

Evaluation of Commercial, Microbial-Based Products to Treat Paraffin Deposition in Tank Bottoms and Oil Production Equipment – Final Report

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Title: Evaluation of Commercial, Microbial-Based Products to Treat Paraffin Deposition in Tank Bottoms and Oil Production Equipment

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Objective:

We aimed to determine the mechanism(s) of action of commercially available, microbial formulations used to treat paraffin deposition in the oil field. There are many conflicting reports by producers on the efficacy of microbial treatments to remedy paraffin deposits, and it is not known why microbial treatments work under some conditions but not others. Knowledge of the mechanism(s) used by microorganisms to remediate paraffin deposits is a critical first step in understanding how the application of microbial treatments for paraffin removal can be optimized in the oil field. Knowing the mechanism(s) of action of these products will allow the independent producer to determine the conditions under which they are likely to succeed and to determine if and when the purchase of microbial commercial paraffin treatments represents a wise expenditure of investment dollars.

Introduction:

Paraffins are naturally-occurring components of crude oils, but often form solids within oil reservoirs and on oil production equipment when oil is harvested from hot subsurface temperatures to the cooler surface environments. Microbial treatment is one approach that can be used to help alleviate this problem, and has been deemed more environmentally-friendly, safer to use, and more cost-effective than more traditional thermal or chemical methods of paraffin treatment. However, there are numerous conflicting reports by producers on the efficacy of microbes to remedy paraffin deposits; it is not known why microbial treatments work under some conditions but not others. Although not scientifically documented, several hypotheses have been proposed as to how microbes may act in oil reservoirs to treat paraffins. These include the “cracking” of

long-chain hydrocarbons into shorter chains (a mechanism with no known biological basis), the production of biosurfactants or bioemulsifiers to help mobilize paraffins, or biodegradation of paraffins into oxidized metabolites which may act as “biosolvents”. Thus, in this project, we sought to investigate such proposed mechanism(s) of microbial paraffin treatment in controlled laboratory studies in order to understand how, why, and when they work. Experiments were conducted in two stages. Stage I experiments consisted of initial screening of paraffinic oils and microbial products by using a highly-sensitive assay which measures changes in the physical properties of oils. Any positive results from Stage I screenings were then tested further for possible mechanisms of action in Stage II experiments. Two paraffinic oils were studied in this project, and pertinent results are presented below.

Results of Stage I Experiments:

The two oils chosen for study were designated Alaska Oil A and Alaska Oil B. These oils were supplied to a proprietary manufacturer of a widely-used commercial microbial treatment product who recommended a unique microbial formulation which should be effective for the treatment of each oil. The Stage I experiments entailed incubating the given oil with artificial brine medium in the absence (negative control) or presence of a proprietary microbial product chosen specifically to treat the oil. Tests were conducted at room temperature (~25°C) and at 60°C either in the presence or absence of oxygen. All test variables were established in triplicate and were incubated for 3 or 7 days. After incubation with slow, end-over-end mixing, oil layers were removed to conduct the wax appearance temperature (WAT) test. This highly-sensitive assay uses cross-polarized microscopy to measure the temperature at which paraffin crystals begin to form when a given oil is cooled under controlled conditions. For the purposes of this research, microbial paraffin treatments which lowered the WAT by a minimum of 5% over that of the parallel microbial-free controls were considered successful for the preliminary screening assays.

Table 1 shows the WAT results obtained during Stage I screening experiments conducted for both Alaska Oils A and B. Statistical analysis (ANOVA test) of all of the WAT data from Alaska Oil A Stage I experiments showed that there was no significant difference in the WAT values for any microbe-amended incubation under any condition relative to the microbe-free (negative) controls. These results suggested that the proprietary microbial product selected to treat this oil was ineffective, thus this oil was not studied further.

