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EFFECT OF DISPERSANTS ON OIL BIODEGRADATION UNDER SIMULATED MARINE CONDITIONS

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ABSTRACT*: A study was undertaken on the dispersion, microbial colonisation and biodegradation of chemically-dispersed weathered Forties crude oil under simulated marine conditions in laboratory microcosms. The measurements of droplet size, number and microbial colonisation were made using new techniques developed by the project team. Rapid growth of indigenous micro-organisms capable of degrading both crude oil and dispersants was observed in the presence of chemically-dispersed oil. These organisms colonised the dispersed oil and biodegraded the aliphatic and aromatic hydrocabons. These processes was stimulated by the addition of inorganic nutrients. Some colonised droplets agglomerated into neutrally-buoyant "clusters" (100 µm- 2 mm diameter) consisting of oil, bacteria, protozoa, and nematodes. After substantial hydrocarbon biodegradation these clusters sank to the bottom of the microcosms. No biodegradation or cluster formation was noted in "killed" controls in which biological activity had been inhibited. Different dispersants promoted microbial growth to differing extents. These results suggest that the addition of dispersants can increase the rate of oil biodegradation under natural conditions by promoting the growth of indigenous hydrocarbon-degrading bacteria, as well as increasing the surface area of oil available for microbial colonisation.*

Introduction

The study of the interaction of small oil droplets (1-10 µm droplets) with micro-organisms under simulated marine conditions has been limited by the lack of techniques to monitor droplet size and microbial colonisation without unduly disrupting the sample. Techniques used previously include passage of droplets at high flow rate through small apertures (using a Coulter Counter), which may cause droplet shear, or by allowing oil droplets to float to the surface in an osmotic gradient, which may encourage coagulation and fail to measure small droplets which may be maintained in solution by Brownian motion (Delvigne and Sweeney, 1988; Krigsvoll et al., 1991; Varadaraj et al., 1995). The presence of micro-organisms and other contaminants in the seawater interferes with the accuracy of a Malvern particle analyser. Therefore, we have developed a new technique derived from the work of Delvigne & Sweeney (1988), which reduces these difficulties and allows microbial colonisation of small droplets to be determined (Swannell et al., 1997). Using this technique and others, we have found that increased oil dispersion in marine microcosms occurred when the hydrocarbon-degrading population increased sufficiently to stabilise the oil droplets dispersed by physical processes (i.e dispersion promoted by a tangential air flow over the slick and Brownian motion), a process that was stimulated by the addition of inorganic nutrients (Swannell et al., 1997). As the production of extracellular biosurfactants was not detected, the stabilisation of the physically-dispersed oil droplets was attributed to a combination of the bacteria providing a physical barrier preventing the re-coalescence of the droplets, and an alteration of oil droplet buoyancy. Microscopic examination of the dispersed droplets showed many were colonised by bacteria, and that with time these colonised droplets agglomerated into large neutrally-buoyant "clusters" of oil, bacteria and other micro-organisms. Substantial biodegradation of the oil dispersed in the water column was observed in the biologically-active microcosms after 28 days, a process that was, as expected, stimulated by the addition of high levels of inorganic nutrients (Atlas and Bartha, 1972).

Research on the effect of dispersants on the fate of dispersed oil has often been conflicting, with some workers proposing that dispersants had little effect on oil biodegradation, some suggesting a positive effect and others noting a negative effect (Varadaraj et al., 1995). It has been suggested that dispersants tend to increase oil biodegradation by increasing the surface area for microbial attack, and encouraging migration of the droplets through the water column making oxygen and nutrients more readily available (Mulyono et al., 1994). However, some dispersants were also found to have a toxic effect on microbial processes retarding the rate of oil decomposition (Mulyono et al., 1994; Varadaraj et al., 1995). Varadaraj et al. (1995) studied the capability of dispersants for increasing the surface area of dispersed oil and affecting the growth of hydrocarbon-degraders, and concluded that both of these factors influenced the effectiveness of a dispersant. They found that increasing the sorbitan content of their dispersant stimulated oil biodegradation by acting as a nutrient source for the growth of hydrocarbon-degraders. Varadaraj et al. (1995) conducted their work in 300 ml bottles shaken in a orbital shaker in order to maintain constant experimental conditions and allow replication. This paper discusses research conducted in natural seawater at 15 litre scale in a Mackay apparatus designed to be more representative of marine conditions (particularly in terms of nutrient content and temperature). We studied the effect of four dispersants that were used operationally during the Sea Empress incident (MPCU, 1996), in order to clarify their effect on oil dispersion, growth of indigenous hydrocarbon-degraders and oil biodegradation, and considered the implications of these findings for the operational use of dispersants at sea.

