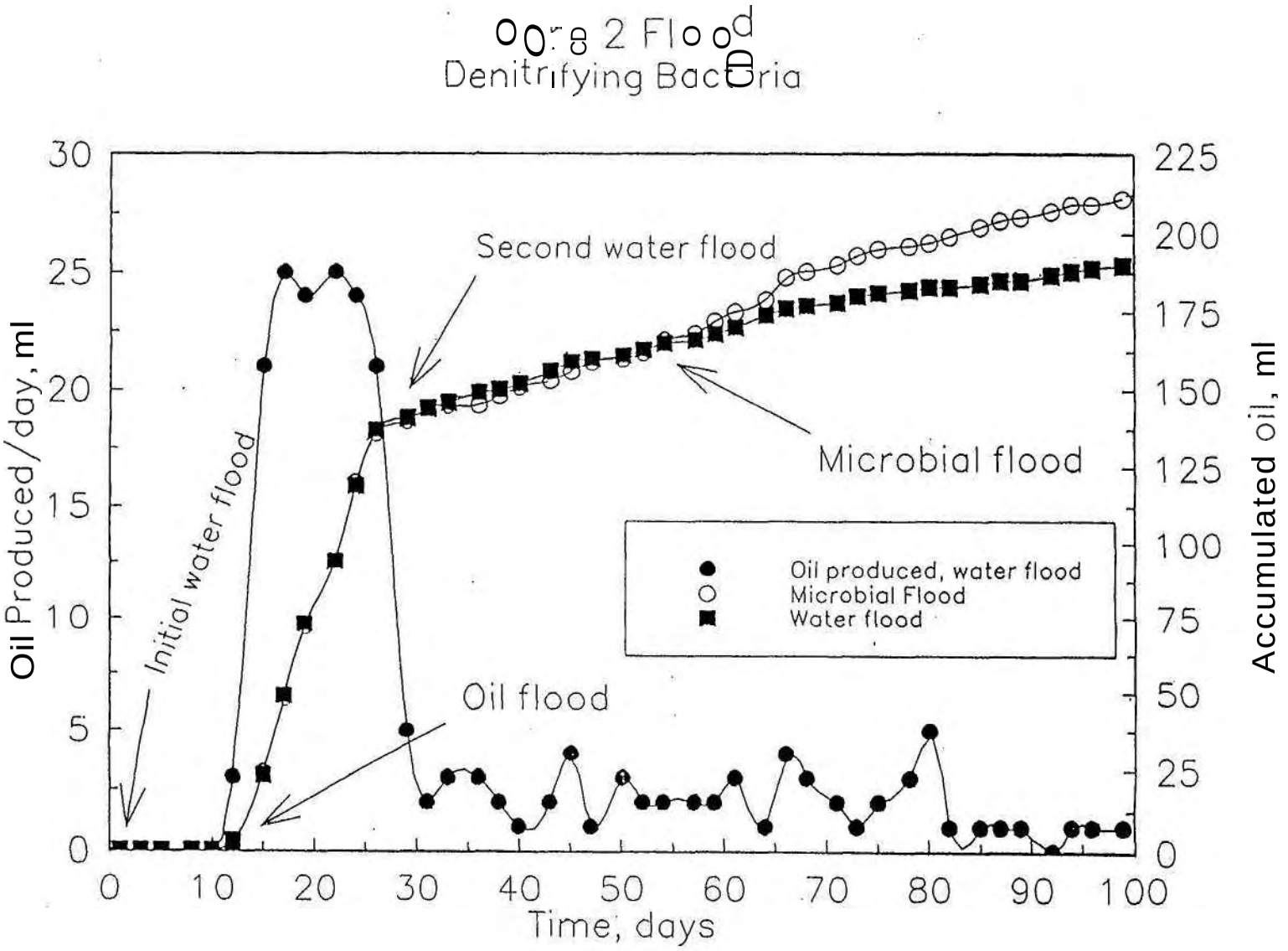


FIGURE 7

DENITRIFYING BACTERIAL FLOOD OF A SANDSTONE CORE



nitrate causes the cultures to become nitrogen starved; under these conditions excess carbon is transformed into viscous extracellular polysaccharides. Cultures grown with acetate and propionate and acetate:propionate mixtures readily showed the formation of viscosifying agents (150-500 centipoise). If potassium nitrate is used in place of ammonium nitrate, cultures using acetate, propionate and a mixture of acetate/propionate (25:2) as sole sources of carbon and energy produced highly viscous cultures. These viscosifying agents can also be induced with ammonium nitrate if the level is very carefully controlled such that the cells become starved for nitrogen. It should therefore be possible to use these organisms to produce viscosifying agents *in situ* and therefore get better water "push" during water flooding.

Thus, denitrifying bacteria found in oil field produced water will produce highly viscous cultures when starved for ammonia. The limitation of only nitrate addition and no ammonia as a source of nitrogen drastically reduces the nature of the mixed microbial population. With only nitrate almost all of the organisms belong to the genus *Pseudomonas* and related genera, whereas the addition of ammonia to the flood waters induces a mixed population containing many diverse types of microorganisms.

We also know that as the temperature increases, the ability of denitrifying bacteria to perform dissimilatory nitrate reduction becomes more limited, such that at temperatures over 60°C nitrogen gas is formed at lower concentrations with the majority of the nitrate being reduced to ammonia. Under these conditions, a reduction of in *situ* produced viscosifying agents occurs. However, at temperatures less than 60°C dissimilatory nitrate reduction is predominant and, in the absence of ammonia, causes the population to produce highly viscous solutions.

