



## Bacteria for Improvement of Oil Recovery: A Laboratory Study

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*In microbial enhanced oil recovery (MEOR) technique, microorganisms and/or their products (gases, chemicals) are used in the enhancement of oil recovery. In the present study, MEOR is tested for Garzan (26° API) crude oil, produced from Southeast Turkey. This work consists of shut-in pressure tests and microbial water flooding experiments. In shut-in pressure tests, the oil is placed in a stainless steel cell and a certain amount of microbial solution (Clostridium acetobutylicum) is introduced. During the soaking period, the pressure increase is monitored. Results of the measurements carried out after this stage show that gas (mainly CO<sub>2</sub>) production by the bacteria decreases the oil viscosity effectively. In microbial flooding experiments, an unconsolidated, preflooded reservoir model is subjected to microbial treatment and then flooded with water under varying conditions. The bacteria used in these experiments were Clostridium acetobutylicum and mixed soil bacteria. When compared with a conventional water flood, the results of microbial runs showed that the residual oil recovery increased more than 100% and the pH of the medium decreased.*

**Keywords** bacterial gas production, microbial enhanced oil recovery, viscosity reduction

### Introduction and Theory

Microbial enhanced oil recovery (MEOR) technique enables the improvement of oil recovery by injection of microorganisms and/or their products into depleted oil reservoirs. The theory for utilization of bacteria in the enhancement of oil recovery was first proposed by Beckman in 1926 (Hitzman, 1983). The interest shown in MEOR gradually increased after the pioneering work of ZoBell in 1947 (ZoBell, 1947), and many countries launched laboratory and field tests utilizing this technique. Although the results were promising, the research in this area lost its impetus during the 1970s due to economic reasons. During the last 2 decades, tests on MEOR have been revived throughout the world in the search for a high performance and cost-effective EOR method.

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The mechanisms by which the bacteria can improve the oil recovery are as follows:

- (a) **Biodegradation of Crude Oil:** A proposed mechanism of MEOR is utilization of bacteria that can degrade crude oil and consume its heavy fractions. As a result of this process, oil becomes a lighter and more valuable product as a result of a decrease in viscosity (Bryant and Burchfield, 1989). *Pseudomonas*, *Arthrobacter*, and other aerobic bacteria are especially effective in the degradation of crude oil (Bushnell and Haas, 1941; Bryant, 1990). However, this degradation is confined to lighter portions of petroleum—especially paraffins—and bacterial treatment is beneficial for removal of paraffins from the wellbore, which can restrict the flow seriously (Pelger, 1992).
- (b) **Gas Production:** The bacterially produced gases (such as CO<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub>, and CH<sub>4</sub>) improve the oil recovery in 2 ways: it either dissolves in the crude oil and thus reduces its viscosity or increases the pressure in the reservoir (Donaldson and Clark, 1982). The source of this produced gas is in-situ fermentation of carbon sources such as glucose by usually anaerobic bacteria (Jack, 1983). The most important gas-producing bacteria are *Clostridium*, *Desulfovibrio*, *Pseudomonas*, and certain methanogenes (Bryant and Burchfield, 1989).
- (c) **Production of Chemicals:** Chemicals that can be useful in the improvement of oil recovery such as organic acids, alcohols, solvents, surfactants, and polymers are produced by a wide array of microorganisms (Bryant and Lockhart, 2001).
- (d) **Selective Plugging:** Apart from these techniques, bacteria can be used in selective plugging (permeability modification) operations. In this method, polymers or bacteria themselves are used to reduce the permeability of highly permeable zones or of water channels that form in heterogeneous reservoirs. Thus the unswept formations are invaded by the water and sweep efficiency increases (Production Operations, 1997). *Bacillus*, *Xanthomonas*, and *Leuconostoc* strains are reported to be effective in such processes (Yakimov et al., 1997; Jenneman et al., 1994).
- (e) **Other Techniques:** Other uses of bacteria in the petroleum industry include the control of unwanted bacteria (such as sulfate-reducing bacteria) in oil fields (Hitzman and Sperl, 1994) and biodegradation of hazardous wastes caused by petroleum-related activities for the controlling and removal of environmental pollution (Ronchel et al., 1995).

There are some environmental factors that affect the performance of MEOR operations. These are temperature, permeability, pH, salinity of the medium, and oxygen content (Donaldson and Clark, 1982). As all oil reservoirs are essentially devoid of oxygen, anaerobic bacteria are generally preferred in field applications.