Materials and methods

Model Design and Construction. The design of the marine microcosms was based on the apparatus of MacKay and Szeto (1980), which was built to simulate oil dispersal at sea (Figure 1). Three microcosms were built and held under identical conditions. Each microcosm was filled with 15 litres of natural sea water (obtained from near the Eddystone Lighthouse, UK). The water in the microcosms was maintained at 15ºC, a temperature representative of summer marine conditions around the UK, and kept in the dark to prevent the growth of photosynthetic micro-organisms.

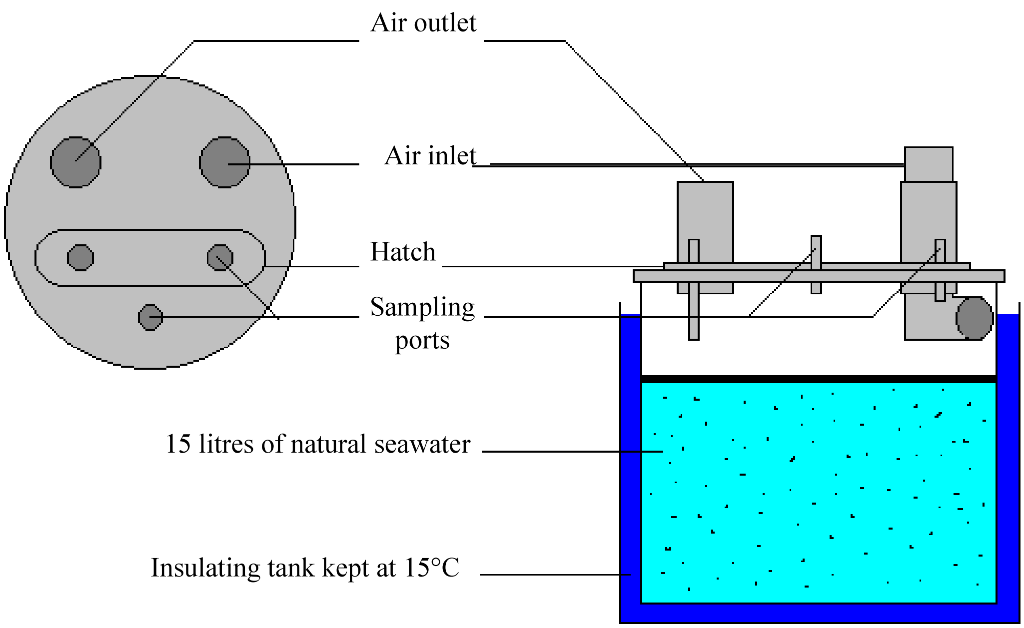


Figure 1. Schematic diagram of the completed experimental apparatus.

An oil slick of weathered Forties crude oil (4 ml) was chemically dispersed with Corexit 9500, Enersperse 1583, Finasol OSR-51 or Slickgone LTSW (10 % w/w of the oil weight). The simulated oil weathering was performed by distillation of crude oil at 250°C, a process which removed approximately 25% by weight (Walker *et al.*, 1992). Volatile hydrocarbons have been shown to be toxic to bacteria and hence may inhibit oil biodegradation (Floodgate, 1984). Under natural conditions, these compounds tend to evaporate rapidly or dissolve and disperse away from the slick, but in the MacKay apparatus the soluble hydrocarbons will be unable to disperse, potentially adversely affecting the results.

