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Chemical Flocculation of Microorganisms in the Reservoir During Meor

Chukwuma G.J. Nmegbu, Jossy Spiff

Abstract*—Clogging of the pores of a porous medium can be caused chemically by formation of a precipitate, physically by entrainment of suspended particles or biologically by the formation of biomass by microbes. All these mechanisms are potentially relevant to enhanced oil recovery. This paper investigates the level of flocculating activity in a Berea core sample dosed with Bacillus and Pseudomonas species, Nutrient agar and Salt diluent. Three of such samples were prepared and incubated for 12, 24 and 48hours. From scan pictures of the core, slimy substances were seen suggesting the production of biofilms, crystalline substances were observed inferring the production of biopolymers by the microorganisms and colloids observed, proving the production of biosurfactants. Heat was also generated during the process and the basic characteristics of the crude oil changed as seen in the flow of the crude oil. Thus, the chemical flocculation of these microorganisms in the reservoir aids in the recovery of substantial amounts of crude oil.

***Index Terms*—Bioflocculants, Chemical flocculation, MEOR, Microbial Flocculation.**

I. INTRODUCTION

Petroleum hydrocarbons may exist in vadose and saturated zones as a free liquid or ganglia of residual hydrocarbons. Even if the free liquid hydrocarbon can be removed, substantial amounts of residual hydrocarbons remain entrapped by capillary forces, thus reducing the overall oil recovery efficiency. MEOR (Microbial Enhanced Oil Recovery) targets these entrapped hydrocarbons. MEOR is the use of microbes in the petroleum reservoir to enhance the amount of oil that can be produced. It is a family of processes that involves the use of microorganism for enhanced oil recovery. As a result of the metabolic activities of these microorganisms, they excrete natural and non-toxic bioproducts which cause a series of very desirable changes in the physicochemical properties of the crude. These microbes in MEOR are typically hydrocarbon-utilizing, non-pathogenic microorganisms that are naturally found in petroleum reservoirs or are introduced. Research has proven that bacteria are the most promising of these microbes due to its natural tendency to attach to rock surfaces rather than free float in liquid [1]. Flocculation is a process where colloids come out of suspension in the form of floc or flakes. The action differs from precipitation in that, prior to flocculation, colloids are merely suspended in a liquid and not actually dissolved in a solution. In the flocculated system, there is no formation of cake since all flocs are in the suspension. Microorganisms, due to their metabolic activity excrete natural and nontoxic bioproducts such as biogas, bioacids, biopolymers and biosurfactants [2] of all these, the surfactants are most likely to assemble in a bulk solution [crude oil] into aggregates and this aggregates are referred to as floc [3]. A good example of a flocculating biosurfactant is polyacrylamide often used as an industrial coagulating and flocculating agent [4],[5]. The dynamic process resulting from the synthesis of extracellular polymer by living cells (microorganisms) is known as Bioflocculation. Bioflocculation has been investigated extensively and a correlation has been established between the accumulation of extracellular bioflocculants and cell aggregation [6],[7]. Aggregation of microorganisms is effected by an interaction of polymers excreted by the microbial cell or exposed at their surface [7],[8]. Bioflocculants are essentially chemicals known as polymers produced by microorganisms during their growth in the reservoir. Their flocculating activities results in the chemical flocculation of microorganisms in the reservoir during MEOR. A similar approach is the process of flocculation of microorganisms in biological waste treatment [5],[9],[10]. Tenney and Stumn [10] proposed that the flocculation of microorganisms in biological waste treatment is affected by the interaction of polymers excreted by the microorganisms exposed at the microbial surface under suitable physiological conditions. According to the hypothesis, flocculation of the dispersed organisms is interpreted as a process whereby polymers produced by the microorganisms absorb to and bridge between cell surfaces. Without flocculation of the microorganisms, only the fraction of the organic matter which has been oxidized will be removed. Thus, overall efficiency of a biological treatment process depends on flocculation [11],[12]. This research was undertaken to provide experimental corroboration for a polymer mechanism for a polymer mechanism in bioflocculation and a better understanding of some of the factors that influence aggregation of microorganisms and other colloids in biological waste and by extension, in Microbial Enhanced Oil Recovery (MEOR).

