THE INFLUENCE OF MICROORGANISMS ON OIL-MINERAL FINE INTERACTIONS IN LOW-ENERGY COASTAL ENVIRONMENT

Ronan Jézéquel and François-Xavier Merlin
CEDRE
Technopole Brest-Iroise BP 72
29280 Plouzané, France

Kenneth Lee
Fisheries and Oceans Canada
Maurice Lamontagne Institute
P.O. Box 1000
Mont-Joli, Quebec G5H 3Z4, Canada

ABSTRACT: *When spilled in the environment, oil frequently interacts with fine mineral particles to form aggregates. This process contributes to the natural shoreline restoration process as the residual oil remains mobile and becomes more accessible to oil-degrading biota. Studies have been undertaken to accelerate this mechanism of natural oil dispersion as an oil spill countermeasure (e.g., surf-washing). Mixing energy (waves) has been considered a key factor controlling the formation of oil-mineral fine aggregates. While most laboratory studies have used strong mixing energy to induce the formation of oil-mineral fine aggregates, they have also been observed to occur in the environment under moderate levels of agitation.*

To test this hypothesis, experiments were undertaken in mesocosms, recreating a sandy shoreline environment subject to identical levels of moderate wave activity and tidal movement. Microcosm experiments with oiled sediments amended with nutrients and bacteria have been conducted over a 4-week period with and without the addition of clay. Results and observations show positive influence of microbial activity on the formation of oil-mineral fine aggregates, which subsequently accelerates the rate of oil removal from the sand by the tide.

Introduction

When spilled in the environment, especially in coastal systems, oil frequently interacts with fine mineral particles to form aggregates. This process contributes to natural shoreline restoration (Bragg *et al.*, 1995); oil associated with fines is more readily dispersible. The resulting lower oil concentrations are more suitable for biodegradation, and the aggregates are believed to provide a favorable habitat for oil-degrading bacteria (Lee *et al.*, 1997).

Different studies have explored the interaction of oil and mineral fines and their potential benefit in spill cleanup operations (e.g., surf-washing) (Lee *et al.*, 1997). Mixing energy is considered a key factor in generating oil-mineral aggregates. Some laboratory simulations have required strong mixing energy to generate the aggregates, and it is hypothesized that the addition of surfactants (chemical dispersants) can enhance the formation of low-energy aggregates.

Oil-mineral fine interactions have been demonstrated to occur *in situ* with even the moderate levels of agitation found within sheltered sites. A possible explanation of this phenomenon may be the influence of biosurfactants generated by natural biota. To test this hypothesis, a study was undertaken in mesocosms, simulating a sheltered sandy shoreline subjected to controlled tidal movements and wave action, to compare the behavior of oil with and without the addition of fine minerals.

Experimental design

Mesocosm experiments were conducted to assess the influence of clay particles on the biodegradation rate of the oil and the degree of sediment restoration in a low-energy environment, such as a sheltered sandy beach. These mesocosms were similar to those used in previous studies at CEDRE's (Centre de Documentation de Recherche et d'Experimentations sur les Pollutions Accidentelles des Eaux) test facility located in Brest, France (Jézéquel *et al.*, 1998).

To generate identical wave activity, six replicate mesocosms, each consisting of an aquarium filled with a bed of sorted medium sand (300 to 1,000 µm) and fresh Atlantic seawater, were fixed on a large oscillating table (periodic movement: 35 s). To simulate tidal movement, each mesocosm was connected to another aquarium (renewal tank) by a small hose with a peristaltic pump to facilitate water exchange twice daily (Figures 1 and 2). The seawater in the renewal tank was replaced twice a week to simulate the dilution process that occurs in the natural environment.

The experiment was conducted according to the following controlled test conditions: clay particles absent [C0] and clay particles present [C1]. Each treatment condition was replicated in triplicate.