*Clostridium acetobutylicum* (American Type Culture Collection, ATCC-824), which is the main type of bacteria used in this study, is an anaerobic microorganism that utilizes 12% molasses and 5 g/l yeast extract in an aqueous solution as a substrate. On fermenting these nutrients, this culture forms gases (CO<sub>2</sub> and H<sub>2</sub>), acetic and butyric acids, and solvents such as acetone and butanol (Ballangue et al., 1987). At the first stage of the fermentation, gases and acids are produced, and at the second stage, with partial conversion of acids to solvents, acetone and butanol are formed (Lepage et al., 1987).

## Study

This work consists of 2 sets of experiments: shut-in tests and microbial water flooding experiments.

### Procedure

**Shut-in Tests.** These tests were performed in order to see the effect of the bacterial culture used (*Clostridium acetobutylicum*) on the density and viscosity of the crude oil. The maximum pressure attained due to bacterial activity was also recorded.

In each experiment, a cylindrical, stainless steel cell having a volume of 375 cc was filled with Garzan oil (26° API), produced from Southeast Turkey. Bacterial solution was then injected into the cell with a syringe under N<sub>2</sub> purging and the cell was placed in an oven operating at the optimum survival temperature of the bacteria used (37° C). After a certain period, the cell was opened and measurements mentioned above were performed. The conditions for shut-in tests are presented in Table 1.

**Microbial Water Flooding Tests.** These flooding tests were carried out in order to observe the efficiency of bacterial treatment on oil recovery from a depleted reservoir.

The system consisted of the flooding apparatus shown in Figure 1. The one-dimensional cylindrical model (length: 45 cm; inner diameter: 4.2 cm) was made of stainless steel and had a 207 cc pore volume when filled with crushed limestone between 14 and 35 mesh sizes (mean particle diameter = 0.975 mm). The porosity of the model was 33% and the permeability was 8.3 darcies. It was saturated with brine having a 70,000 ppm salinity and then with Garzan oil. In order to obtain a depleted reservoir model, it was flooded with water until water breakthrough occurred. After this stage, microbial solution at different slug sizes was injected into the model under N<sub>2</sub> purging (to attain anaerobic conditions) and then the shut-in period was started. Pressure and temperature data were recorded during this period by a data logger.

At the end of the predetermined shut-in period, the system was flooded with water having a pH of 7.0. The list of experiments, together with conditions, are presented in Table 2. In addition to these tests, pure nutrient medium was injected as slug into the

**Table 1**  
Conditions and results for shut-in runs

Test	Bacterial solution		Shut-in period (day)	Maximum pressure (bar)	Oil viscosity (cp)	
	Amount (cc)	Initial inoculum (cc)			Before test	After test
G-1	0	0	4	0.1	70.2	70.9
GM-I	10	10	4	1	85.3	76.2
GM-II	20	10	4	1.7	72.1	53.8
GM-III <sup>a</sup>	20	10	4	1.6	71.2	52.4
GM-IV	20	5	4	1.8	75.3	54.6
GM-V	20	20	4	1.7	74.9	55.1
GM-VI	30	30	4	2.2	79.7	54.0
GM-VII	40	40	4	2.6	86.4	56.3
GM-VIII	20	20	7	1.8	74.6	71.4
GM-IX	75	75	4	3.7	81	35.7
GM-X	20	20	2	1.5	83.6	69.3

<sup>a</sup>Reproducibility for GM-II.

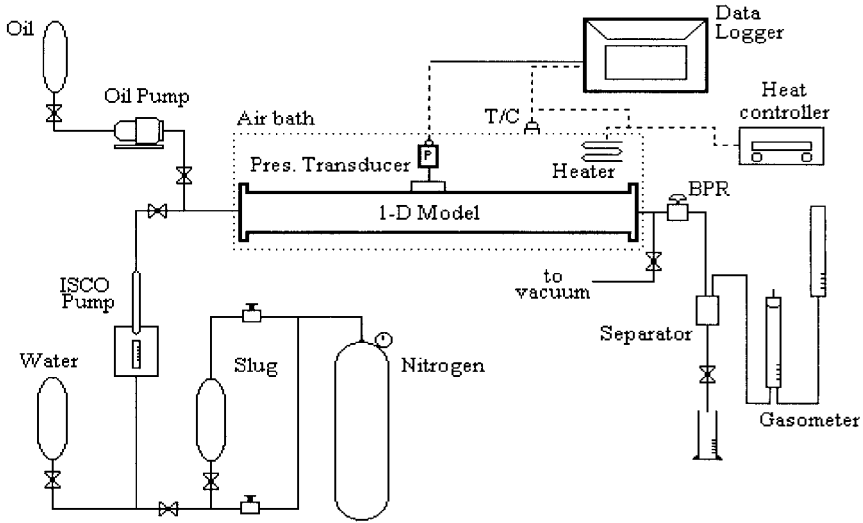


Figure 1. Bacterial water flooding test setup.

