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TOPICAL REPORT

**USE OF LABORATORY PROCEDURES
FOR SELECTION OF MEOR FORMULATIONS**

by

R.S. Bryant, J. Douglas, and K. Bertus

Work performed for
U.S. Department of Energy
Under Cooperative Agreement
DE-FC22-83FE60149



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Project BE3, Milestone 7, FY88

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ABSTRACT

In order to optimally design a field experiment, laboratory studies must simulate the field conditions as closely as possible. When injecting a microbial solution into a petroleum reservoir, it must survive and produce the chemicals that are necessary for improving oil mobilization. To obtain these microbial cultures, a series of compatibility experiments should be performed with all reservoir fluids, and under the environmental conditions of temperature and pressure in the selected reservoir. Porous media tests, such as microbial coreflooding and micromodel studies, can serve as excellent tools for determining the oil recovery mechanisms of microbial formulations and comparing the effectiveness of different microbial formulations. As part of a field experiment sponsored by the U.S. Department of Energy, the above laboratory studies were used to develop the microbial formulation for a waterflood field test in Delaware-Childers field. This paper will discuss the design of laboratory compatibility studies, coreflooding and other porous media tests, and covers tasks 1 through 5 of the FY88 BE3 annual research plan.

INTRODUCTION

The depletion of petroleum reserves in the United States and the continuing dependence upon imported oil has caused great concern to the United States Government and to the oil industry.¹ Microbial enhanced oil recovery (MEOR) processes are rapidly emerging as technologies for independent oil producers, as well as major oil companies. Microbial methods for improving oil recovery are attractive because the cost of the injectant is relatively low in comparison with that of other enhanced oil recovery methods. MEOR processes use microbial cultures (usually bacteria) that can survive and grow at the temperatures and salinities found in petroleum reservoirs. This is a favorable economic process because the growth of the organisms can be sustained under reservoir conditions by injection of a relatively inexpensive and readily available nutrient such as molasses.

Although several single-well field trials have been conducted using microbial formulations, there is very little evidence from either laboratory or field data to establish the mechanism of oil recovery by microorganisms. A number of mechanisms have been proposed for the enhancement of oil recovery, including production of gases, surfactants, acids and solvents. One of the constraints to determining the key mechanisms for MEOR is that little or no quantitative data are available on the amounts or rates of production of gases, surfactants, acids, and solvents under reservoir conditions. Also, the relationship between the production of metabolites and oil recovery efficiency has not been established. Additional laboratory studies are needed to develop procedures for optimizing microbial formulations for oil recovery applications. A coordinated effort between laboratory and field experiments must be maintained to further develop MEOR technology.

This work is a continuation of the ongoing research program at NIPER in the area of microbial enhanced oil recovery. NIPER has currently assembled one of the world's best equipped MEOR laboratories. The research program has focused on two major areas: optimization of microbial activity in porous media for improving oil recovery, and development of criteria necessary to implement microbial technology in oilfield applications in an environmentally safe manner.

A series of experiments was performed to determine and quantify which metabolites are important for oil recovery. The target metabolites will include carbon dioxide, surfactants, acids, and alcohols that are produced by strains of microorganisms used in MEOR tests.

TYPES OF MICROBIAL EOR METHODS

The most commonly applied microbial method to date has been single-well cyclic stimulation in stripper wells (figure 1). A volume of a microbial formulation and nutrient is injected into a producing well; the well is usually shut in for several days or weeks, then production is resumed. Nutrient may be injected occasionally to sustain the microbial activity. Improvement in oil recovery from single-well injection can result from removal of asphaltic deposits from the near-wellbore region, or from mobilization of residual oil in the limited, relatively small volume of the reservoir that is treated. Single-well stimulation treatments can be applied with only minor

modifications to existing field facilities and are relatively inexpensive.²⁻³ An alternate method that has a much greater oil recovery potential is a microbial-enhanced waterflood (figure 2). A microbial formulation is slugged into injection wells and pushed through the formation with the injected water. Injection of nutrient is required to sustain the growth of the microbial formulation as it moves through the formation. Microbial-enhanced waterflooding has not been extensively field tested, but some projects are being conducted to determine its technical feasibility.⁴⁻⁶ This method has a greater economic potential than single-well treatments because of the larger volume of the reservoir that can be treated and therefore a larger amount of oil can be recovered.² Microbial formulations that produce viscous biopolymers and biomass are also being developed for permeability modification treatments.⁷⁻⁸ In reservoirs that have high permeability zones, it may be feasible to block these zones by injection of a microbial formulation that will divert the flow of water into unswept areas of the reservoir, thereby increasing sweep efficiency.

SELECTION OF MICROBIAL SPECIES

There are two major requirements for a successful microbial EOR formulation: (1) they must be able to thrive under the reservoir conditions of temperature and pressure, and salinity and mineral content of the water in that formation; and (2) they must also produce the necessary products that will enhance oil recovery -- surfactants, alcohols and acids, or polymers. One unique aspect of in situ MEOR is that the microbes tend to aggregate at the oil/water interface and produce the chemicals there. This probably is a key to enhanced oil mobilization by microorganisms.

Since a major criterion is that the microbes must grow under reservoir conditions, many microbial species used for enhanced oil recovery are first isolated from petroleum reservoirs or around sites of petroleum contamination. Once the microorganisms have been isolated from these sites, they are transported to a laboratory where they can be evaluated for desirable characteristics.

Microorganisms have a very short generation time, usually doubling their population every 20 to 30 minutes; therefore, their mutation rate is also high. Microbiologists can adapt these bacterial strains by growing them under

a series of nutritional variations and conditions in order to enhance their capabilities for producing certain products or tolerating certain environments. As an example, bacteria from several different petroleum reservoirs and sources have been used in microbial coreflooding studies with Berea sandstone for several years at NIPER.⁹⁻¹¹ These bacteria appear to transport through sandstone with permeabilities as low as 100 millidarcies, and seem to thrive better in Berea sandstone cores than other microbial species that have not been adapted to rock.¹²

One important aspect of microbial enhanced oil recovery processes that is sometimes overlooked is the pathogenicity of microorganisms. Certainly any microorganism to be considered for field injection should be tested to ensure that it is not pathogenic. Probably the easiest method of pathogenicity testing is to expose mice to the microbial formulation, both by feeding and injection. A complete biochemical characterization of the microbial species will determine whether the microbe in question is pathogenic. Not only is there a potential danger if the microbe is pathogenic, but the products of microbial metabolism may be toxic and this possibility should be investigated.

COMPATIBILITY TESTING

Compatibility testing of microbial formulations and reservoir fluids is crucial to the success of any MEOR field project. Not only must reservoir fluids be tested, but they should be tested under the same conditions of temperature and pressure as those of the target reservoir. Some examples of compatibility tests that we recommend include: (1) testing of the microbial formulation with the nutrient at several decreasing concentrations since the nutrient will be extremely diluted by formation water and/or injection water in the case of a waterflood; (2) compatibility testing with reservoir brine and crude oil at the temperatures of the formation; (3) compatibility testing with formation rock, preferably reservoir cores, but at least rock that is representative of the formation, i.e. a sandstone reservoir can be simulated with Berea rock; (4) some type of long-term stability testing of the microbial formulation with the reservoir fluids and rock, such as 2 to 3 weeks, in order to determine if the microbial formulation can survive under reservoir conditions; and (5) we recommend that in a larger field project, such as an ongoing waterflood operation, a single-well injectivity test should be done to

determine if the microorganisms can survive during a shut-in period, and if there are any adverse effects on injection rates or pressures.

Substances that are toxic or inhibitory for the microbial formulation may be present in a reservoir. If a mixture, or consortia of microorganisms is used, all species must be cultured together in the reservoir fluids under reservoir conditions. Earlier compatibility studies using a consortia of bacteria have shown that certain bacteria are able to out-compete and overgrow other species present.⁹ It is imperative that these preliminary tests for compatibility are performed before a microbial injectant is selected for field application.