A washed sandy sediment in all mesocosms was treated with identical amounts of oil (110 °C topped Arabian crude oil): 24 L of sediment was mixed in a cement mixer with 400g of oil for 20 minutes; then 4 L of the polluted sediment was put in each mesocosm 4 days before the beginning of the experiment.

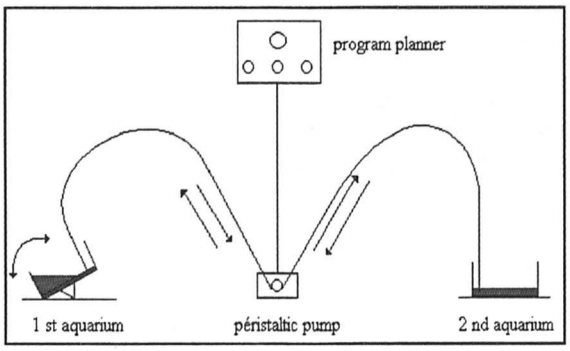

Figure 1. Experimental design of mesocosm table.

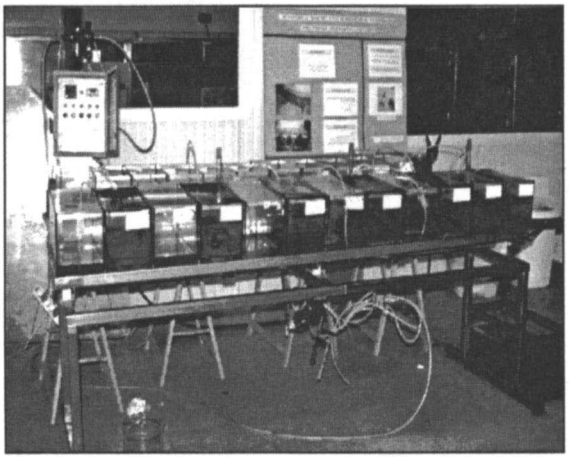

Figure 2. Oscillating table.

Each mesocosm was amended with 100 mL of a bacterial inoculum (Biolen Ecosoil 1106; Gamlen Industries s.a.) containing 17 g of oil-degrading bacteria in fresh seawater (1 g of oil-degrading bacteria = 1.2 x 109 CFU).

To maintain specified test conditions, clay and nutrient were re-added to the mesocosms, as appropriate. The clay was mainly montmorillonite; 5 g was added twice a week. Nutrients were also added twice a week: 30 g of Max-Bac (Grace Sierra International B.V.) dissolved in 100 mL of fresh seawater.

Bacteria and nutrient solutions added to the mesocosms were filtered (12 µm) to avoid the addition of any fines to the sediment.

Measurements. The following variables were monitored and/or quantified over a 4-week experimental period: bacterial numbers, hydrocarbon in the water column, oil content in the sediment, aggregates in the water column, oil composition from sediment, and aggregates.

Bacterial numbers. The total number of hydrocarbon-degraders was estimated by plate counts using Marine-Agar amended with topped Arabian crude oil as the carbon source. Samples of sediment and tidal water were extracted from each mesocosm at various times. Using sterilized instruments, 2 mL of sediments and 10 mL of water column were collected at the beginning, middle, and end of the experiment.

Hydrocarbon content in the water and aggregates. At each water change (twice a week), the discarded water was recovered for chemical analyses. Mineral fines in suspension were isolated over a 3-day period by sedimentation. After this time, a 100-mL sample of the tidal water was extracted with methylene chloride to quantify variations of hydrocarbon concentrations between the treatments using a UV spectrophotometer.

Aggregate structures were characterized by phase-contrast and UV epifluorescence microscopy prior to hydrocarbon extraction. Samples were stained with acridine orange, a fluorescent stain for nucleic acids, to highlight bacteria.

Hydrocarbons in aggregates were extracted with methylene chloride in an ultrasonic bath and concentrated by evaporation and gravimetric quantification to determine the ratio of hydrocarbon weight to aggregate weight.

