Using Paraffin Degrading Bacterial (PDB) Consortium To Study The Degradation Behaviour Of Oil From An Onshore Oil Well

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Abstract : Paraffinic crude oil flows freely at higher temperature, as temperature reduces it tends to crystallize, or deposit around the down hole tubular, or at the face of producing zones in the well bore regions. It results in complete blockage of crude passage and possesses severe production problems causing huge economic loss. This problem is overcome by using microbes as tertiary oil recovery and this technique termed as Microbial Enhanced Oil Recovery (MEOR). Oil rich in paraffin is degraded with Paraffin Degrading Bacteria (PDB) so as to mitigate deposition of paraffin in well tubing's, decreasing the pour point of oil and increase its flow rate. Wells in which production of oil decreases are treated with bacterial culture so as to enhance oil recovery from the field. Laboratory studies in Indian Institute of Reservoir Studies (IRS) at Ahmedabad showed significant reduction in the viscosity and the pour point of the crude oil.

Keywords: EOR, IRS, MEOR, Paraffinic crude, PDB, Tertiary oil recovery, Viscosity

1. INTRODUCTION

Paraffin deposition resulting in loss of production is a perennial problem in the oil fields, globally. The problem gets even worse during the winter with falling temperatures. These problems range from clogging reservoir flow paths and causing premature abandonment of reserves, to increasing maintenance costs of pumps, rods, tubing, flow-lines, separators and creating tank bottoms in the surface facilities. The paraffinic wax constituent of petroleum crude begins crystallizing depending on temperature and pressure. Freezing out of heavier fraction of the paraffin from the crude is found at low temperatures of flow. High temperature of the reservoirs keep these paraffinic compounds solubilized but below Wax Appearance Temperature of crude, the surfaces of the well tubing, surface flow line get coated with layers of wax. (SPE 129002) Paraffins are normal or straight carbon chain alkanes with carbon chain lengths of C18+. The alkanes in this range solidify at temperatures from 80 to over 200^{o}F. Paraffin is amorphous but may appear to have structure in slow growth examples. It is natural constituent of most crude oil. At reservoir conditions paraffin occurs in the soluble (liquid) state in the crude oil, but at surface conditions they tend to crystallize (Solidify). No difficulties result as long as it is in soluble state. However, deposition in formation pore system can create significant problems. Paraffin wax produced from crude oil consists primarily of long chain, saturated hydrocarbons (linear alkanes/ n-Paraffins) with carbon chain lengths of C18 to C75+, having individual melting points from 40 to 700C. This wax material is referred to as ―microcrystalline wax.‖ Fig. 1 shows the generic molecular structures of n-Paraffins, iso-paraffins, and naphthenes. As the temperature of the crude drops below a critical level and/or as the low-molecular-weight hydrocarbons vaporize, the dissolved waxes begin to form insoluble crystals.

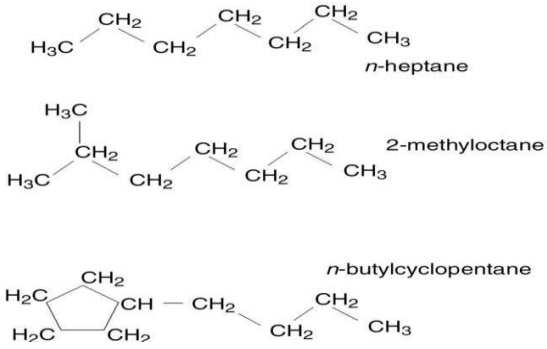

Figure 1: *Structures of hydrocarbon classes involved in wax deposition.*

(Courtesy: http://petrowiki.org/Wax_problems_in_production)

Mechanism of Paraffin Degradation Bacteria:

Saturated un-branched acyclic hydrocarbons will attach on well walls (solidification points at 95-104^{0} F). During the deposition process, other material such as sand, scale and oil becomes encased in the scale. The paraffin degradation bacteria produce a natural surfactant, penetrating and loosening the deposits from pipe walls and the nearby formation, while assimilating mineral deposits (i.e. calcium, magnesium, etc.) which neutralize and condition paraffin deposits making them less sensitive and divalent ion concentrations. The process of adding bio-activator to the paraffin degrading bacteria accelerates the bacterial reaction with the straight chained bonds of paraffin, breaking it down into simple, shorter chained alkanes, lowering viscosities and increasing influx rates. The developed microbial consortium also deposits bio-film at the inner surface of the tubing which does not allow the crystals of wax to get aggregated for deposited due to creation of this very smooth bio-film.

The bacteria in the film degrade the fresh wax crystals getting generated on the surface and also prevent agglomeration of such wax crystals by forming a coat around tiny wax crystals. The bacteria also degrade the

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higher molecules of wax into smaller molecules which easily get dispersed in to the crude oil. This was not allowing the paraffin to get deposited. (SPE 129002)

Characteristic Paraffin Degrading Bacterial Microbes:

- Thermophilic- Active up to 90^0C.
- Halophilic- Resistance to salinity up to 3%
- Microaerophilic and thermophilic
- Gram positive and rod shaped
- pH ranges 6-8
- Carbon source - glucose and wax
- Nutrient Media - Minimum Salt Media(MSM) Biocatalyst
- Metabolites- solvents, bio surfactants, bio polymers and bacterial cell gases
- Inoculation- 36 hours at 55^0C at 180 RPM at Shaker Incubator

EXPERIMENTAL WORK

Experimental Procedure: An Oil sample collected from ONGC well no # M-39 (Gujarat) for Degradation Studies on Oil using Paraffin Degrading Bacterial (PDB) Consortium

Preparation of MSM Media:

Step- 1: Take 500 ml distilled water and from this distilled water take 350ml, 50ml and 50 ml into three conical flasks for preparation of glucose, buffer and PDB media.