POROUS MEDIA TESTING

Microbial verification of enhanced oil production in porous media is a valuable laboratory tool for field projects. The use of porous media simulates the field conditions more effectively than flask testing. Several types of porous media can be used. Information obtained prior to the initiation of a field project can help to optimize the injection protocol for the field. To adequately use physical models of porous media for microorganisms, some assumptions must be made about the type of oil recovery mechanism that will be desired for the field. There are several different types of microorganisms that can have applications for enhanced oil recovery (table 1) but all of these will not be used for one field project. As an example, in a microbial-enhanced waterflood field project sponsored by the U.S. Department of Energy, Microbial Systems Corp., and INJECTECH, Inc. and conducted by NIPER, our target of interest was the trapped residual crude oil left in the water swept zone. We therefore assumed that our microbial formulation would assist in releasing that trapped crude oil by producing surfactants and other chemicals.¹³ In another MEOR waterflood project sponsored by the U.S. Department of Energy, injection water channeling in the field appears to be a major problem for oil production, and a microbial formulation will be designed to divert injection water to the by-passed areas of the reservoir. This means that the microorganisms used for this test will be primarily polymer producers.¹⁴⁻¹⁵

A laboratory testing program using porous media must be designed to assist in simulation of the types of recovery mechanisms desired. If microbial

TABLE 1. - Microbial species used in enhanced oil recovery

Scientific Name	Aerobic or anaerobic	Products
<i>Clostridium sp.</i>	anaerobic ¹	gases, acids, alcohols, surfactants
<i>Bacillus sp.</i>	facultative ²	acids, surfactants
<i>Pseudomonas sp.</i>	aerobic ³	surfactants, polymers, can degrade hydrocarbons
<i>Xanthomonas sp.</i>	aerobic	polymer
<i>Leuconostoc sp.</i>	facultative	polymer
<i>Desulfovibrio sp.</i>	anaerobic	gases, acids - sulfate-reducing
<i>Arthrobacter sp.</i>	facultative	surfactants, alcohols
<i>Corynebacterium sp.</i>	aerobic	surfactants
<i>Enterobacter sp.</i>	facultative	gases, acids

¹Non-oxygen-requiring.

²Can grow with or without oxygen.

³Oxygen-requiring.

surfactant production is going to be used, then microbial coreflooding tests, micromodels, and other chemical tests such as phase behavior and surface and interfacial tension measurements will be helpful. Phase behavior and surface and interfacial tension measurements are used to evaluate the properties of the surfactant. Useful information regarding oil emulsification and the relative oil mobilization efficiency of the surfactant can be obtained.

If a microbial solution is desired for fluid diversion or water mobility control, then parallel or sandwiched cores should be used to demonstrate fluid diversion between a low-permeability and a high-permeability rock. For either EOR method, measurements of incremental oil recovery can be used to compare the efficiencies of different microbial formulations and nutrients, as well as injection protocols, to select the best combination for the target reservoir.

RESULTS FROM BE3 MICROBIAL EOR RESEARCH IN FY88

Studies were conducted during fiscal year 1988 to determine what microbial products are effective for mobilizing residual crude oil in porous media. Several microbial coreflood effluents were sampled and analyzed on a Perkin Elmer 5990A gas chromatograph using a Poropak QS glass column. The coreflood apparatus is illustrated in figure 3. Tables 2 and 3 present the results from several coreflooding experiments. As anticipated, it appears that every microbial species will not only traverse through porous media at different rates, but its products will also be transported differently. All cores were flooded at 1 ft/d. Cores B6, B12, and B14 had pore volumes of approximately 60 mL, whereas cores B18 and MP2 had pore volumes of approximately 140 mL. As an example, core B12, injected with a mixed consortia of four microorganisms (see table 3), produced only ethanol after 2 hr and 6 hr, although methanol was present in a higher concentration than ethanol in the 4-hr effluent, and a compound we designated as unknown because it did not fit the retention times of any of our standards appeared in the 8-hr sample. Cores B14 and B18 were injected with two different species of *Clostridium*; both 2-hr effluents showed that only acetic acid was present. However, the compounds changed in the 4-, 6-, and 8-hr samples. The alcohols appeared to lag behind the acetic acid in those cores injected with only *Clostridium*. In core MP2, which was injected with two components of NIPER Bac 1, one species of *Clostridium* and *Bacillus licheniformis*, isopropyl alcohol, and ethanol appeared in the 0-hr effluent, which indicated that some of the compound had migrated during shut-in. This result was logical since the cores were re-fed twice with 0.2 PV of molasses (with the core outlet end open), and the microorganisms were probably pushed further into the core. When *Bacillus* and *Clostridium* were injected, more compounds were formed, and isopropyl alcohol tended to predominate.

An unfired Berea sandstone core, 4 ft in length, was used to examine the microbial products during the waterflood. The pore volume of the core was 522 mL. The coreflood apparatus was essentially the same as shown in figure 3, except intermediate pressure taps were present on the 4 ft coreholder and used for sampling. The distance between each tap was 0.25 ft with the first part being 0.125 ft from the injection end. Core B25 was injected with 0.1 PV of a mixture of *Bacillus licheniformis* and NIPER 6, a *Clostridium* species, and 0.2 PV of 4% molasses (fig. 4). The core was shut in for 3 days, then re-fed with 0.2 PV of 4% molasses; shut in for 3 more days,

TABLE 2. - Core experiments with individual microbial species

Core	Injectant	k md	S _{owf} %	S _{ocf} %	Er %	Pressure psi	Microbial CFU/ml	
							Aerobic	Anaerobic
B6	0.1 PV NIPER 2	267	35.26	35.01	0.7	10	1.5 X 10 ²	2.8 X 10 ²
B7	0.1 PV Mo1.	278	37.9	37.9	0	10	6.3 X 10 ¹	7.3 X 10 ¹
B9	0.1 PV NIPER 3	204	36.4	33.3	8.5	16	8.8 X 10 ⁴	7.6 X 10 ⁴
B10	0.1 PV NIPER 4	261	33.9	32.5	4.1	11	1.8 X 10 ²	5.7 X 10 ²
B11	0.1 PV NIPER 1	293	38.3	33.0	13.8	14	2.3 X 10 ⁴	2.6 X 10 ⁴
B12	0.1 PV BAC 1	271	34.7	30.4	12.4	15	7.6 X 10 ³	3.3 X 10 ⁴
B13	0.1 PV Mo1.	372	30.0	29.5	1.7	20	1.1 X 10 ⁴	5.6 X 10 ³
B14	0.1 PV NIPER 5	379	33.8	24.3	28.2	45	5.0 X 10 ⁵	2.7 X 10 ³
B15A*	0.1 PV NIPER 5	341	38.0	30.8	18.8	38	2.3 X 10 ⁵	7.9 X 10 ⁴
B16*	0.1 PV Bac 1	362	35.7	31.9	10.6	20	4.6 X 10 ⁴	2.8 X 10 ⁴
B18*	0.1 PV NIPER 3	262	38.4	33.3	13.3	30	3.0 X 10 ¹	7.0 X 10 ²
B19*	0.1 PV NIPER 4	312	37.8	36.1	4.6	25	1.4 X 10 ⁴	4.6 X 10 ²
B20*	0.1 PV NIPER 1 & 5	274	38.1	31.3	17.8	45	3.2 X 10 ³	1.7 X 10 ³
MP2*	0.1 PV NIPER 1 & 3	394	37.8	32.8	13.2	40	7.5 X 10 ³	8.9 X 10 ³

Nutrient was OKC molasses, 4% concentration.

NIPER 1 = *Bacillus licheniformis*.

NIPER 2 = *Bacillus* species.

NIPER 3 = *Clostridium* species.

NIPER 4 = Gram-negative facultative rod.

Bac 1 = Mixed culture of NIPER 1, 2, 3, and 4.

NIPER 5 = *Clostridium* species (OSU).

k = absolute permeability to brine in millidarcies.

S_{owf} = residual oil saturation after waterflooding (% PV).

S_{ocf} = residual oil saturation after microbial treatment (% PV).

psi = maximum increase in pressure during core incubation.

Er = recovery efficiency $\frac{S_{owf} - S_{ocf}}{S_{owf}} \times 100\%$.

* = These cores had larger pore volumes of approximately 140 mL, where as all others had pore volumes of approximately 60 mL.

CFU/ml = Colony forming units/mL.