Oil content in the sediment. Each aquarium was divided into three areas (top, middle, and bottom); each area was then divided into a 10 × 14 sample grid. For random sampling three samples of sediment were taken from each aquarium and mixed to obtain a representative mixture of the sediment. Following the addition of internal standards, the mixtures were extracted with methylene chloride using a soxhlet, and concentrated by evaporation for gravimetric analysis.

Changes in oil composition from the sediment and the aggregates were determined by gas chromatography (GC) following sample extraction with methylene chloride, concentration by evaporation, solvent exchanged into hexane and separation into aliphatic and aromatic fractions on an alumina-silica gel column.

Results

Bacterial numbers. Figure 3 summarizes the differences in bacterial numbers measured in the experiment. Differences between treatments were significant: an increase in bacterial numbers was correlated to the addition of clay particles (C1>C0).

Hydrocarbon concentrations in the water column. Figure 4 and Table 1 summarize the differences in TPH between the two treatments at each sampling time. There was no significant difference between treatments due to high levels of variability caused by the presence of suspended particulate oil in the water column. Therefore, it was difficult to show a generalized difference in the analytical results.

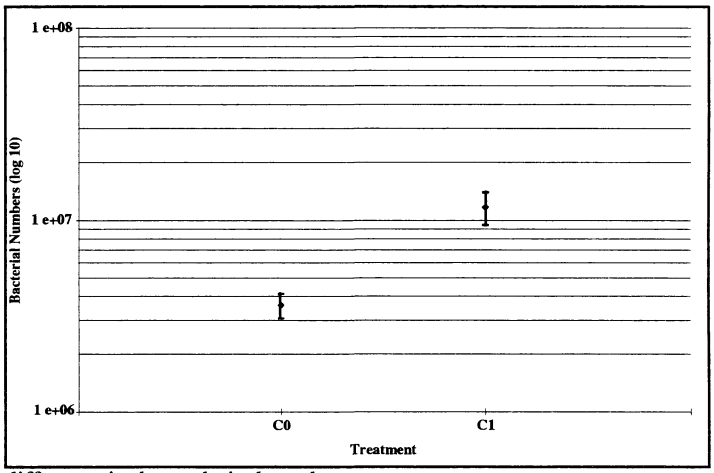

Figure 3. Bacterial numbers (log 10) for C0 and C1 treatment (number/g sediment) at the end of the experiment

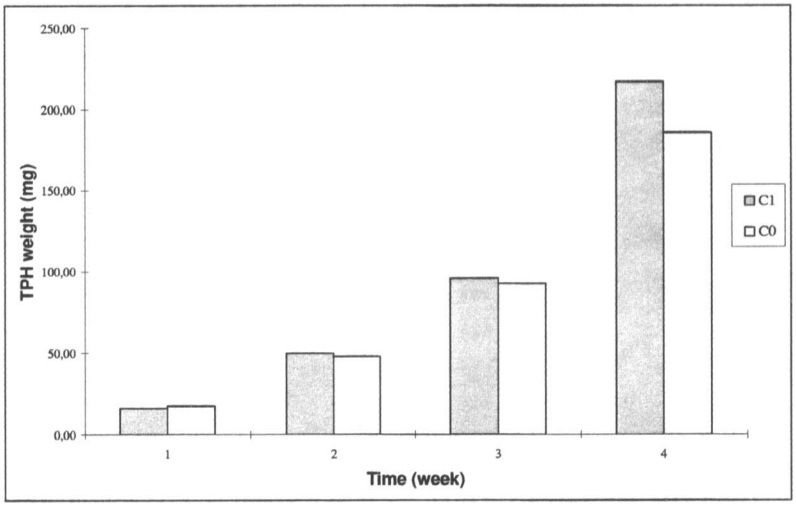

Figure 4. TPH weight cumulative (g) extracted from the water column during the 4 weeks.

Table 1. TPH Weight cumulative (g) extracted from the water column.