II. MATERIALS AND METHODS

A. Cultivation of Bacteria

The microorganisms were isolated from the soil and cultivated by ten-fold serial dilution described by Ofume [13]. 1.0g soil was introduced into 9ml of normal saline (diluents) in a test tube to give 10^{-1} dilution. Further serial dilution was made by transferring 1ml of the 10^{-1} dilution to another test tube up to 10^{-3} dilutions. From 10^{-3} dilution, 0.1ml aliquot was introduced onto the surface of sterile bent glass rod. The inoculated plates were incubated at 37˚C for 24 hours and colonies that developed were examined and based on their cultural characteristics discrete colonies, *bacillus* and *pseudomonas* organisms were presumptively isolated and purified by sub-culturing on to a fresh sterile solid nutrient agar and incubated at 37˚C for 24 hours. Identification of the bacterial isolates (*Bacillus subtilis* and *Pseudomonas*) species was done by reference to Bachnan and Gibbons [3].

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***** Correspondence Author (s)
Chukwuma G.J. Nmegbu, Department of Petroleum Engineering, Rivers State University of Science and Technology, Port Harcourt, Nigeria
Jossy Spiff, Department of Petroleum Engineering, Rivers State University of Science and Technology, Port Harcourt, Nigeria.

B. Preparation of Bacillus and Pseudomonas Species

The bacterial cultures were prepared by inoculating pure culture of the identified bacteria into 500ml of sterile nutrient broth medium. The inoculated medium was incubated at 37˚C for 24 hours and served as culture for treatment of crude oil during the experiment.

Table 1 Summary of Morphological/Cultural Characteristics and Biochemical Reactions Used for Presumptive Identification of the bacteria isolates

Bacteria Species	Morphological/ cultural characteristics	Biochemical reactions
Bacillus Subtilis	Rods, short, endospores formed, motilic, eiroum positive, occur in pairs, colonies are translucent and creamy.	Strict aerobes, oxidative metabolism, catalase positive, starch hydrolyzed, nitrate reduced, acid nor gas from glucose
Pseudomonas species	Rods, moderate colonies, motile, gram negative, colonies are greenish orange	Aerobes, oxidative metabolism, oxidase positive, catalase positive, nitrate reduced, acid from glucose

Table 2 Medium Composition: Nutrient agar

Constituent/ Parameter	Value
Peptone	10.0g
Meat extracts	10.0g
Sodium Chloride	8.0g
Agar	15.0g
Distilled water	1 litre
pH	7.0

C. Preparation of Salt Diluent

The diluent used was prepared by weighing 50 grams of Sodium Chloride and dissolving it in one litre of de-ionized water in a flat bottom flask to give 0.05 g/ml. A glass funnel was inserted into the MEOR design and the Sodium chloride concentration was poured into the MEOR design in order to saturate the core sample.

Table 3 Water Saturation of Brine in different samples

Sample	Volume of Brine Injected (Ml)	Volume Of Brine Recovered (Ml)	Volume of Brine in Core Sample (Ml)	Water Saturation
A	600	585	15	30.25
B	750	710	40	6.47
C	550	355	195	1.65

D. MEOR Procedure

The experimental procedure described below was conducted for three core sizes – 2.00mm, 3.35mm and 4.75mm core sizes.

1. Fill the core holder with 2.00mm grain size.
2. Attach the core holder to the entire MEOR design.
3. Lock all other inlets and outlets leaving only one inlet open.
4. Saturate the sand pack with 0.05g/ml of sodium chloride, using a glass funnel to pour it into the MEOR design.
5. Allow the brine to drain out.
6. Pump the crude oil into the sand pack and flush the brine.
7. Pump the brine and displace the oil. Measure the oil saturation.
8. Introduce the crude oil and apply pump pressure of 5psi.
9. Scan the core holder containing the sand pack and crude oil.
10. Open the outlet and allow the crude to flow for 3 seconds to get several beakers of crude oil.
11. Allow the crude to flow again for 12 seconds while pressure is added.
12. After the flow, scan the holder.
13. Through the inlet of the MEOR design, introduce 100ml of fresh bacteria solution into the sand pack containing the crude oil.
14. Scan the core holder containing the crude oil and the microorganism after the shut in.
15. Seal off the inlet and outlet valve and incubate for 24 hours at room temperature.
16. Scan the core holder after incubating for 24 hours.
17. Flow the crude for 12 seconds after 24 hours of incubation to get several beakers of crude oil.
18. Measure the flow rate of the crude oil.
19. Measure the viscosity.
20. Measure the specific gravity.
21. Scan the core holder containing the sand pack, crude oil and microbes.