TABLE 3 - Qualitative gas chromatographic analyses of core effluents

Core	% PV	Products ¹	% Area ²
B6	0.25	None	
<i>Bacillus</i> NIPER 2	0.5	Formic acid Ethanol	29.1 67.5
	0.75	Formic acid Butanol	93.5 0
	1.0	Ethanol	94.1
B12	.25	Ethanol	94.3
NIPER Bac 1 mixture	.5	Formic acid Methanol Ethanol	8.6 47.5 39.0
	.75	Ethanol	67.4
	1.0	Acetone Unknown Ethanol	0 46.8 46.1
B14	.25	Acetic acid	99.4
NIPER 5	.5	Formic acid Methanol Unknown Ethanol	3.6 1.7 43.8 42.6
	.75	Formic acid Acetic acid Isopropanol	20.0 30.2 40.7
	1.0	Acetic acid	96.5
B18	.25	Methanol Acetic acid	0 98.2
NIPER 3	.5	Formic acid Methanol Isopropanol	26.9 34.2 34.6
	.75	Acetone Formic acid Methanol Ethanol Acetic acid	1.0 19.9 13.8 19.9 43.8

TABLE 3 - Qualitative gas chromatographic analyses of core effluents
(continued)

Core	% PV	Products ¹	% Area ²
MP2 NIPER 1 & 3	1.0	Formic acid	9.3
		Methanol	20.6
		Ethanol	0
		Acetic acid	68.2
	0	Formic acid	6.3
		Methanol	4.1
		Ethanol	32.6
		Acetic acid	20.0
		Isopropanol	37.1
	.25	Formic acid	3.3
		Methanol	2.9
		Ethanol	29.3
		Acetic acid	22.3
		Isopropanol	40.1
		Butanol	2.1
	.5	Formic acid	13.4
		Methanol	14.3
		Ethanol	14.6
		Acetic acid	12.4
		Isopropanol	37.5
Butyric acid		5.9	
.75	Formic acid	3.0	
	Methanol	1.9	
	Ethanol	19.9	
	Acetic acid	13.3	
	Isopropanol	25.8	
	Butanol	0	
	Propionic acid	0	
	Isobutyric acid	0	
	Butyric acid	29.7	

¹Based solely on retention times of standard solutions.

²% area represents % of integrated area under the peaks.

and waterflooded at 1 ft/d. Four sample ports which were equally spaced along the core were used and designated as port 1 (nearest the injected end) through port 4 (near the outlet end). Tables 4 and 5 and figures 5 through 11 show the results of this coreflood experiment. Figure 5 shows a reduction in residual oil saturation of almost 10%. Most of the oil was recovered before the first pore volume of fluid had been injected. Figures 6 and 7 present the microbial counts during the coreflood. Figures 8 through 11 illustrate the gas chromatographic results of the coreflood. These results indicate that the microbial products moved ahead of the microbial cells, and that the microbial population had colonized throughout the core by the end of the waterflood. The greatest response of microbial products was seen in the 23-hr samples, which represented about 0.13 PV into the waterflood. The injection port had an almost equal amount of acetone and ethanol but methanol production was higher. In ports 2 and 3, the methanol again predominated; and in port 4, near the outlet end, acetone and propanol were the dominant compounds. It is significant that at the fluid formation Port 3, 15% methanol was obtained.

As part of NIPER's laboratory research for the DOE,¹⁴ a microbial formulation used in the Mink Unit Field Project was further investigated to determine the actual mechanisms of oil recovery. Table 6 gives the names and products of the microorganisms used for that study. Each component of NIPER Bac 1 was investigated in Berea sandstone coreflood experiments, and Berea sandstone plugs were used to determine wettability alterations. The results from these tests are shown in table 7. These data indicate that only two components of NIPER Bac 1, NIPER 1 and NIPER 3, were useful in recovering oil. NIPER 1 was shown to alter the wettability of Berea sandstone from +0.255 to a more water-wet state (+0.888). Two other *Clostridium* species, NIPER 5 and 6, were also investigated, and it was determined that although they do not alter the wettability as much as NIPER 1, their residual oil recovery efficiencies are higher. Concurrent glass micromodel studies were performed to examine the microbial solutions at a more microscopic level. We have demonstrated that microbial residual oil recovery efficiencies correlated very well to the ability of the microbial solution to move residual crude oil in glass micromodels.⁹ Interestingly, we observed that the *Clostridium* species, NIPER 5 and NIPER 6, moved more crude oil out of the micromodel than any of the other species. Since NIPER 5 and NIPER 6 produce carbon dioxide and surfactant, it appears that the combination of these two formulations can

move trapped crude oil more effectively than a microorganism that produces only surfactant.

TABLE 4. - Total gas chromatographic response of core B25

Port ¹	% Pore Volume ²	Weight % ³
1	0	1.571
2		0.565
3		1.655
4		0.145
2	.023	0.04
3		1.116
4		0.356
1	.131	11.463
2		11.066
3		21.913
4		0.543
1	.171	0.041
2		0.146
3		1.107
1	.273	0.282
2		0.223
3		1.159
1	.307	0.334
2		0.391
1	.409	0.254
2		0.125
3		0.129
4		0.803
1	.443	0.248
2		0.651
3		0.776
4		0.785
1	.546	0.037
2		0.239
3		T
4		0.66

TABLE 4. - Total gas chromatographic response of core B25 (continued)

Port ¹	% Pore Volume ²	Weight % ³
1	.580	0.011
2		0.389
3		1.302
4		0.19
1	.989	0.649
2		0.059
3		0.311
4		1.083
1	1.125	1.401
2		0.633
3		0.875
4		0.111
1	1.262	0.211
2		1.024
3		0.162
4		0.168
1	1.398	0.32
2		0.015
3		0.571
4		0.155

¹Port 1 was 0.125 PV from the injection end;
Port 2 was 0.375 PV from the injection end;
Port 3 was 0.625 PV from the injection end;
Port 4 was 0.75 PV from the injection end;

²% pore volume designates the start of the waterflood. 0.5 PV of fluid was injected prior to the waterflood.

³Total weight % of compounds produced.

TABLE 5. - Results of gas chromatographic analysis of core B25

Port ¹	% Pore ² volume	Compound	Weight % ³
1	0	Acetone	1.117
		Isopropanol	.439
		Butyric acid	.015
2	0	Ethanol	.029
		Acetone	.238
		Isopropanol	.014
		Propanol	.136
		Acetic acid	.104
		Isobutanol	.027
		Butanol	.017
3	0	Acetone	1.6
		Butyric acid	.055
4	0	Acetone	.13
		Butanol	.015
2	.023	Methanol	T ³
		Ethanol	T
		Acetone	.026
		Isopropanol	.009
		Butanol	T
		Propionic acid	.005
3	.023	Methanol	T
		Acetone	.416
		Isopropanol	.139
		Propanol	.521
		Butanol	.01
4	.023	Ethanol	.03
		Acetone	.104
		Isopropanol	.085
		Propanol	.137
1	.131	Methanol	6.196
		Ethanol	2.666
		Acetone	2.601
2	.131	Methanol	5.049
		Ethanol	2.146
		Acetone	3.743
		Propionic acid	.035
		Butyric acid	.093

TABLE 5. - Results of gas chromatographic analysis of core B25 (continued)

Port ¹	% Pore ² volume	Compound	Weight % ³
3	.131	Methanol	15.708
		Ethanol	5.964
		Propionic acid	.078
		Butyric acid	.163
4	.131	Methanol	T
		Ethanol	T
		Acetone	.315
		Propanol	.228
1	.171	Ethanol	.041
2	.171	Methanol	.06
		Acetone	.044
		Propionic acid	.042
3	.171	Methanol	T
		Ethanol	T
		Acetone	.441
		Isopropanol	.155
		Propanol	.3
		Butanol	.005
		Propionic acid	.116
1	.273	Acetone	.106
		Isopropanol	.036
		Propanol	.083
		Isobutanol	.057
2	.273	Methanol	T
		Ethanol	T
		Acetone	.096
		Isopropanol	.029
		Isobutanol	.058
		Propionic acid	.04
3	.273	Methanol	T
		Ethanol	T
		Acetone	.199
		Isopropanol	.294
		Propanol	.489
		Isobutanol	.062
		Butyric acid	.115

TABLE 5. - Results of gas chromatographic analysis of core B25 (continued)

Port ¹	% Pore ² volume	Compound	Weight % ³
2	.307	Methanol	T
		Acetone	.266
		Isopropanol	.068
		Isobutanol	T
4	.307	Acetone	.045
		Propanol	.009
		Butanol	T
		Propionic acid	.181
		Butyric acid	.156
1	.409	Acetone	.186
		Isopropanol	.068
2	.409	Acetone	.102
		Isopropanol	.023
3	.409	Methanol	T
		Acetone	.088
		Isopropanol	.041
4	.409	Methanol	T
		Ethanol	T
		Acetone	.349
		Isopropanol	.146
		Propanol	.212
		Isobutanol	T
		Propionic acid	.03
Butyric acid	.066		
1	.443	Acetone	.153
		Isopropanol	.058
		Propanol	.037
2	.443	Acetone	.29
		Isopropanol	.093
		Propanol	.268
3	.443	Acetone	.212
		Isopropanol	.064
		Propanol	.2
		Propionic acid	T
		Butyric acid	T

TABLE 5. - Results of gas chromatographic analysis of core B25 (continued)