	Week 1	Week 2	Week 3	Week 4
C1	16.25 (±0.40)	49.87 (±6.83)	96.08 (±11.59)	217.11 (±46.46)
C0	17.71 (±10.93)	48.10 (±15.71)	92.78 (±38.06)	185.62 (±72.62)

Aggregates in the water column–quantitative analyses. There was a significant difference between the treatments with clay and without clay with respect to the weight of aggregates in the water column: weight of aggregates collected for the C0 treatment was clearly lower than weight of aggregates for the C1 treatment due to the addition of clay (2*5 g) (Figure 5). The concentration of oil extracted from these aggregates in each mesocosm was approximately the same (TPH weight/aggregates weight = 0.15). Thus, the addition of clay tended to enhance the rate of oil removal from the sand to the water column.

Qualitative analyses. Observations of aggregates made at the end of the experiment by phase contrast microscopy showed more compact aggregates and larger oil droplets for the C0 treatment than for the C1 treatment. The addition of particles of mineral fines increased the surface/volume ratio of the oil, making it more accessible to bacterial attack and oxygenation. This observation is in good agreement with previous studies (Weise *et al.*, 1997).

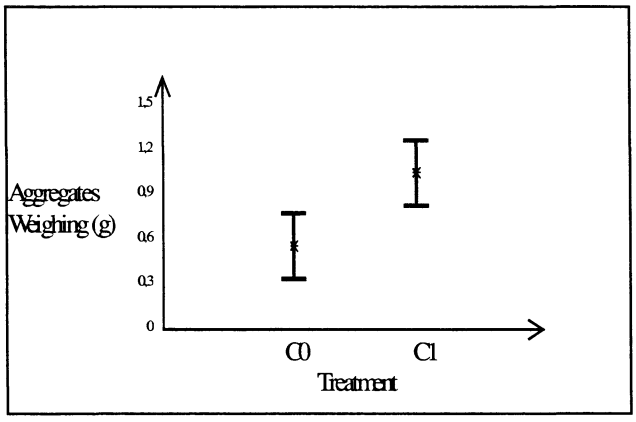

Figure 5. Aggregates weighing (g) for each treatment (error bars represent 95% confidence limit).

Acridine orange stained observation of aggregates with UV epifluorescence microscopy highlighted the differences in the size and composition of the aggregates. For the C1 treatment, droplets of oil were approximately 25 µm in diameter surrounded by an organic/inorganic matrix composed of clay and bacteria; for the C0 treatment without mineral fines, the aggregates had larger oil droplets (80 µm) associated with an organic matrix. These observations correspond to the "droplet aggregates" previously described (Lee *et al.*, 1998).

Hydrocarbon chemistry-analyses of oil in the sediment. Results showed a greater degree of n-alkane biodegradation in mesocosms containing clay particles. This is illustrated by Figure 6, comparing the oil composition (saturated fraction analysed by GC) of the original oil (week 0) with the oil extracted from the treated sediments. A similar observation was also made from the *n*-C17/pristane ratio data (Figure 7).

Analyses of oil in the aggregates. In oil extracted from the aggregates, the degree of biodegradation as measured by the n C17/pristane ratios was greater for the treatment with clay (Figure 8).

For each treatment (C0 or C1), oil extracted from the aggregates (collected at each water change) was more biodegraded than oil in sediment. Figure 9 shows the comparisons of the oil composition (saturated fraction analysed by GC) for the original oil (week 0), for the oil extracted from a sediment sample of the C1 treatment at week 4, and for the oil extracted from the aggregates collected from the C1 treatment at week 4. Figure 10 shows the comparison of the degree of n-alkane biodegradation for oil extracted from the sediment and the aggregates after 4 weeks.

Discussion

This experiment was designed to determine whether the presence of clay particles associated with the presence of active microorganisms could help in the natural restoration of a coastal site.

Despite the fact that pristane has been shown in the literature to be an unreliable biomarker, its use in this study was sufficient to enable comparison of the extent of biodegradation between treatments with and without clay.