In order to obtain an homogeneous addition of the dispersant to the oil slick, oil was retained by a PTFE ring (diameter: 50 mm) on the water surface. The dispersant was added evenly over the surface of the oil. The air flow was switched on, the ring was removed and the dispersant/oil mixture was allowed to spread over the surface of the seawater in the microcosm. Oil dispersal was encouraged by pumping air through the tangential inlet (Figure 1) at a velocity of 5.0 ± 0.5 m/s. This created a gyration of the liquid in the microcosms, keeping most free oil away from the walls, as well as simulating physical dispersion caused by wind and wave action.

For each dispersant, 3 microcosms were treated as follows:

1. seawater containing oil, dispersant and low levels of nutrients (1 mg N-NO_3/litre) reproducibly simulating estuarine and coastal water conditions in the UK. The level of nutrients in the natural seawater was influenced by season and the carriage time to the laboratory, and therefore additional $NaNO_3$ was added if necessary to obtain a constant starting concentration.

2. seawater amended with oil, dispersant and high levels of nutrients containing sufficient nutrients (10 mg/litre N as $NaNO_3$ and 1 mg/litre P as KH_2PO_4) to encourage rapid

biodegradation of all the added oil (Bartha and Atlas, 1987). This microcosms was as a "Positive Control", studying the effect of dispersants under conditions more conducive to the microbial biodegradation of oil. However, concentrations of 10 mg N/litre have been found in estuarine waters in the UK.

3. a "Killed Control" containing seawater amended with oil, dispersant and low levels of nutrients in which the microbiological activity was completely inhibited by the addition of mercuric chloride (300 mg/l: Russel *et al.*, 1992).

In addition, an experiment was conducted as above without dispersant application, to study the relationship between physical disperion, microbial growth, droplet colonsiation and oil biodegradation. The concentration of nitrate was determined using a UV spectrophotometric method described by Greenberg *et al.* (1992). The samples (10 ml) were collected and analysed immediately or stored at -20°C prior to analysis.

Oil droplet analysis

A new method (termed the "Chamber Slide Method") was developed from that of Delvigne and Sweeney (1988). Technique modifications were designed to eliminate the effect of osmotic pressure and the long settling times of the earlier technique, which could influence droplet distribution (Swannell *et al.*, 1997). Clusters were counted and measured using a calibrated binocular microscope.

Microbial biomass and activity measurements

The total number of hydrocarbon-degraders was estimated at various times in the experiments using the Most Probable Number technique described by Croft *et al.* (1995), derived from the "Sheen Screen" method described by Brown and Braddock (1990) and modified by Venosa *et al.* (1993), using weathered Forties crude oil as the carbon source. Simultaneously, the population of dispersant-degraders was estimated by the same method using the dispersant as the carbon source, at a similar concentration as provided in the microcosms. The samples (3 ml) were removed aseptically 1 cm below the surface of the oil slick and were processed within 16 hours of removal from the marine microcosms. The MPN plates were incubated at 15°C and growth was determined after 15 days.

The success of the Killed Control at inhibiting the microbial growth was assessed by inoculating agar plates containing solidified Marine Broth (Difco), at the same time as samples were taken for MPN determinations. The plates were incubated at 15°C for 15 days and the development of any colonies was recorded.

Oil residue analysis

Samples of seawater (300 ml) and sedimented "clusters" of oil and bacteria were taken from each microcosm. Seawater samples were taken at 10 cm below the water surface. The samples were taken into clean glass jars and stored at - 20ºC prior to analysis by gas chromatography with mass spectrometry detection (GC/MS) in accordance with a method described by Swannell *et al.* (1997).

Results And Discussion

The addition of dispersants greatly increased the level of oil dispersion in our experimental apparatus, encouraging the rapid formation of a much higher number of small droplets than was noted in microcosms subjected to physical forces only

Table 1. Influence of dispersants on the indigenous microbial population and on the number of oil droplets. LN= Low nutrients, HN= High nutrients, SD= unbaised standard deviation of the mean, CL= Confidence Limits. *maximum measurable droplet number using the chamber slide technique.