Port ¹	% Pore ² volume	Compound	Weight % ³
4	.443	Methanol	T
		Acetone	.313
		Isopropanol	.124
		Propanol	.331
		Butyric acid	.017
1	.546	Acetone	.027
		Isopropanol	.01
2	.546	Acetone	.168
		Isopropanol	.071
3	.546	Acetone	T
		Propionic acid	T
4	.546	Acetone	.291
		Isopropanol	.118
		Propanol	.251
1	.58	Acetone	.011
2	.58	Acetone	.288
		Isopropanol	.088
		Propanol	.013
3	.58	Methanol	T
		Acetone	.653
		Isopropanol	.204
		Propanol	.445
		Propionic acid	T
4	.58	Methanol	T
		Acetone	.123
		Isopropanol	.032
		Butyric acid	.035
1	.989	Methanol	.031
		Ethanol	.006
		Acetone	.272
		Isopropanol	.09
		Propanol	.239
		Propionic acid	.005
		Butyric acid	.006

TABLE 5. - Results of gas chromatographic analysis of core B25 (continued)

Port ¹	% Pore ² volume	Compound	Weight % ³
2	.989	Acetone	.059
3	.989	Acetone	.145
		Isopropanol	.047
		Propanol	.119
		Isobutanol	T
4	.989	Ethanol	T
		Acetone	.492
		Isopropanol	.152
		Propanol	.439
		Isobutanol	T
1	1.125	Acetone	.708
		Isopropanol	.169
		Propanol	.524
2	1.125	Acetone	.293
		Isopropanol	.081
		Propanol	.259
3	1.125	Methanol	T
		Acetone	.43
		Isopropanol	.133
		Propanol	.312
		Butyric acid	T
4	1.125	Methanol	T
		Ethanol	T
		Acetone	.054
		Isopropanol	.012
		Propanol	.045
		Isobutanol	T
		Propionic acid	T
		Butyric acid	T
1	1.262	Acetone	.11
		Isopropanol	.03
		Propanol	.071
		Butyric acid	T
2	1.262	Ethanol	T
		Acetone	.572
		Isopropanol	.12
		Propanol	.332

TABLE 5. - Results of gas chromatographic analysis of core B25 (continued)

Port ¹	% Pore ² volume	Compound	Weight % ³
3	1.262	Acetone	.13
		Isopropanol	.032
4	1.262	Ethanol	T
		Acetone	.09
		Isopropanol	.018
1	1.398	Acetone	.175
		Isopropanol	.043
		Propanol	.102
2	1.398	Acetone	.015
3	1.398	Acetone	.571
		Propanol	T
4	1.398	Acetone	.133
		Isopropanol	.022

Note: Experiment was terminated after 1.4 pore volumes.

¹Port 1 was 0.125 PV from the injection end;

Port 2 was 0.375 PV from the injection end;

Port 3 was 0.625 PV from the injection end;

Port 4 was 0.75 PV from the injection end;

²% pore volume designates the start of the waterflood. 0.5 PV of fluid was injected prior to the waterflood.

³T=Trace amount; <0.005%.

TABLE 6. - Microorganisms used for MEOR mechanistic studies

Designation	Type of microorganism	Chemical products
NIPER 1	<i>Bacillus licheniformis</i>	Surfactant, acids
NIPER 2	<i>Bacillus</i> species	Surfactant
NIPER 3	<i>Clostridium</i> species	Surfactant, acids, alcohols, gases
NIPER 4	Gram-negative facultative	Gases, acids
NIPER Bac 1	Mixture of the above four	
NIPER 5	<i>Clostridium</i> species	Surfactant, acids, alcohols, gases
NIPER 6	<i>Clostridium</i> species	Surfactant, acids, alcohols, gases
NIPER 7	Gram-negative facultative	Carbon dioxide

TABLE 7. - Residual oil recovery efficiencies and wettability values for several microbial cultures

Microbial culture	Er, % ¹	log (A1/A2) ²
Control (Berea only)	-	+0.255
NIPER Bac 1	12.4	+0.787
NIPER 1	13.8	+0.888
NIPER 2	0.7	+0.127
NIPER 3	13.3	+0.216
NIPER 4	4.1	+0.273
NIPER 5	28.1	+0.428
NIPER 6	30.8	+0.312
NIPER 1 & 3	17.8	+0.657

¹Residual oil recovery efficiency (%) (see table 2).

²Value of $\frac{\text{area of water imbibition curve}}{\text{area of oil drainage curve}}$, the more positive a value, the more water-wet the core.

CONCLUSIONS

The oil recovery mechanisms for microorganisms used in this study are complex, particularly because each microbe produces several different metabolites that can influence crude oil mobilization. We have observed that some microorganisms that produce potentially effective metabolites for MEOR do not survive or mobilize trapped crude oil in porous media. A laboratory testing program using porous media is essential for optimizing a microbial formulation for enhanced oil recovery. Combinations of certain bacteria such as *Bacillus* and *Clostridium* species do not always synergistically improve oil recovery. A single species of *Clostridium* may be just as efficient.

We have developed laboratory procedures to quantify microbial metabolites in porous media. These data will provide valuable information regarding the transport of microbial metabolites. When a combination of *Bacillus* and *Clostridium* was used in a coreflood, alcohol production dominated over acid production, and the greatest concentration of metabolites was produced at the fluid front in the core.

In most instances, when the wettability of Berea sandstone was altered to a more water-wet state, the residual oil recovery efficiency ranged from 12% to almost 31%, while if the wettability value was less than the initial value of Berea sandstone alone, the recovery was less than 5%. Alteration of wettability probably contributes to the overall oil recovery mechanism, but other properties such as interfacial and surface tension lowering also contribute.

These studies indicate that there is a potential for improved oil recovery by microorganisms, and that laboratory selection and optimization is crucial to the process.

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CYCLIC MICROBIAL RECOVERY

A solution of microorganisms and nutrients is introduced into an oil reservoir during an "injection phase." The injection well is then shut in for a "soak period" allowing the microorganisms to produce carbon dioxide gas and surfactants that help to mobilize the oil. The well is then opened and oil and products resulting from the "soak period" are produced. This process may be repeated.

Schematic portrays one well during the 3 phases of this process.
Flow pattern is stylized for clarity.

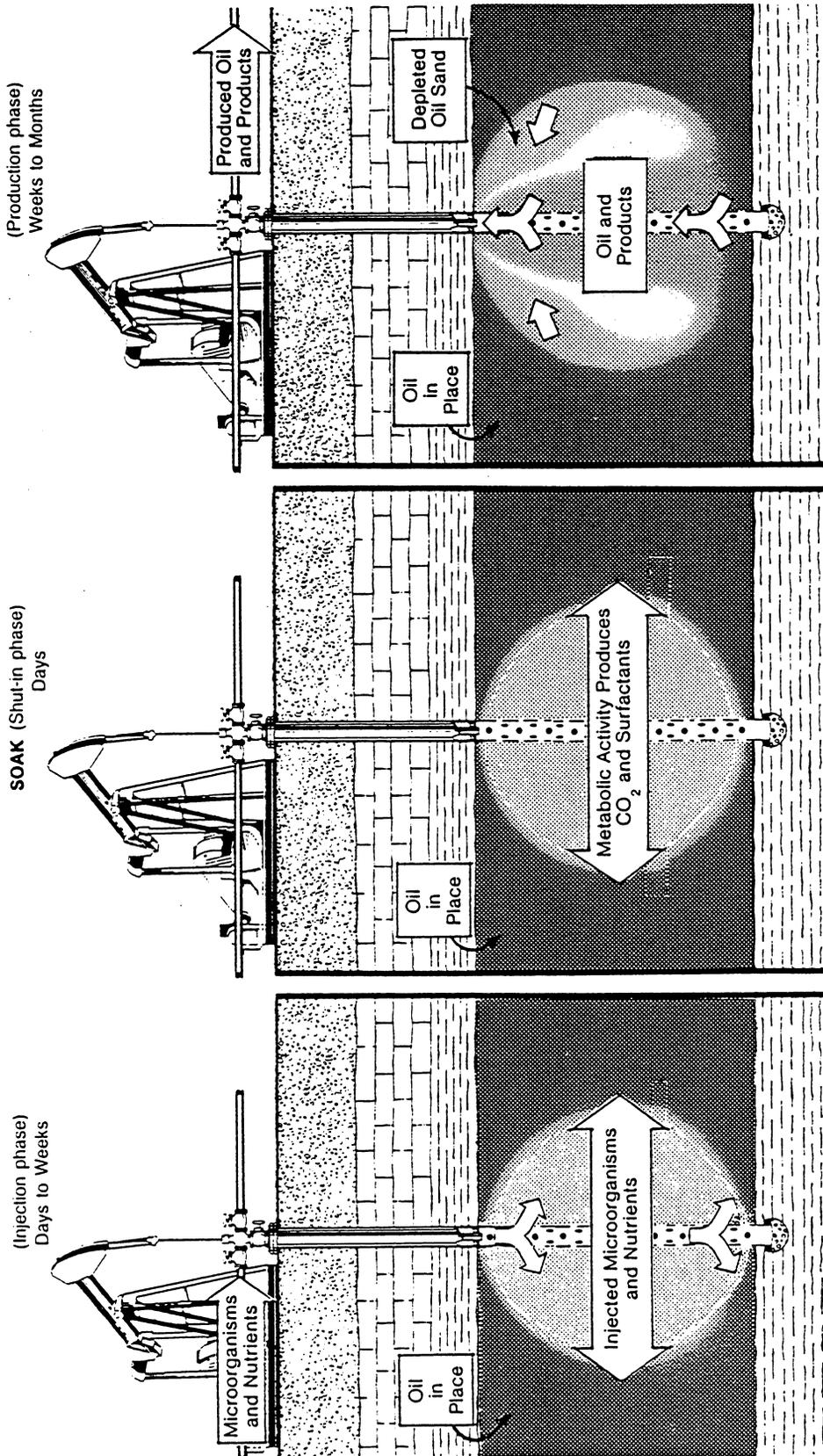


FIGURE 1. - Cyclic microbial recovery.