In core studies, the injection of microorganisms and a

nitrogen poor medium rapidly causes the increase in pressure such that flow is quickly stopped at 200 psi. The reintroduction of a nitrogen balanced medium into the injection stream causes the pressure to drop to less than 100 psi within a week of flooding. When these organisms were introduced into a Berea sandstone core, the production of viscosifying agents caused a rise in the pressure on the core necessary to maintain a 1 ml/hr flow rate to >250 psi (the limit of our existing system). The core became plugged and we were not able to reverse the plugging by the introduction of ammonia which we believe would happen if we could force new nutrients into the core.

VII. DISCUSSION.

In reservoirs undergoing souring, significant populations of sulfate reducing bacteria occur. These microorganisms may be supported by minerals native to the reservoir and simple dissolved organic material in the formation water. Even without significant dissolved organic material, SRB are capable of autotrophic growth in oil reservoir environments where sulfate and other mineral nutrients are available. They are also capable of integrating sulfate reduction and ammonification of nitrate. Often sulfate is absent from formation waters, and therefore, the growth of sulfate reducing bacteria is not favored. However, when waterflooding operations begin, new substances (such as sulfate) are introduced into the reservoir (especially if sea water is used to make up volume and maintain pressure) and SRB then become active and powerful geochemical agents.

We determined the ability of heterotrophic denitrifying bacteria to grow in typical reservoir fluids and to produce gas and other products in sand packs and in cores and the effect of denitrifying organisms on oil release from these cores. We also showed that heterotrophic denitrifiers may produce viscosifying agents *in situ.* Since the fuel for denitrification by

heterotrophic bacteria is dissolved organic material in water, *T. denitrificans* will then become active when these are depleted. In reservoirs where there is significant reduced sulfur (probably primarily as H_2S) and little dissolved organic material *T. denitrificans* may become an important tool in MEOR technologies.

In sour or souring carbonate-containing reservoirs that have negligible levels of simple dissolved organic materials, active populations of denitrifying Thiobacilli can be supported by the introduction of nitrate and low concentrations of other mineral nutrients. Under these conditions SRB and the denitrifying Thiobacilli form a mutually supporting microbial population. This work has led to US patent #5,044,435.[14] There are almost always metal sulfides present in oil formations and we have shown that denitrifying bacteria are capable of solubilizing these, releasing the sulfide for metabolic purposes. Other Thiobacilli are well known for this property. These microorganisms may be very beneficial for MEOR processes, both for oil release and sweetening of the reservoir.

In typical reservoirs which have complicated mixtures of available nutrients such as simple dissolved organic substances (volatile fatty acids) and reduced sulfur compounds, the introduction of nitrate will produce several effects. First, heterotrophic denitrifying bacteria will rapidly become predominant and will slowly remove sulfide, including both dissolved sulfide as hydrogen sulfide and some crystalline metal sulfides through solubilization. The growth of these organisms rapidly inhibits the growth of SRB and inhibits new sulfide formation. This sulfide metabolism is apparently not involved in energy metabolism.

Second, these microorganisms, given enough nutrients, will completely oxidize the simple dissolved organic compounds to CO_2

with concomitant production of nitrogen gas. The contribution of CO_2 produced by these organisms leading directly to oil mobilization may be great. CO_2 floods are an accepted and valuable EOR technique. The possibility of the complete removal of dissolved organic materials from the reservoir is quite real and would allow organisms such as *T. denitrificans* to then become established.

And third, they may form highly viscous solutions if the available nitrogen is limited. All of these effects would be positive factors for MEOR processes. An additional possible advantage is that, since the alterations to the natural environment are minimal and simple, it may be possible to avoid well bore clogging by microorganisms since the simple inorganic nutrients could more readily penetrate into the rock formations.

This work has demonstrated the potential of a new MEOR technology to recover additional oil from reservoirs. In addition to the information developed on the microbial populations present in reservoirs, their interactions and energy sources that already exist or are introduced into reservoirs is important. It can now be theorized that the VFA content of the reservoir is a key factor in the souring of a reservoir. In addition it has been shown that the microbial population dynamics and interrelationships can be modified by simple mineral additions or deletions. These interactive and sequential populations will play an important role in any MEOR process and more importantly these interactions are now occurring in the fields even in the absence of deliberate manupulative MEOR techniques.

The data show that when sulfate containing waters are introduced into reservoirs with a VFA content it can be expected that hydrogen sulfide generation will result. Thus, the level of

sulfide could be governed by the content of the VFA. This may explain why a flood has a progressive increase in sulfide content with time, but then the level of sulfide reaches a plateau even though high levels of sulfate are present. This importance of the VFA content of flood waters has not been recognized previously and may be the key factor in determining which fields will suffer with sulfide and microbial problems. Our work has taken advantage of this VFA factor and have used it to develop a new technology in which a favorable microbial population is developed which out-competes an undesirable population. This replacement effect can be induced by the addition of only a simple inorganic compound (nitrate). The denitrifying population which developes can be manipulated by the ammonia content and offers the potential to produce reservoir profile modifications. In addition, the denitrifying autotrophic *Thiobacilli* have the capability of attacking carbonates in reservoirs and produce high levels of CO_2 and N_2. These effects of rock dissolution and gas generation should have positive effects on increasing oil release and recovery.

Thus, in developing this new MEOR technology many factors and observations pertinent to reservoir ecology and oil production practices were uncovered and investigated. It is believed the application of this new MEOR technology will have a significant impact on oil field operations and offers industry a methodology for treating sour reservoirs and concurrently recovering more oil.

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