Treatment	Mean Maximum Growth Rate of HC-Degraders (10^3MPN/ml/day)		Onset of Maximum Growth Rate (Days)		Mean Maximum Population of HC-degraders (10^3MPN/ml ± SD)		Mean Maximum Population of Dispersant-degraders (10^3MPN/ml ± SD)		Maximum Oil Droplet Concentration (10^3Droplets/ml ±95%CL)	
	LN	HN	LN	HN	LN	HN	LN	HN	LN	HN
Physical Dispersion	3.4	137.0	21	1	30.5 (±22.4)	993 (±630)	N/A	N/A	89.6 (± 9.0)	354.0 (± 35.0)
Corexit 9500	109.0	978.0	2	3	233.0 (±100)	2330 (±111)	530.0 (±495.0)	600.0 (±520.0)	5040*	4260 (±426.0)
Enersperse 1583	25.2	48.2	13	9	85.3 (±28.6)	287.0 (±40.4)	66.7 (±21.9)	413.0 (±111.0)	5040*	5040*
Finasol OSR-51	112.0	614.0	6	6	513.0 (±508)	2026 (±254)	208.0 (±148.3)	1160 (±911.0)	738.0 (± 73.9)	1790 (±179.0)
Slickgone LTSW	92.5	443.0	17	17	657.0 (±287)	4600 (±399)	603.0 (±290.)	4770 (±1110)	2300 (±230)	1400 (±140.0)

Differences in oil dispersion patterns between the dispersants were noted under the conditions of the experiment, however in all cases the number of oil droplets decreased dramatically in the biologically-active microcosms in 28 days, whereas the number of small droplets remained approximately the same or increased in the “Killed Controls” (for examples see Figures 2 & 3).

(A)

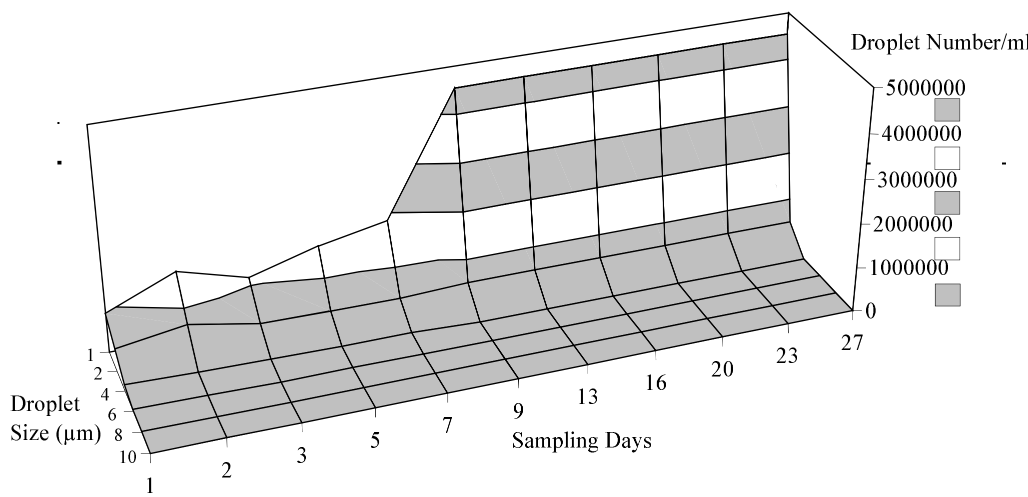
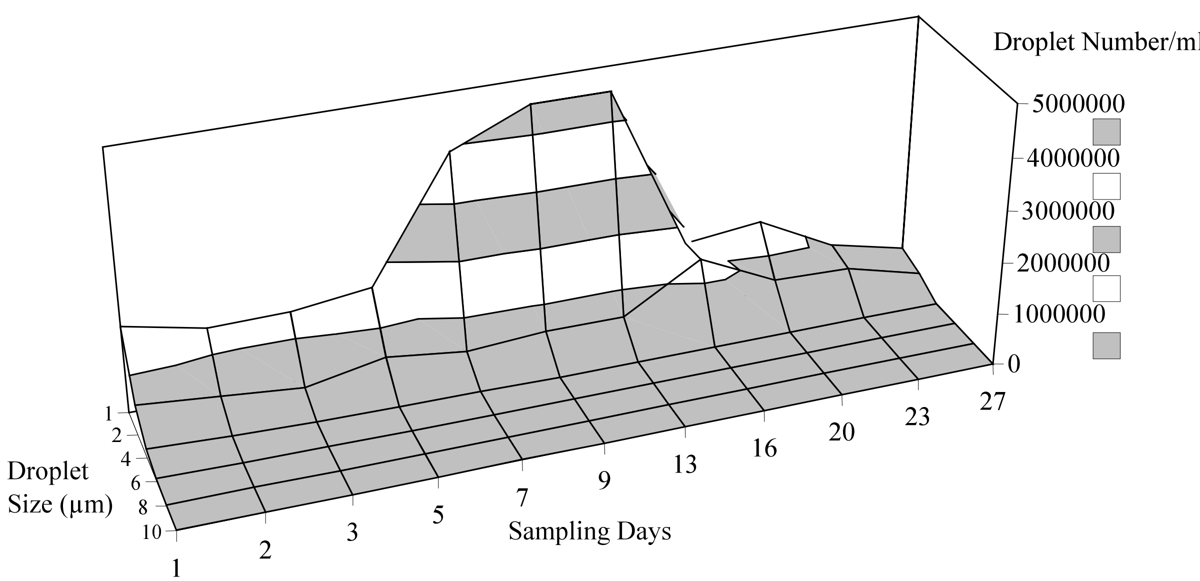