MICROBIAL FLOODING

Recovery by this method utilizes the effect of microbial solutions on a reservoir. The reservoir is usually conditioned by a water preflush, then a solution of microorganisms and nutrients is injected. As this solution is pushed through the reservoir by drive water, it forms gases and surfactants that help to mobilize the oil. The resulting oil and product solution is then pumped out through production wells.

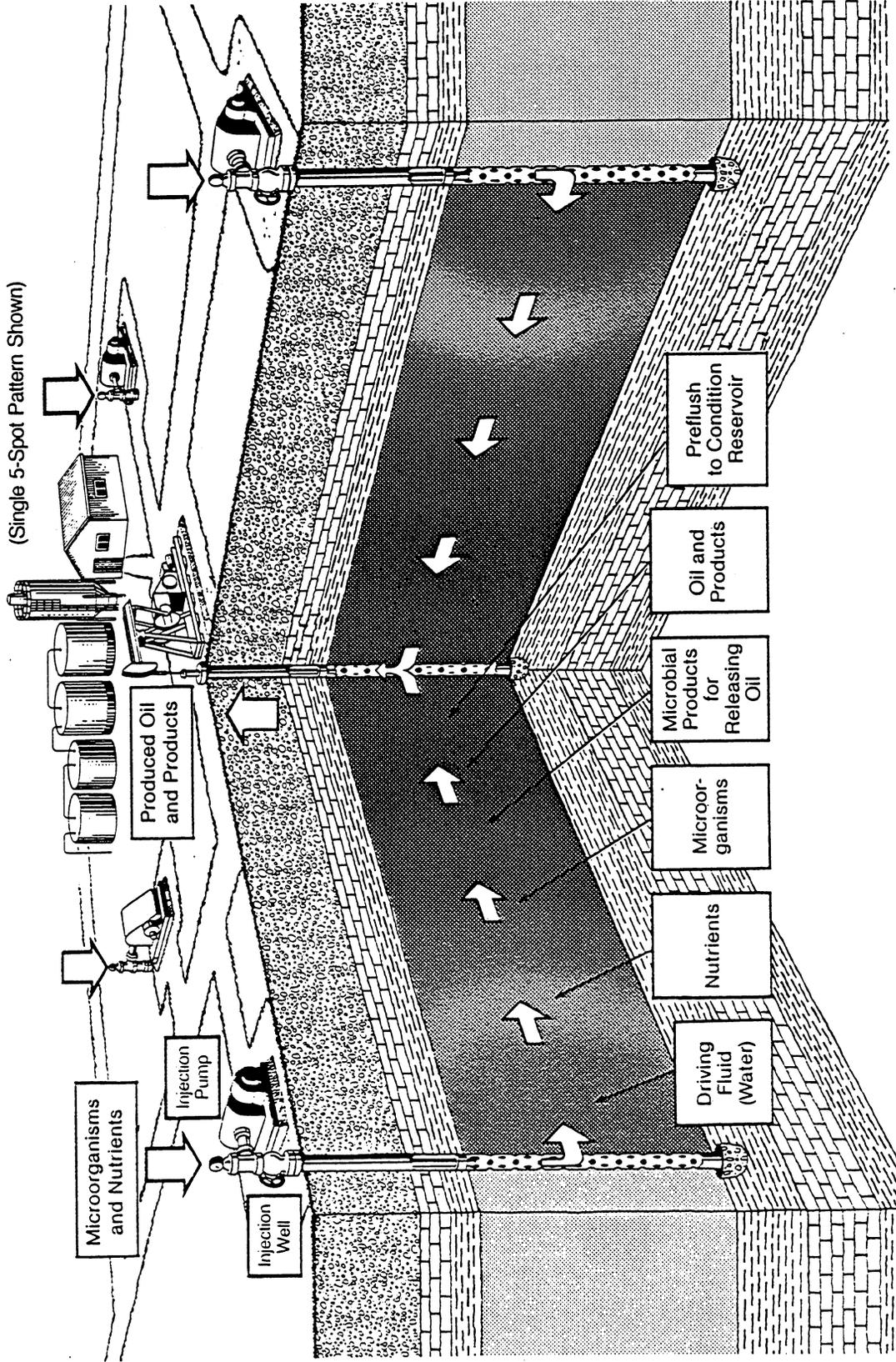


FIGURE 2. - Microbial flooding.

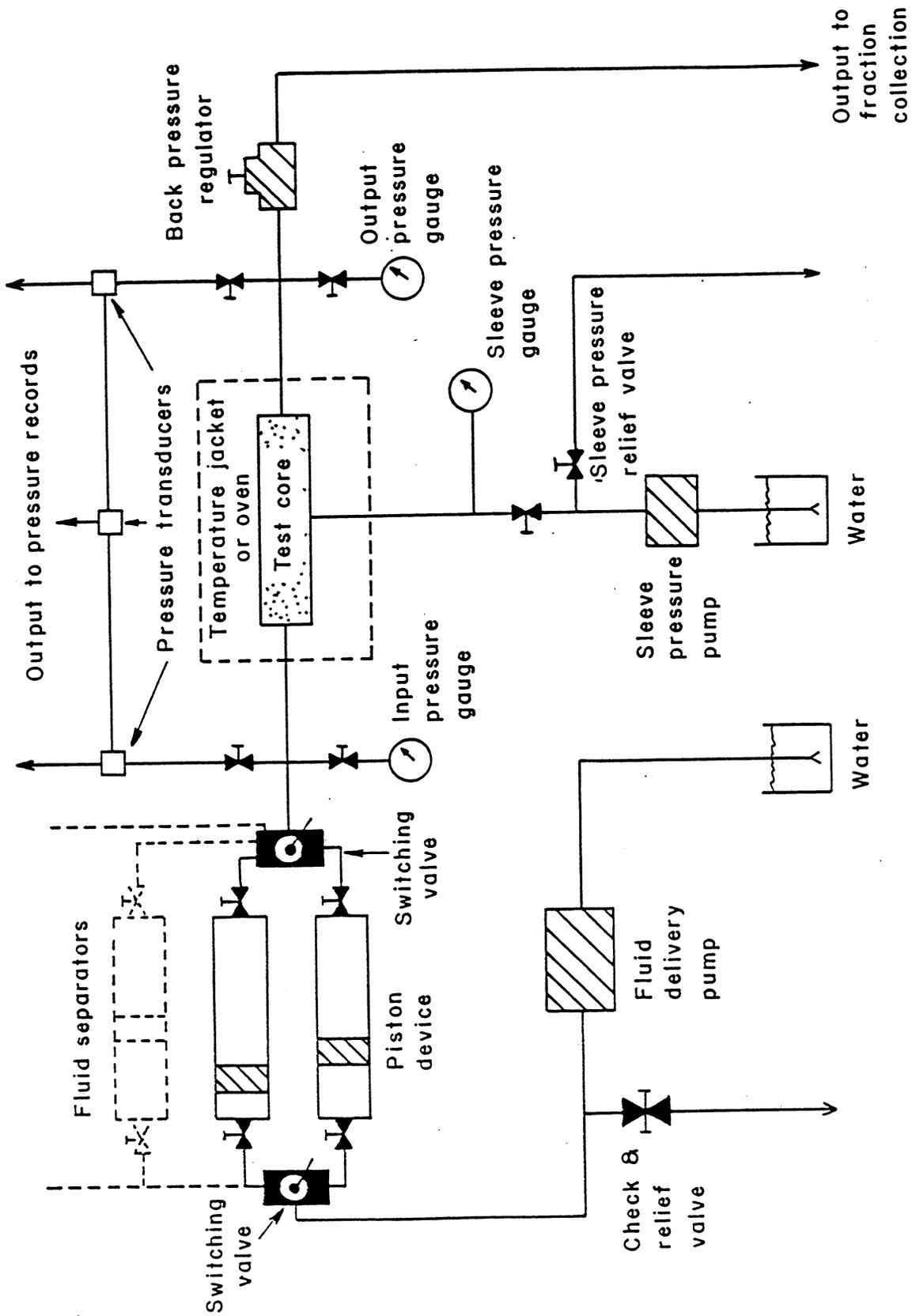


FIGURE 3. - Coreflooding apparatus.