III. Results

For sample A and B, after the microorganisms were added, shut in was done for 24 hours before producing the crude oil and for sample C, after the microorganisms were added, the crude oil was produced after shut in for 12 hours, 24 hours and 48 hours. After shut in, when the crude oil was to be produced, water came out first indicating viscous fingering. There was formation of gas which resulted in an increased pressure consequently increasing the volume of crude oil produced. There were crystalline substances along the flow line, slimy substances in the core holder. Colloids/fines were also observed. Heat was also generated during the process and the basic characteristics of the crude oil changed as seen in the flow of the crude oil. Crystalline substances observed infer the production of biopolymers by the microorganisms. Biopolymers produced from microorganisms are especially suitable in flocculation activities due to their ability to be produced uniformly and reliably. The formation of flocs is induced by the production of biopolymers by microorganisms and the interactions between these flocs are greatly influenced by these polymers. These polymers are the dominant contributors to the bio-clogging process and thus, flocculation activities. The colloids observed during the MEOR experiment prove the production of biosurfactants by the microorganisms. These biosurfactants vary in their chemical properties and molecular size.

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The yield of such biosurfactant depends, to a large extent, on the nutritional environment of the growing organism. These biosurfactants produced by the microorganisms aids in the chemical flocculation of microorganisms in the reservoir during MEOR due to their ability to form colloids/flocs. From scan pictures of the core, slimy substances were seen, suggesting the production of biofilms – extracellular polymeric material which serves as a growth medium and provide a means of attachment. These biofilms are responsible for the attachment of cells to one another, thereby forming flocs.

Table 4 Porosity of Different Core Sizes

Grain Size (ml)	Mass of Core Sample (g)	Mass of Grain Sample (g)	Volume of Water Displaced (ml)	Density (g/ml)	Porosity
4.75	2365	121	36.5	3.3151	0.5605
3.35	2510	176	70.5	2.4965	0.3807
2.00	2485	146	57.5	2.5391	0.3971

Table 5 Properties of the Test Fluid and system before MEOR

System Property	Measured value
Density at ambient conditions	0.8463g/cm^3
Oil viscosity at ambient conditions	0.41cp
Oil Formation Volume Factor at ambient conditions	1.559vol/vol ST
Solution gas at ambient conditions, R_s	955scf/stb
Gas Oil Ratio (GOR)	979scf/stb
Mean single phase compressibility	14.98 * 10^{-6}/psi
Gas gravity at ambient condition	0.883
Outlet pressure	14.7 psi
Inlet pressure	22psi

Table 6 Properties of Crude Oil after MEOR

Sample	Viscosity (cp)	Density (g/cm^3)
A	0.22	0.6350
B	0.20	0.6000
C	0.18	0.4825

The reduction in the densities and viscosities of the samples is seen from table 6. This is a direct reflection of the impact of the inherent microbial activity.

Table 7 Flocculating Activities and Flocculating rates of microbes added to the various core samples

Sample	$(O.D_{530})_C$	$(O.D_{530})_S$	Flocculating Activity *	Flocculating Rate**
A	4.22	2.88	0.11	31.75
B	4.22	2.80	0.12	33.65
C	4.22	2.76	0.13	34.60

$$*\text{Flocculation Activity} = \frac{1}{(\text{OD}_{530})_S} - \frac{1}{(OD_{530})_C} \quad (1)$$

$$**\text{Flocculation rate} = \frac{(\text{OD}_{530})_C - (\text{OD}_{530})_S}{(OD_{530})_C} \times 100 \quad (2)$$

It can be observed from Table 7 above that Sample C (which had the least water saturation) showed greatest flocculating activity of the three samples.