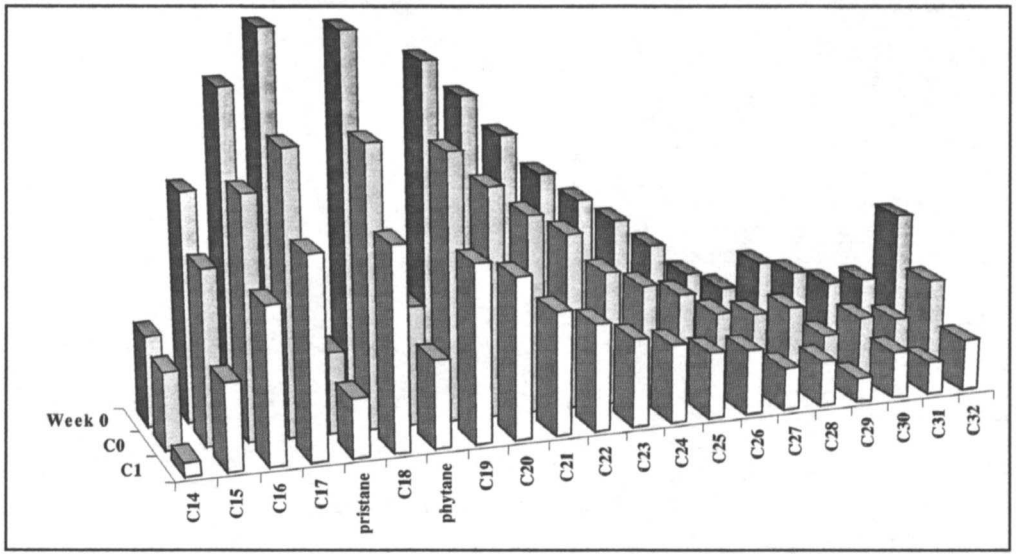

Figure 6. Comparison of chromatograms from oil extracted from original oil (week 0), with C0 and C1 treatments after 4 weeks.

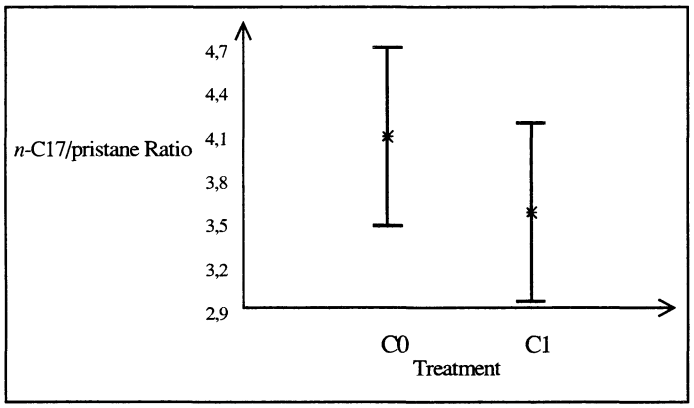

Figure 7. Oil degradation in the sediment (error bars represent 95% confidence limit).

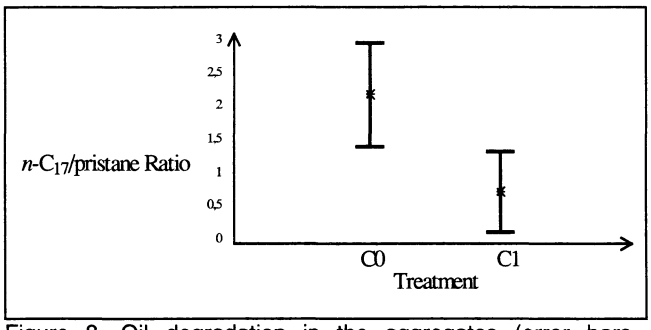

Figure 8. Oil degradation in the aggregates (error bars represent 95% confidence limit; at 70% confidence limit differences became significant between treatments).