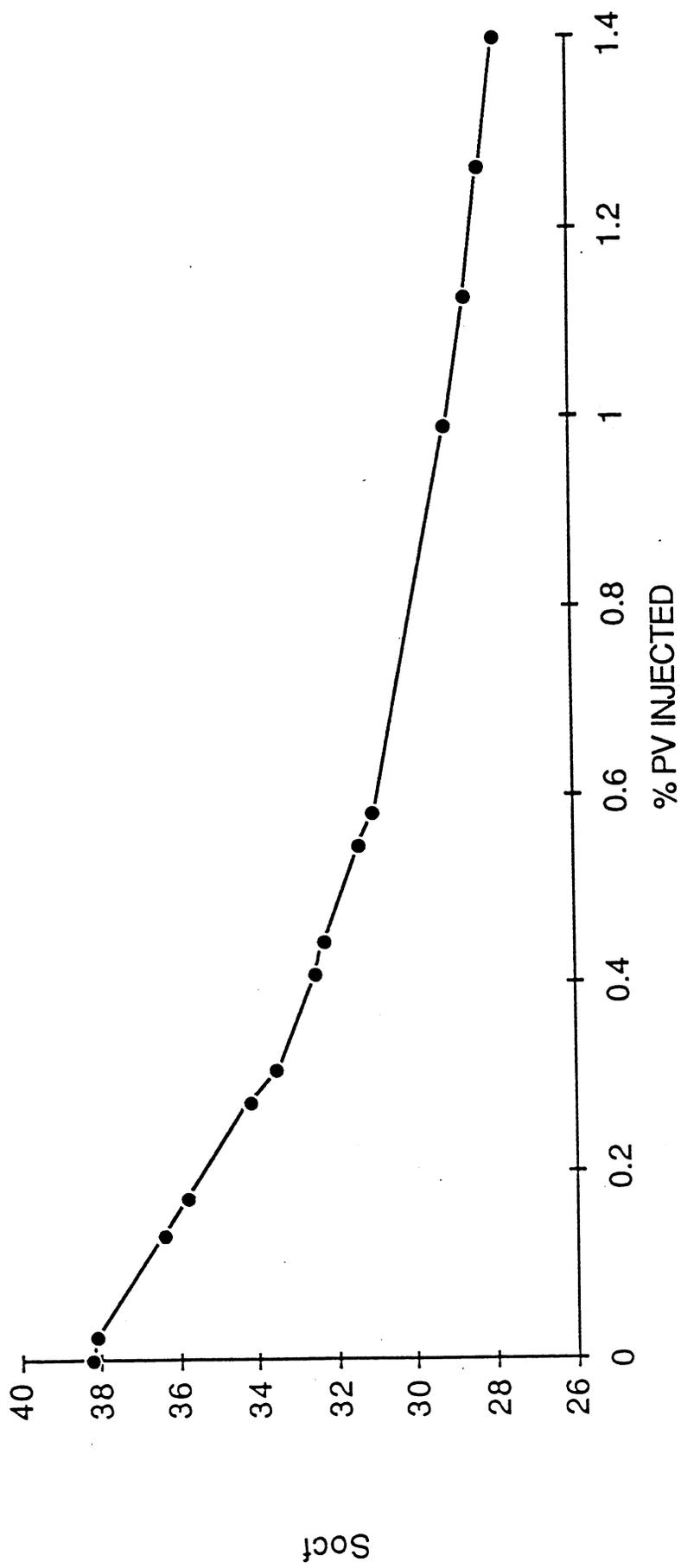


FIGURE 5. - Residual oil saturation decrease of core B25.

AEROBIC CFU/ML

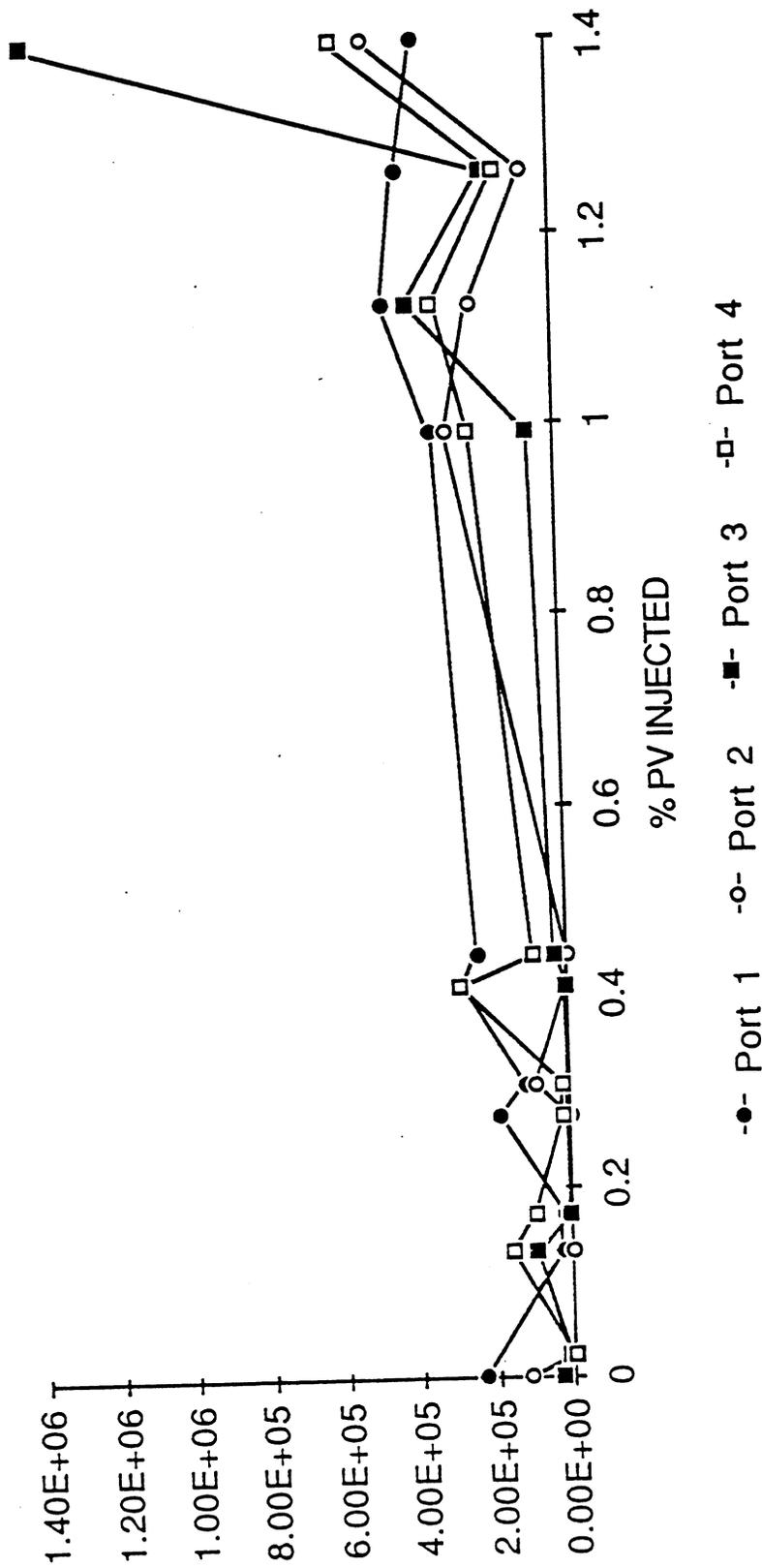


FIGURE 6. - Aerobic microbial counts for core B25.