In contrast, some changes in the WAT values obtained for Alaska Oil B were evident. At 60°C, ANOVA tests showed that there was a significant difference in the WAT values between microbe-free controls and microbe-amended incubations in the presence of oxygen (Table 1). For this oil, aerobic treatment with microbial formulation at 60°C resulted in an 8.5% decrease in the WAT relative to microbe-free controls. In the absence of oxygen, a 4.9% difference was observed between microbe-free and microbe-amended incubations, barely meeting the 5% criterion for successful treatment. When we examined oil removed from microbe-free tests incubated at 25°C (e.g., negative controls only) we found at least an 11.5% difference among the WAT values obtained, well above the 5% criterion (data not shown). This variability at 25°C was thought to be

due to a phenomenon known as cold seeding in which paraffins drop out of the oil and move into an emulsion layer when the oil is below its cloud point resulting in irreproducible oil samples. Thus, we concluded that the WAT test could not accurately be performed on oils incubated at room temperature (25°C). Nevertheless, the positive results observed at 60°C with Alaska Oil B led us to establish Stage II experiments in order to determine the mechanism(s) of action of the microbial formulation which caused the significant reduction in the WAT values.

Table 1. Wax appearance temperature results for Alaska Oils A & B in Stage I experiments

Incubation	WAT (°C)	ANOVA test
Alaska Oil A		
25°C aerobic control	38.1	no significant
25°C aerobic + microbes	37.8	difference
60°C aerobic control	39.7	no
60°C aerobic + microbes	39.9	significant
60°C anaerobic + microbes	42.1	difference
Alaska Oil B		
60°C aerobic control	28.3	significant
60°C aerobic + microbes	25.9	difference (8.5%)
60°C anaerobic control	28.2	no significant
60°C anaerobic + microbes	26.8	difference (4.9%)

Results of Stage II Experiments:

Stage II experiments with Alaska Oil B were designed to elucidate the mechanism(s) of action of microbial formulations in treating paraffins. Thus, a more detailed mechanism experiment was begun by setting up incubations with Alaska Oil B at 60°C under 6 different conditions. These included the following amendments: (1) whole formulation (*positive control*); (2) cells only (*are the microbial cells only needed for treatment?*); (3) supernatant only (*is there some chemical component in the medium associated with the cells that contributes to paraffin treatment?*); (4) whole formulation plus chloramphenicol (*do cells need to grow for successful treatment?*); (5) heat-killed whole formulation (*are living cells necessary for treatment?*); and (6) no microbes added (*negative control*). All incubations were carried out in triplicate under both aerobic and anaerobic conditions. Although the initial screening experiments showed a statistically-significant decrease in the WAT under aerobic conditions only, anaerobic incubations were also established since the WAT values were near our 5% criterion for successful treatment and anaerobic conditions are more representative of oil reservoirs. These Stage II experiments were incubated for approximately 9 weeks in order to mimic usual successful field treatment times (2 to 3 months). During this time, we assayed for several possible mechanisms of microbial treatment. These included: (1) biosurfactant production (surface tension measurements); (2) bioemulsification activity (assay using

incubation supernatant and hexadecane; Trebbau and McInerney, 1996, *J. Indust. Microbiol.* 16: 1-7); (3) paraffin “cracking” or biodegradation (oil analysis by high-temperature gas chromatography [HT-GC]); (4) metabolite production (organic solvent extraction and gas chromatography-mass spectrometry [GC-MS]); and (5) effect on wax deposition (cold finger test).

Table 2 shows the surface tension results obtained under aerobic and anaerobic conditions at time 0 and after 9 weeks in all of the above-described incubations. Under aerobic conditions, no significant differences were observed in the negative control or heat-killed incubations. However, statistical analysis (ANOVA tests) showed a small yet statistically-significant lowering of the surface tension after the 9-week period in incubations with whole formulation, cells only, supernatant only, and whole formulation in the presence of chloramphenicol. A similar effect was also seen under anaerobic conditions (Table 2). The fact that a decrease in surface tension was observed when either live or heat-killed microbial formulations were added to the oils indicated that some component(s) within the formulation other than the cells was contributing to the observed changes. Similarly, some weak bioemulsification activity was also observed (data not shown), but again, this was evident whether live or heat-killed formulations were incubated with Alaska Oil B.