model to be compared with the reference run in order to see whether this medium alone has an effect on oil recovery.

At the end of each experiment, oil recovery values and pH of the water produced were measured. Water samples were also tested for the presence of bacterial activity, and live bacteria were detected in all of these samples.

Apart from these tests, bacteria in their substrate medium were brought together with 50.000 g of crushed limestone in closed bottles in order to see the effect of bacterial products on carbonate rocks.

## Results

### Shut-in Tests

The shut-in pressure behavior results obtained from 10 microbial runs (GM-I to -X) and 1 reference run (G-1), which was performed by injecting distilled water instead of microbial solution, are presented in Table 1.

During the shut-in experiments, it was observed that the pressure in the cell increased gradually in all the bacterial experiments. CO<sub>2</sub> and H<sub>2</sub> gases produced by *Clostridium acetobutylicum* apparently had caused this increase.

The maximum shut-in pressures for these experiments are presented in Table 1. The pressures reached in all microbial experiments are far greater than that in G-1. As the size of microbial slug increases, the maximum pressure also increases. Duration of the shut-in period does not affect the maximum pressure for the given variables as the maximum pressure is reached in about 2 days. The pressure behavior for these tests (Figure 2) shows that the microbial activity caused a sharp increase in pressure for the first 1,000 min of the experiment and that after about 3,000 min the pressure stabilizes.

It can be seen that the initial amount of the bacteria in the injected solution is not as important in terms of maximum shut-in pressure (GM-II-V) as the total injected amount (GM-I, -II, -VI, -VII, -IX).

**Table 2**  
Conditions and results of water flooding tests

Test	Bacteria	Slug size (PV) <sup>a</sup>	Water inj. (PV) <sup>a</sup>	Inj. rate (cc/hr)	Shut-in (day)	OOIP <sup>b</sup> (cc)	Prod. oil (cc)	Recovery (% OOIP)	pH <sup>d</sup>
GW-1	None	0.25	2	100	4	93	18	19.4	9.4–10.6
GMW-1	<i>Clostridium acetobutylicum</i>	0.25 (1/3)	2	100	4	94	41	43.6	6.5–6.9
GMW-2	<i>Clostridium acetobutylicum</i>	0.125 (1/3)	2	100	4	95	33.5	35.2	6.8–7.4
GMW-3	<i>Clostridium acetobutylicum</i>	0.25 (1/1)	2	100	4	98	41	41.8	6.7–7.1
GMW-4	<i>Clostridium acetobutylicum</i>	0.25 (1/3)	3	100	4	96	47	46.9	6.4–7.0
GMW-5 <sup>c</sup>	<i>Clostridium acetobutylicum</i>	0.25 (1/3)	2	100	4	95	40.5	42.7	6.6–7.0
GMW-6	<i>Clostridium acetobutylicum</i>	0.25 (1/3)	2	100	2	97	37	38.1	6.7–7.4
GMW-7	<i>Clostridium acetobutylicum</i>	0.25 (1/3)	2	50	4	96	47.5	49.5	6.6–7.4
GMW-8	<i>Clostridium acetobutylicum</i>	0.25 (1/3)	2	200	4	93	16.5	17.7	6.4–7.2
GMW-9	<i>Clostridium acetobutylicum</i>	0.25 (1/3)	2	100	8	99	42.5	42.9	6.0–6.4
GMW-10	<i>Clostridium acetobutylicum</i>	0.375 (1/3)	2	100	4	96	45.5	43.2	
GMW-11	<i>Clostridium acetobutylicum</i>	0.125 × 2 (two-step)	2	100	5	92	42.5	46.2	
GMW-12	Soil bacteria	0.25 (1/3)	2	100	4	94	26.5	28.2	

<sup>a</sup> Pore volume.