ANAEROBIC CFU/ML

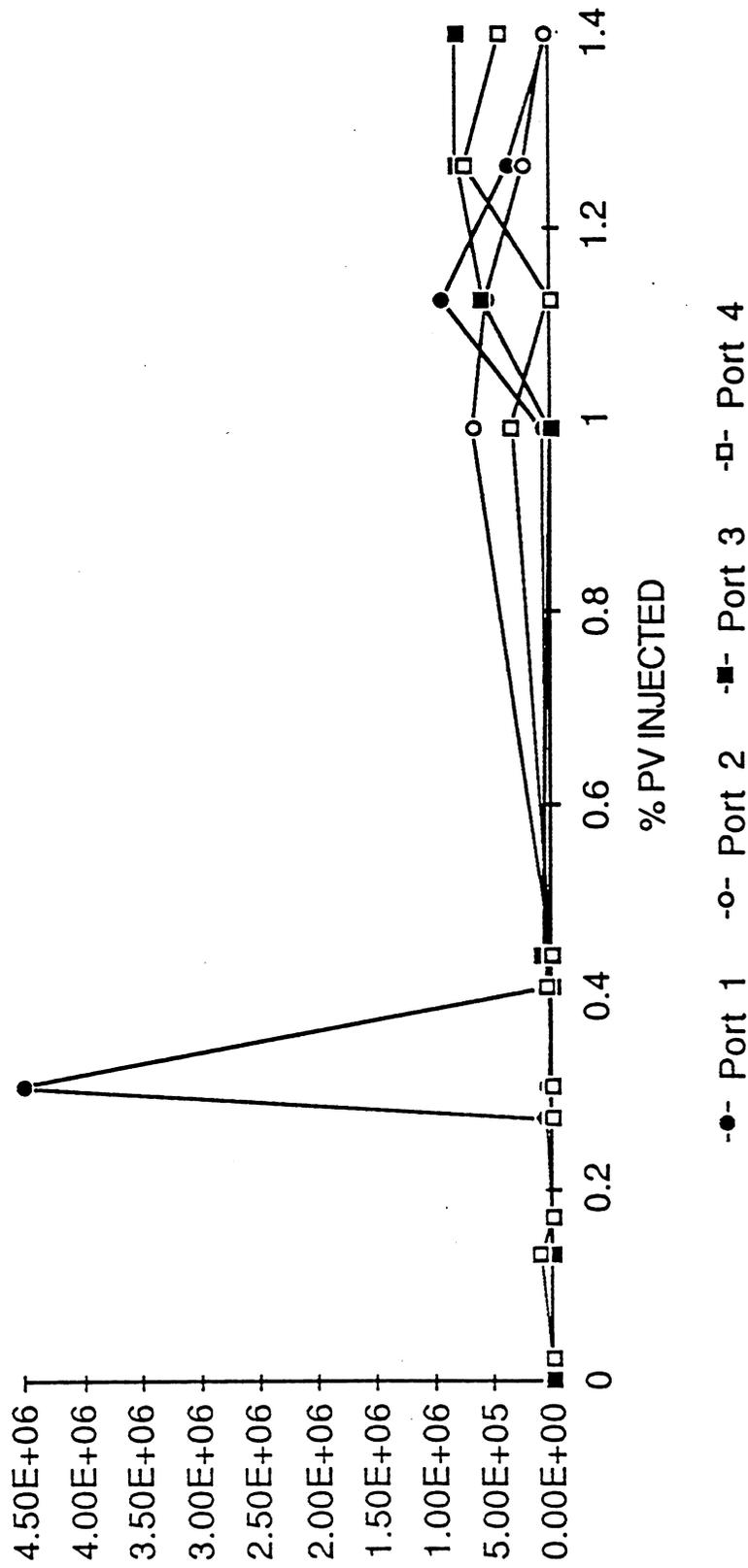


FIGURE 7. - Anaerobic microbial counts for core B25.

TOTAL GC RESPONSE

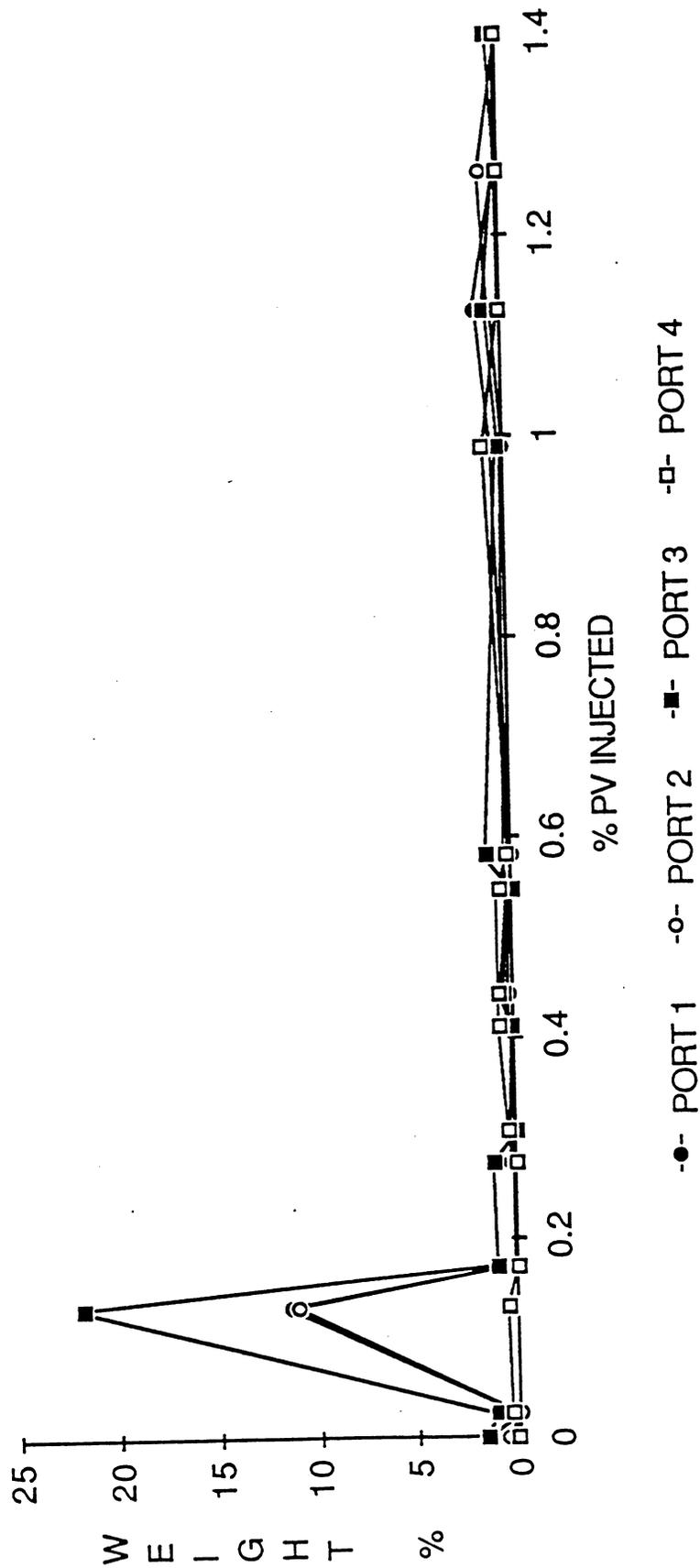


FIGURE 8. - Total solvent production of core B25.