Step - 2: Preparation of Solution

- Glucose Solution Preparation
- Buffer Solution Preparation
- PDB Media solution Preparation

Step- 3: Sterilize these solutions in the auto clave instrument at standard conditions of 120^oC temperature and 15 psi pressure to remove the contamination from the surroundings.

Step- 4: After sterilization process put these solutions in the Laminar Air Flow instrument. Before putting these solutions clean the surface of laminar air flow with Ethanol.

Step- 5: Switch on the UV light and wait until 45mins to kill bacteria inside the laminar air flow. After45mins switch off UV light and Switch on fans. Before switch off the UV light you must switch on the fans.

Step-6:Mix these solutions carefully into one flask in the presence of flame and take the precaution of; never put the ethanol bottle near the flame because it is highly inflammable and also remove the cotton plugs from the flasks far away from the flame.

Step- 7: Add 5ml trace minerals, 0.5ml vitamins to this solution in the presence of flame and stir the solution for proper mixing of these trace minerals and vitamins in the solution. Then sample MSM media was prepared.

Step- 8: Inoculate 50ml PDB bacterial culture (i.e., 10%) to this MSM media in the presence of flame and stir the proper mixing of the culture.

Step- 9: Incubate this solution in the electrical shaker at 180 rpm and 55^0C temperature for 36hrs.Then 500ml Culture sample was prepared.

Step-10: Continue the procedure from step-1 to 7 for preparation of the 4 liter media solution according their chemical quantities required.

Step-11: For 4liters distilled water, the solutions prepared as 400ml Glucose solution, 400ml Buffer solution, 2800ml PDB media solution and 400ml PDB bacterial culture.

Step-12: Divide this 4lits into four 1liter conical flasks and add 10ml trace minerals, 1ml vitamins in each 1liter flask and stir the solution for proper mixing of these minerals, vitamins in the solution. Now bulk MSM media was prepared.

Step- 13: Inoculate100ml PDB bacterial culture to this MSM media in the presence of flame and stir the proper mixing of the culture. Then 4liters PDB bulk culture was prepared

Effect of microbial PDB Culture on oil:

Step-1: Take the prepared 4liters of PDB bulk culture into four one 1liter conical flasks. Each one liter flask having 700ml PDB main culture, 100ml glucose solution, 100ml buffer solution and 100ml PDB inoculum.

Step-2: Add 1.5 to 2% oil into the each 1liter MSM media flask.

Step-3: Incubate this culture in the electrical shaker incubator at 180rpm and 55oC temperature for 5days.

Step-4: After 5 days of incubation the microbial growth present on the PDB culture.

Step-5: After the presence of microbial growth, separate the oil by using separating funnel and test the required parameters of oil.

Separation of Degraded Oil:

The PDB treated oil was separated by the separating funnel under the action of gravity and kept undisturbed for a couple of hours which allows complete separation of two clear phase, with oil at the above layer and water at the below. In this process water is separated from the oil by gravity action and oil is collected. After that dehydration process was started to that separated oil.

Figure 2: *Separating Funnel Dehydration of Separated*

Degraded Oil

Reflux was do condensing for 10 to 12 hours depending on the nature of oil. If any water present in the oil, was then removed with the help of syringe. This water free oil was then subjected to further analysis.

Figure 3: *Reflux apparatus*

Microscopic Observation after Incubation

After incubation growth of consortium was observed by using MOTIC – microscope.

Measurement of Crude Oil Properties

For measurement of crude oil properties like viscosity, pour point, Anton Paar Rheometer MCR 52 was used in the lab.

Figure 4: *Anton Paar Rheometer*

RESULTS

p^H Changes:

pH of MSM media without and with paraffin degrading culture checked before and after incubation respectively. The pH of the culture treated with oil microbial culture decreased. The decrease in pH of oil microbial culture is because of release of low volatile fatty acids. There is not much change in pH of culture after
incubation with PDB culture.

Table 1: *p^H of Sample of Field*

Sample	Constituents	No of cells/ml
1	Crude Oil-B+MSM+PDB	10^{8-9}cfu/ml

Microbial Counts:

Cell count was determined at stage of inoculation and after incubation of culture. Total number of cells in the inoculum was109cells per ml for in paraffin degrading culture. Following is the cell count after incubation with respective waxy crude.

Table 2: *Cell Count Determination after incubation.*

Sets	Constituents	p^H(before incubation)	p^H(after incubation at 50^0 C for 5 days)
1	Crude Oil +MSM+PDB	7.2	6.8

The growth of respective microbes in the culture with oil could be ascertained by the increase in their cell count as observed under the microscope.

Figure 5: *Microscopic Image of Bacterial growth after incubation*

Study of Viscosity Measurement:

After incubation with the culture, change in the viscosity was seen. Viscosity was measured using Anton Paar Rheometer MCR 52.

Table 3: *Viscosity Changes in Sample of Field (Waxy crude)*

Sets	Constituents	Viscosity at 50^0C (cP)
1	Neat Crude Oil	782
2	Degraded oil	581

Release of bio-surfactants, bio-solvents, volatile fatty acids, gases and degradation of higher length chain to smaller length hydrocarbons is primarily responsible for decrease in viscosity.

CONCLUSIONS

The microbial treatment is effective in enhancing oil recovery and preventing wax deposition of the crude oil. The microbe by virtue of degrading the bonds affects the viscosity of crude oil which is an important parameter for evaluation. The used culture has been able to reduce the viscosity of the oil hence can be used in the field for improving the flow and recovery from the oil well.

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