Table 8 Flow Rates of crude oil before and after MEOR

Grain Size (mm)	Flow Id	Before Meor (ml/Sec)	After MEOR		
			12 hours	24 hours	48 hours
SAMPLE A	Q_{01}	2.23		2.68	
	Q_{02}	1.42		1.58	
	Q_{03}	1.17		1.54	
	Q_{04}	0.83		1.08	
	Q_{05}	0.67		0.92	
	Q_{06}	0.50		0.76	
SAMPLE B	Q_{01}	3.08		4.13	
	Q_{02}	2.42		3.91	
	Q_{03}	2.35		3.62	
	Q_{04}	2.03		3.30	
	Q_{05}	2.00		3.13	
	Q_{06}	1.92		3.04	
SAMPLE C	Q_{01}	4.58	4.83	1.83	1.53
	Q_{02}	3.92	4.17	1.58	1.39
	Q_{03}	2.87	3.33	1.50	1.18
	Q_{04}	2.86	2.92	1.48	0.93
	Q_{05}	2.63	2.75	1.33	0.78
	Q_{06}	2.36	2.34	1.55	0.58

The improved flow rate through the cores after microbial treatment is immediately observed from the Table 8 above. The decline in the production rate after some time is expected of all naturally flowing system.

IV. Conclusion

Bacillus subtilis and *Pseudomonas* species used are potentially ideal for MEOR due to their ability to produce biopolymers, biosurfactants and also ultimately to mobilize oil from the reservoir due to their flocculating activities. These activities resulted in a reduced fluid viscosity and improved oil flow rate. However, it is recommended that further research be carried out on the growth rate and detrimental effects of the bioflocculants in a petroleum reservoir as these areas could have significant impacts on the results.

V. References

[1] D. Momeni and T. Yen, "Introduction to Microbial Enhanced Oil Recovery", Principles and Practice, CRC Press, Boca Raton, Florida, 1990
[2] C. Yokoi, F.L. Toledo, J. Gonzalez and C. Calvo, "Production of bioemulsifiers by Bacillus subtilis, alcaligenes faeculis and enterobacter species in liquid culture", Biores Technol, vol. 99, 1996, pp. 8470-8475
[3] R.E. Bachanan and N.E. Gibbons, "Bergeys Manual of Determinative Bacteriology", 9th Edition, The Williams and Williams company, Baltimore, 1994, pp. 39-596
[4] J.S. Kumar, S. Joshi, Y. Bharucha, A. Nerukar and A.J. Desai, "Biosurfactant production using molasses and why under thermophilic conditions, Biores Tech, vol. 99, 2004, pp.195-199
[5] J.D. Desai, and I.M. Banat, "Microbial production of surfactants and their commercial potential", Microbial, vol 61, 1997, pp. 47 – 64
[6] F.C. Koizumi, M.C. Finald and J.G. Holt, "The shorter Bengegs Manual of Determination of Bacteriology", Baltimore, Vol. 2, 1991
[7] S.H. Tokeda and S.A. Kuran, "Extracellular biopolymeric flocculants", Recent trends and Biotechnology importance, Biotechnology Adv., Vol. 19, No. 5, 1991, pp.371-385

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[8] P.L. Busch and W. Stumn, “Chemical Interactions in the Aggregation of bacteria bioflocculation in waste treatment”, Division of Engineering and Applied Physics, Harvard University, Cambridge, 1968
[9] R.H. Lee, E. Jamshidi, A. Mazahari and H. Bonakdarpour, “Isolation and Production of Biosurfactant from pseudomonas aeruginosa isolated from Iranian Southern oil wells”, Environ Sci Tech, vol. 2, 1995, pp.121-127
[10] M.W. Tenney and W. Stumn, “Chemical flocculation of microorganisms in Biological Waste Treatment”, Journal of Water Pollution Control Fed., vol. 37, 1965, pp.1370 – 1388
[11] W. Slili, S. Mei, L. Wei, L. Guanzhi and H. Peihui, “Laboratory study on MEOR after polymer flooding”, 2001
[12] J.L. Takeda and L.M. Kurane, “The field pilot of MEOR in a high temperature reservoir”, Pet. Sci. Eng, vol. 48, 1999, pp.265-271
[13] J.I. Ofume, “Bacteriological Examination of clinical specimens”, Achua Publications, Ama J.K. Recreation Park, Owerri, Nigeria, 1991

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