TOTAL ALCOHOLS

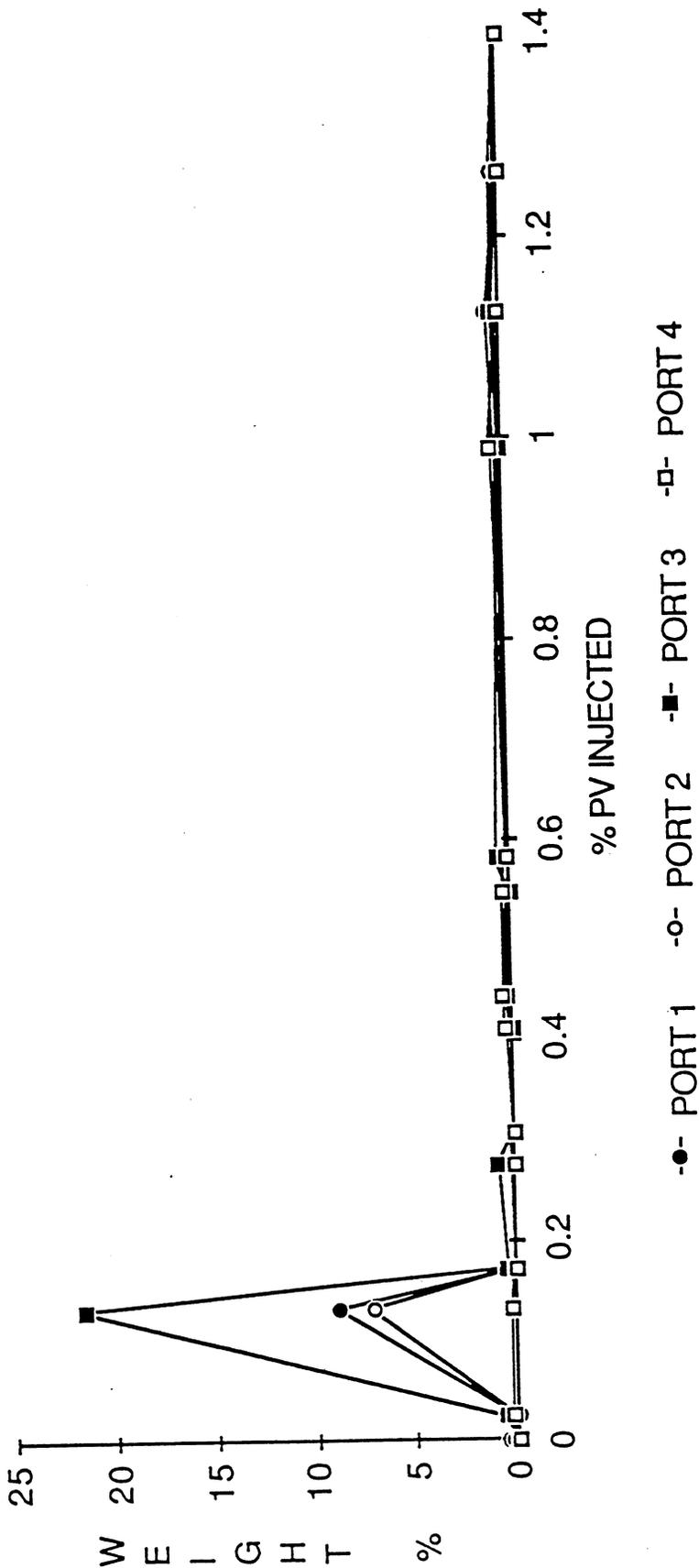


FIGURE 9. - Total alcohol production of core B25.

TOTAL ACIDS

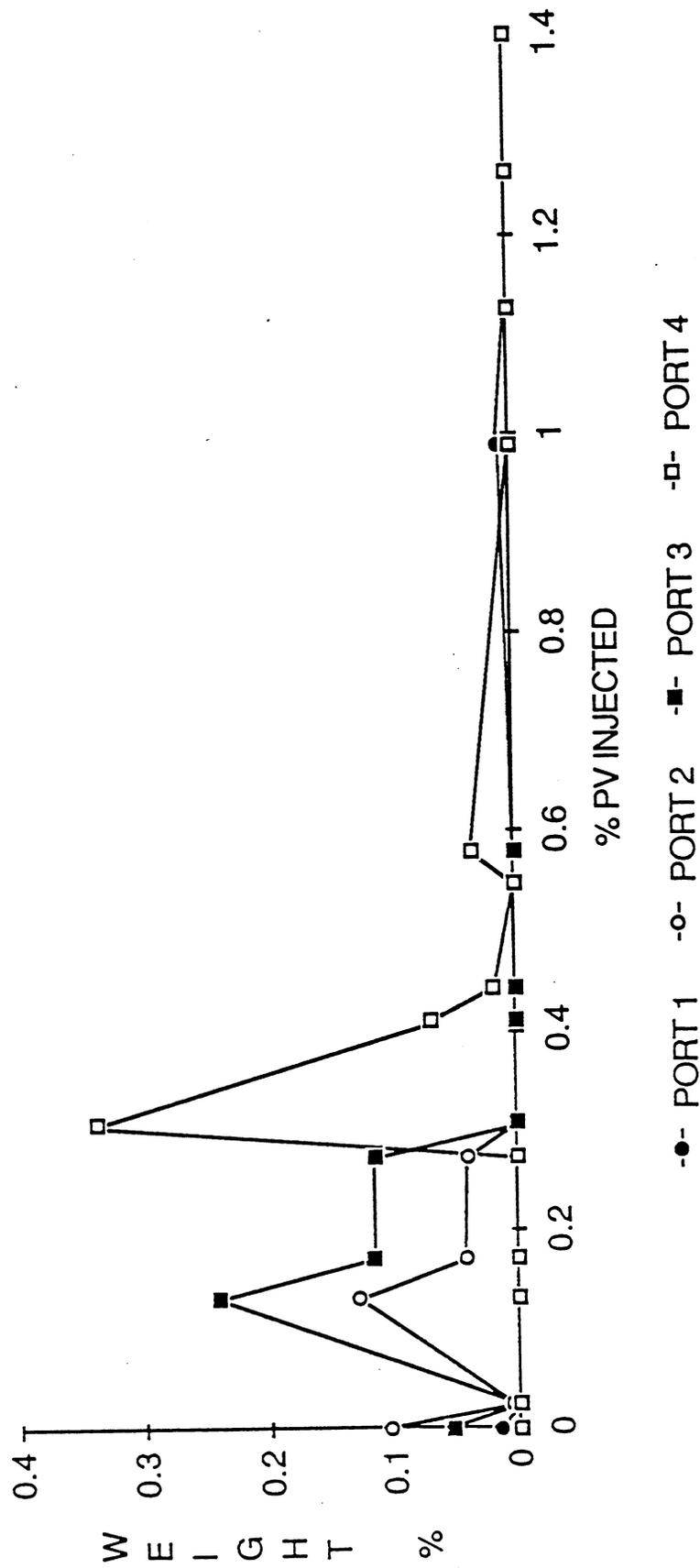


FIGURE 10. - Total acid production of core B25.

ACETONE

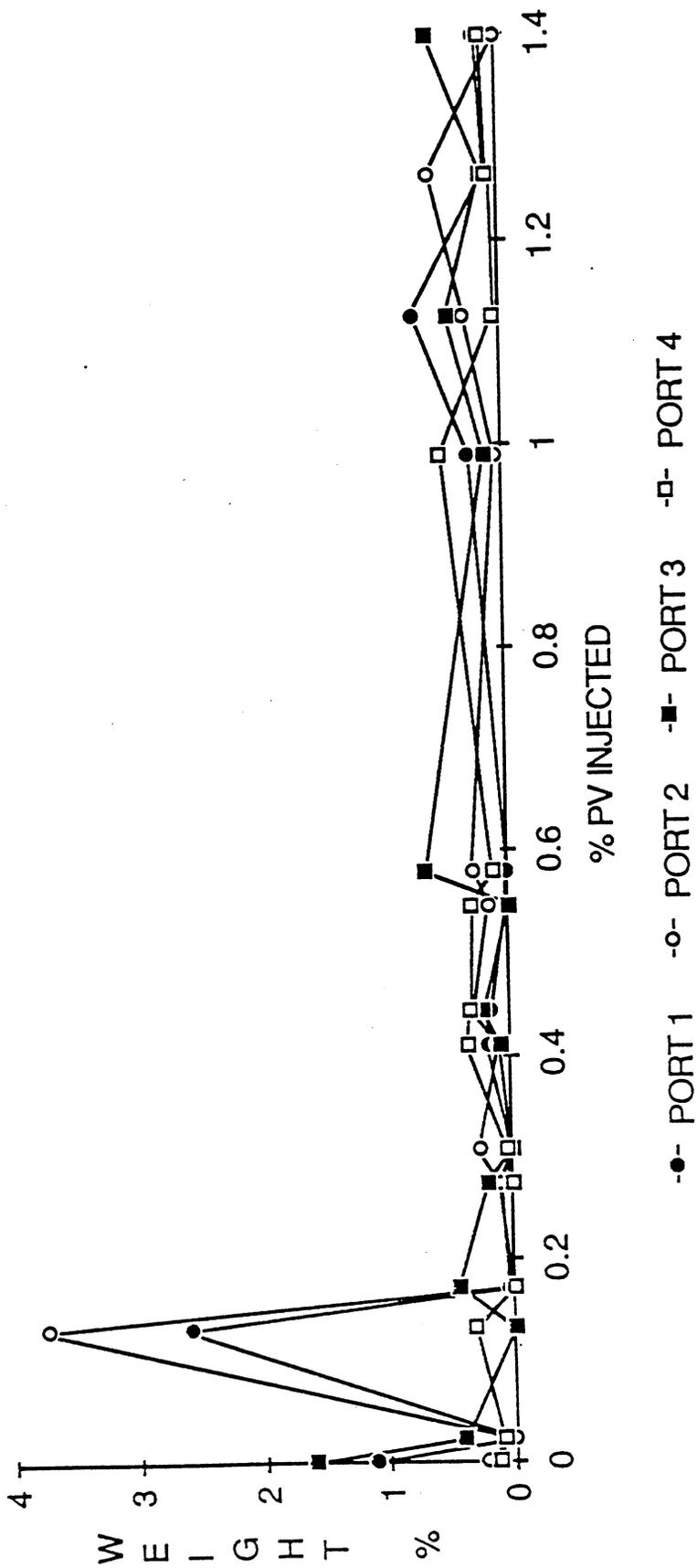


FIGURE 11. - Acetone production in core B25.