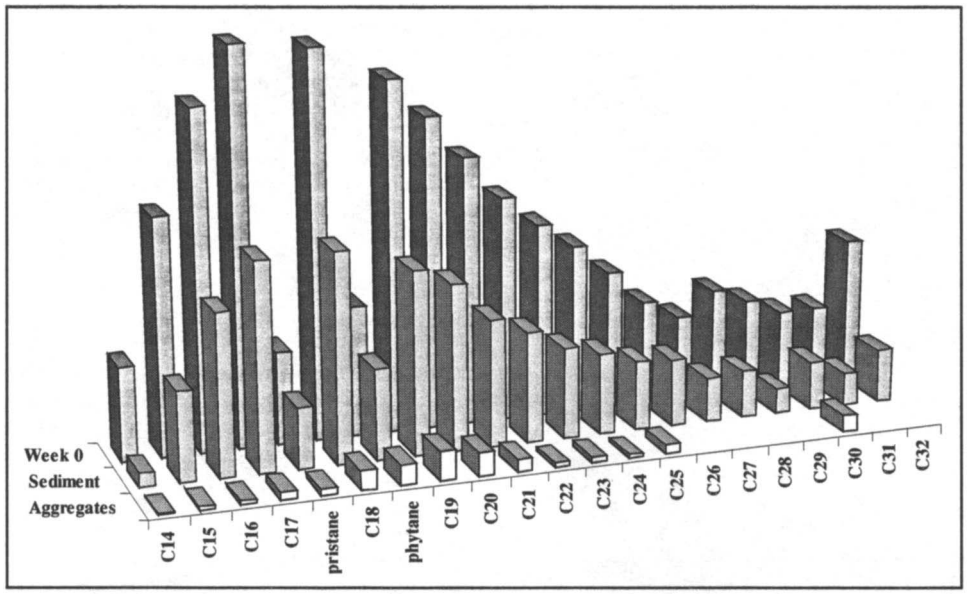

Figure 9. Comparison of chromatograms from oil extracted from the original oil (week 0), from the sediment of a C1 treatment after 4 weeks, and from the aggregates of a C1 treatment after 4 weeks.

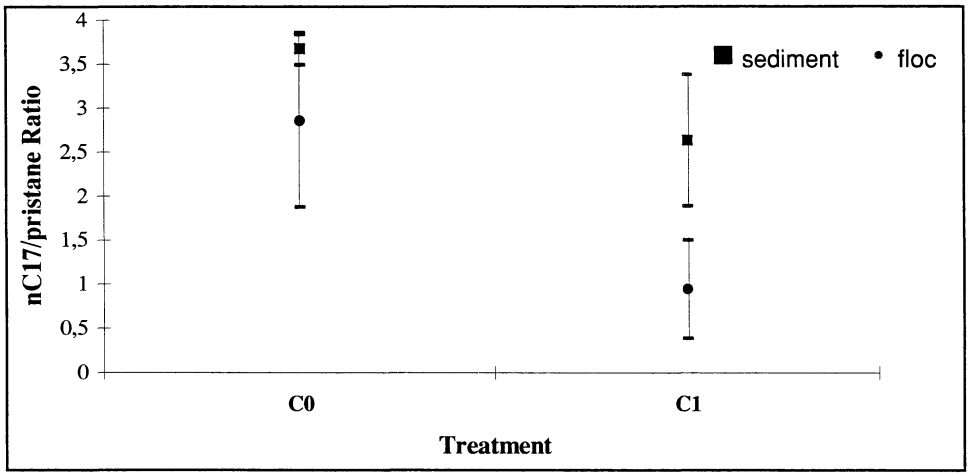

Figure 10. *n*-C17/pristane ratio of oil in the sediment and the aggregates after 4 weeks.