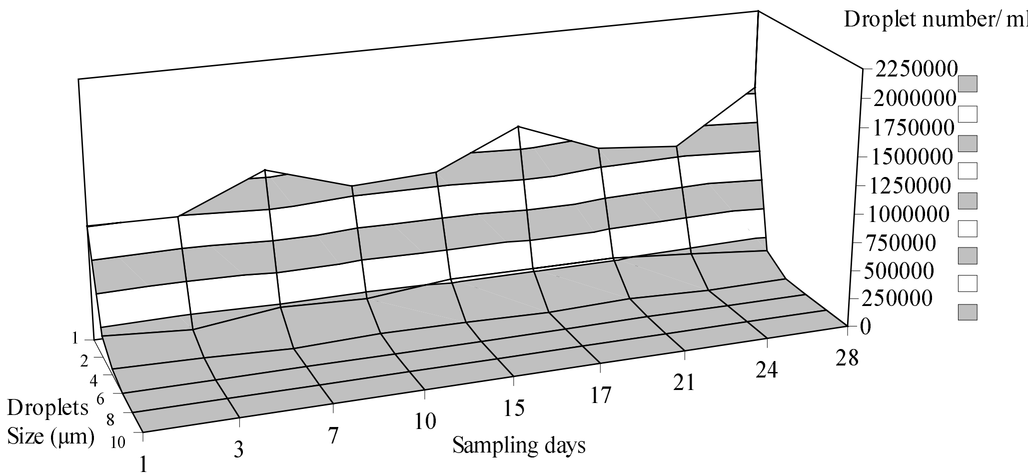
Figure 2. Oil droplet distribution in the Killed Control (A) and in the seawater amended with low levels of nutrients (B),

(B)



both treated with Corexit 9500.

(A)



(B)