<sup>b</sup> Original oil in place.

<sup>c</sup> Reproducibility for GMW-1.

<sup>d</sup> Range of results obtained from multiple samples.

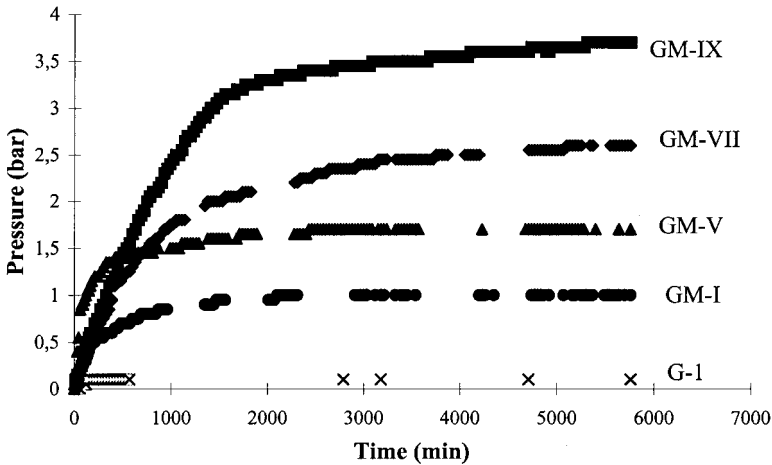


Figure 2. Shut-in pressure behavior for G-1, GM-I, -V, -VII, and -IX.

Oil viscosity is observed to decrease in all the microbial experiments compared to the values obtained before the experiments (Table 1). This decrease can also be attributed to  $\text{CO}_2$  production by the bacteria. It is observed that the initial population of the bacteria injected has no considerable effect on the viscosity reduction. However, when the amount of the total injected solution increases, the reduction of viscosity becomes much more pronounced. It is also seen that when the shut-in period is extended, the decrease in viscosity lessens (GM-VIII). About 10 cc of gas was collected upon opening the cell in this experiment, while there was no gas production in any of the other tests. It seems that the gas molecules diffused in the oil due to microbial activity rise to the top of the cell as a result of segregation as time extends.

It was seen that oil density did not show important changes from initial conditions in microbial experiments. This is an expected outcome, as *Clostridium acetobutylicum* is not a culture that can utilize the crude oil as a carbon source. Thus there is no change in the chemical structure of oil and the gas produced is not sufficient to decrease the density of the crude oil significantly.

### Microbial Water Flooding Tests

The oil recovery results and pH values of the produced water for 1 reference (GW-1) and 12 bacterial (GMW-1 to -12) runs can be found in Table 2.

It can be observed from Table 2 that the microbial runs are much more effective in the recovery of the additional oil than pure water flooding, the only exception being GMW-8, in which the flooding rate was very high (200 cc/h). To see the contribution of the gases produced, the comparative shut-in pressure curves for GMW-1, GMW-11, and GW-1 are plotted in Figure 3. Microbially produced gases cause the pressure to increase to about 5 bar during the shut-in period.

When the slug sizes are compared, it can be seen that all bacterial runs yielded considerable oil recovery increases compared to GW-1 (Figure 4). As expected, the recovered oil values are higher when the amount of bacterial solution used increases. The recovery difference between the slug sizes of 25 and 50 cc (0.125 and 0.25 PV) is greater than that between 50 cc and 75 cc (0.25 and 0.375 PV) slugs.

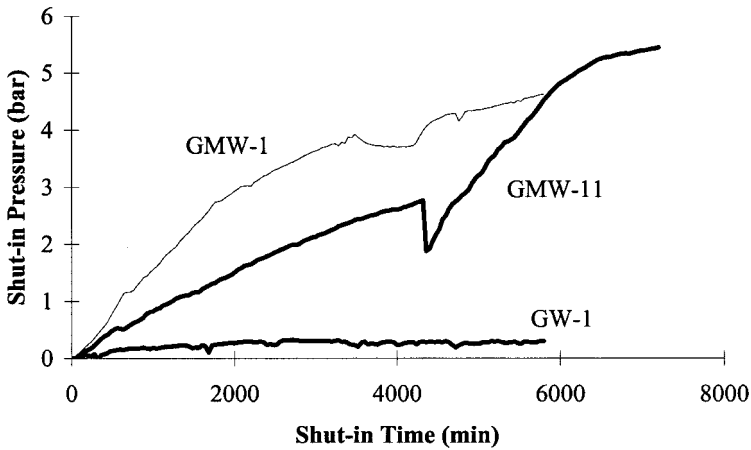


Figure 3. Pressure data in shut-in periods of GW-1, GMW-1, and GMW-11.