Table 2. Surface tension results for incubations of Alaska Oil B in Stage II experiments

Incubation	Surface Tension (dynes/cm)			
	Aerobic	Time 0	9 weeks	ANOVA
Negative control		53.8	54.2	not significant
Heat-killed cells		47.0	48.0	
Whole formulation		53.3	47.8	significant difference
Cells only		55.0	50.9	
Supernatant only		54.4	52.7	
Chloramphenicol		54.9	47.7	
Anaerobic				
Negative control		53.8	56.0	not significant
Heat-killed cells		55.0	50.1	
Whole formulation		72.0	53.0	significant difference
Cells only		55.3	53.0	
Supernatant only		56.8	50.8	
Chloramphenicol		57.5	50.0	

To determine whether paraffin biodegradation was a mechanism of microbial treatment, we examined the oil layers in the incubations for evidence of paraffin decomposition. Oils were sub-sampled from the incubations and analyzed by HT-GC to determine whether decreases in paraffin concentrations were evident or whether the

paraffin profile shifted from one of higher molecular weight to lower molecular weight alkanes. Prior to HT-GC analysis, oil samples were amended with C₂₄D₅₀ as an internal standard for quantification. Table 3 shows the oil-to-internal standard peak area ratio of the paraffins quantified (C₁₀ to C₄₀) for aerobic and select anaerobic incubations. Using this method of quantification, the oil-to-internal standard peak area ratio should *decrease* if a reduction in the paraffins occurred. As can be seen in Table 3, the oil-to-internal standard peak area ratios were remarkably similar in all of the Stage II incubations including the microbe-free controls, indicating that no paraffin biodegradation occurred. In similar fashion, known putative paraffin metabolites (such as long-chain fatty acids or alcohols which would be produced under aerobic conditions, or alkylsuccinates and branched fatty acids which would be produced under anaerobic conditions) were not detected using organic extraction and GC-MS analysis in any of the Stage II incubations.

Table 3. Oil-to-Internal Standard Ratio of Alaska Oil B analysis in Stage II Experiments

Incubation	Oil to Internal Standard Ratio
Aerobic	
Negative control	37.4 +/- 4.3
Heat-killed cells	38.4 +/- 1.5
Whole formulation	38.3 +/- 2.3
Cells only	38.5 +/- 2.9
Supernatant only	35.5 +/- 1.5
Chloramphenicol	42.2 +/- 5.9
Anaerobic	
Negative control	48.5 +/- 2.2
Whole formulation	46.4 +/- 2.1

In all previous experiments, a minimal salts brine medium was used for incubations of the microbial formulations and oil was supplied as the sole carbon and energy source. In field application of the microbial formulations, a nutrient solution is often added to help stimulate the *in situ* activity of the microbes added. Thus, another series of experiments was carried out to determine the effects of adding a commercial nutrient solution to the incubations. To this end, test bottles containing microbial formulation plus nutrients were incubated alongside those containing only the microbial formulation. Incubations were conducted at 60°C under aerobic and anaerobic conditions. Appropriate microbe-free controls were also established. After a 12-week incubation period, supernatants were analyzed for changes in surface tension and oils were assayed for paraffin alterations using HT-GC. As with earlier experiments, HT-GC analyses revealed no difference among oil-to-internal standard ratios in any of the microbe-free or microbe amended incubations indicating that no biodegradation or paraffin “cracking” occurred. Further, a statistically-significant decrease in surface

tension was observed only in the absence of nutrients under aerobic conditions, mimicking previous results.

In addition to the above biology-based assays, we also conducted cold-finger tests to assess whether microbial formulations were functioning in a more physical capacity to prevent wax deposition as opposed to treating wax once it has already been deposited. The cold-finger assays revealed that microbial formulations did not prevent wax deposition (data not shown).

Overall Summary: We had little success in determining an overwhelming mechanism of action of microbial paraffin treatment using commercial formulations since all measurements made showed no or only weak significant differences in microbe-amended versus microbe-free incubations. Initial screening experiments showed a significant reduction in the WAT in one of the oils tested (Alaska Oil B) which prompted more detailed mechanism studies. Paraffin biodegradation or “cracking”, or wax deposition prevention were not mechanisms involved in microbial paraffin treatment. Small yet statistically-significant changes in surface tension and emulsification assays suggested that some component(s) of the microbial formulations had surfactant-like or emulsifying activity. These latter phenomena were not necessarily cell-associated, since incubations with heat-killed cells or “supernatant only” fractions of the microbial formulations showed surface tension reduction. The addition of a commercial nutrient solution did not improve the microbial treatment of paraffinic oils in our controlled laboratory studies.

Publications/Presentations:

Gieg, L.M.; McInerney, M.J.; Suflita, J.M.; Jenneman, G. 2004. *Evaluation of Commercial, Microbial-Based Products to Treat Paraffins in Oil Production Operations*. Oral presentation at 11th Annual International Petroleum Environmental Conference, Albuquerque, NM, October 12-15.