The GC analyses conducted on the oil extracted from the sediment could highlight a significant effect of clay addition in hydrocarbon biodegradation. The oil was more degraded in the mesocosms amended with clay (C1). This was interpreted to mean that clay particles made the oil more bioavailable to the microflora. Concerning the transfer of oil from the sediment to the water column, more oil was found in the water of the mesocosm amended with clay particles, and observations by microscopy suggested this oil was mainly associated with the clay particles. From these findings, there is some evidence that the presence of clay particles enhanced the oil transfer from the sediment to the water column. However, under the experimental conditions of the study, no significant difference in the concentration of oil within the sediment was observed in the test mesocosm with and without clay amendment. This may be due to the short duration of the experiment and/or the relatively high hydrocarbon concentration in the sediment compared to the relatively low amount of oil in the water associated with aggregates.

Bacterial numbers determined by plate count on Marine Agar were slightly higher in the mesocosms amended with clay additions. However, observations by UV epifluorescence microscopy of the aggregates did not show any clear differences in the distribution of bacteria among the oil droplets and mineral fines between the treatments, despite the fact that chemical analyses of aggregates showed a significant effect of clay particles: n-C17/pristane ratio was lower for the C1 treatment than for the C0 treatment as in the sediment. The addition of mineral particles increased the surface/volume ratio of the oil, making it more bioavailable to bacterial attack and oxygenation (Weise *et al.*, 1997).

A significant difference in the extent of oil degradation was observed between the suspended aggregate and sediment samples. For the C1 treatment, oil transferred from the sand to the water column was more degraded than the residual oil remaining in the sediment. This observation is consistent with that of field operations using surf-washing to accelerate dispersion of oil stranded within sediments (Lee *et al.*, 1997).

This experiment showed the positive influence of clay on the transfer of the oil between the sediment and the water column. However, the experimental design was limited by scale and duration to quantify the amount of oil which was transferred.

In terms of oil spill cleanup, the operational benefit that can be expected from the interaction of mineral fines and residual oil still needs to be determined.

Conclusion

This mesocosm experiment conducted in a simulated coastal environment confirmed that fine mineral particles can contribute to the natural restoration process by enhancing the physical removal rates of oil from the sediment and its subsequent biodegradation rates.

There is now a need to understand the actual role of oil-mineral fine particle interaction in habitat restoration following oil spills. The operational benefit that could result from this process still needs to be quantified. Answers to these questions may lead to the development of new cleanup options response.

References

1. Bragg, J.R., and E.H, Owens, 1995, "Clay-Oil Flocculation as a Natural Cleansing Process Following Oil Spills" Parts 1 and 2, in *Proceedings of the 1995 International Oil Spill Conference,* American Petroleum Institute, Washington, D.C.
2. Jézéquel, R., S. Le Floch, F. X. Merlin, J. Drewes, and K. Lee, 1998, "The influence of microorganisms on oil-mineral fine interactions in low energy coastal environments: Preliminary results." in *Proceedings of the 21st Arctic and Marine Oilspill Program (AMOP) Technical Seminar*, Environment Canada, Ottawa, Ontario, pp. 957–962.
3. Lee, K., T. Lunel, P. Wood, R. Swannel, and P. Stoffyn-Egli, 1997, "Shoreline Cleanup by Acceleration of Clay-Oil Flocculation Processes," in *Proceedings of the 1997 International Oil Spill Conference*, American Petroleum Institute, Washington, D.C., Publication No. 4651, pp. 235–240.
4. Lee, K., and P. Stoffyn-Egli, 1998, "Formation and Structure of Oil-Mineral Aggregates in Coastal Environments," in *Proceedings of the 21st Arctic and Marine Oilspill Program (AMOP) Technical Seminar*, Environment Canada, Ottawa, Ontario, pp. 911–921.
5. Weise, A., and K. Lee, 1997, "The effect of clay-oil flocculation on natural oil biodegradation," in *Proceedings of the 1997 International Oil Spill Conference*, American Petroleum Institute, Washington D.C. pp. 955–956.