It can also be seen from Table 2 that the variation in the initial population of microorganisms in the bacterial slug does not affect the oil recovered additionally from the reservoir model.

As can be expected, the efficiency of microbial water flooding decreases with increasing flow rate. Table 2 shows this behavior clearly. The effect of microbial treatment on the oil production diminishes, especially at the highest flow rate.

In regard to shut-in times, the results show that there is little difference in oil production for the 4- and 8-day shut-in period tests. The difference, however, becomes more pronounced when 2- and 4-day periods are compared, indicating that the optimum shut-in period is between 2 and 4 days for this particular system.

When a two-step injection procedure is applied (GMW-11)—that is, first 25 cc of bacterial solution was injected into the model, and after waiting for 3 days, 25 cc nutrient was injected—total oil production didn't show a considerable change. The shut-in

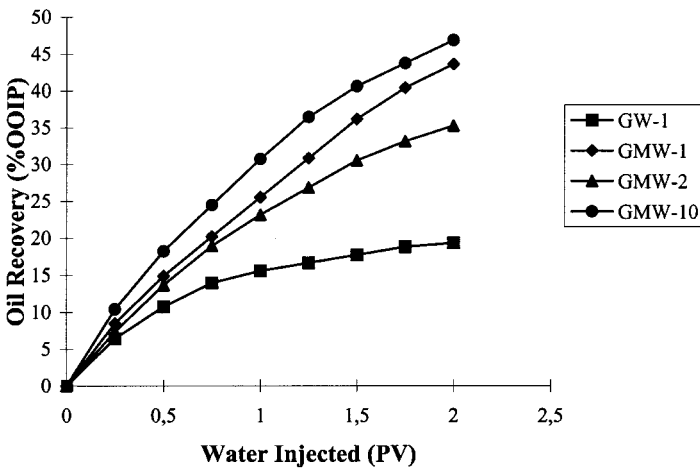


Figure 4. Effect of bacterial slug size on oil recovery.

pressure curve (Figure 3) demonstrates that the final pressure does not vary significantly, although the behavior is different.

From Table 2, it can be stated that both types of bacteria were more effective in oil recovery than the conventional water flooding. It can also easily be seen that *Clostridium acetobutylicum* cultures are much more effective in the oil release than the anaerobic soil bacteria, which in fact have been isolated from the production water of an oil well located in the northwestern region of Turkey.

When Table 2 is examined for the range of pH results, it can be observed that acidity in microbial experiments increased significantly. It can also be seen that the experiment having the longest shut-in period yielded the lowest pH. This can be attributed to the amount of CO<sub>2</sub> and organic acids produced by the bacteria. This acidification causes dissolution of limestone and, consequently, an increase in permeability. This is a fact that has been substantiated by the bacteria-limestone interaction test. (In the bacteria-limestone interaction test, the weight of the limestone after 2 days of contact was measured to be 44.593 g. This decrease in the weight of limestone is attributed to the acidic effect of microbial products.)

Here it must be added that the results obtained from the control test that was carried out with nutrient medium as slug did not differ significantly from those of the reference test, G-1.

## Conclusions

1. *Clostridium acetobutylicum* causes a reduction in oil viscosity due to its vigorous CO<sub>2</sub> production. This gas also causes extensive pressurization.
2. *Clostridium acetobutylicum* is also effective in recovering oil from depleted reservoirs. The oil recovery increase due to microbial activity is more than twofold compared to water flooding alone.
3. For this system, the optimum shut-in period is observed to be between 2 and 4 days, in regard to oil recovery.
4. The optimum slug size seems to be 50 cc (0.25 PV) for this system, as values above this size do not yield important additional oil recovery. The initial amount of bacterial population in the injected slug seems to have no effect on the oil viscosity reduction and oil recovery enhancement.
5. The pH of the medium is lowered by the gas and organic acids produced by *Clostridium acetobutylicum*. This also contributes to improvement of the oil recovery to some extent by the breaking down of carbonate rock.
6. Although not as effective as *Clostridium acetobutylicum*, the soil bacteria used improved the oil recovery.

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