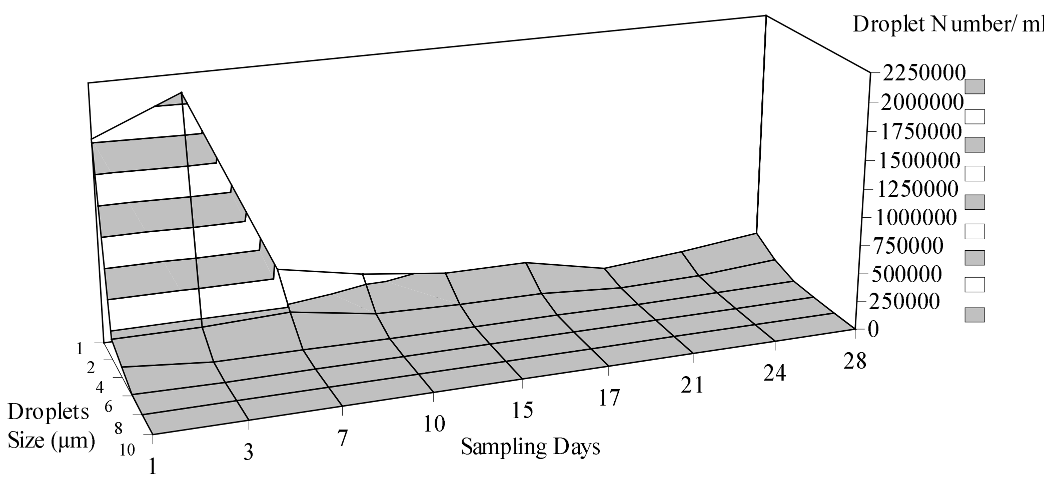


Figure 3.Oil droplet distribution in the Killed Control (A) and in the seawater amended with low levels of nutrients (B), both treated with Slickgone LTSW.

In the biologically-active microcosms amended with low levels of nutrients, the number of oil droplets started to decrease after 3 to 13 days depending on the dispersant (Figure 2 & 3). This probably reflected both biodegradation of oil droplets and the formation of clusters of oil droplets and bacteria too large (<100 µm) to be observable using the Chamber Slide Method. Droplet colonisation was noted between 1–3 days after dispersant application, whereas no colonisation was seen in the “Killed Controls”. Rapid growth of hydrocarbon-degrading and dispersant-degrading bacteria were noted in all biologically-active microcosms (Table 1), although no growth was seen in the "Killed Controls". Not all the droplets were colonised with bacteria initially as is apparent from Table 1, however clusters of oil droplets, bacteria, protozoa and nematodes were noted between 3–4 days after dispersant addition (Figure 4), and were found to vary in size from 10 µm to 2 mm. The concentration of clusters decreased towards the end of the experiment, presumably as a result of biodegradation of oil and sedimentation (Figure 4).

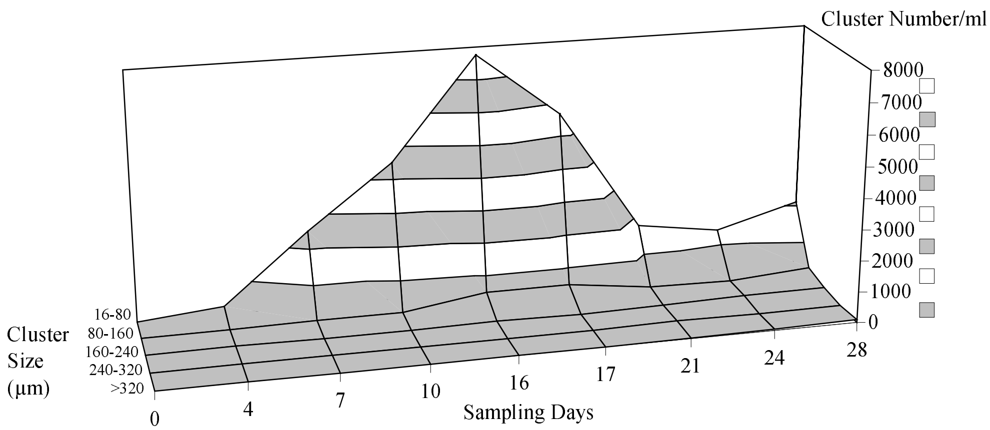


Figure 4. Concentration of clusters noted in the microcosms treated with Dasic Slickgone LTSW.

The clusters consisted of micro-organisms, oil droplets, protozoa and occasionally nematodes. No reduction in oil droplet concentration was noted in the Killed Controls, and no clusters were formed, confirming that the reduction in droplet concentration was microbiologically- mediated.

The dispersants dramatically stimulated the growth of the indigenous hydrocarbon-degrading populations in comparison to that observed when oil was dispersed by physical processes. The maximum growth rate observed with dispersants was at least 7 times and up to 33 times higher in the microcosms amended with low levels of nutrients, than was noted in the absence of dispersant (Table 1). Dispersants encouraging the formation of the largest numbers of small droplets in the microcosms did not necessarily promote the most rapid growth of hydrocarbon-degraders. For example, in the presence of a low level of nutrients, Finasol OSR-51 encouraged a higher maximum growth rate than Enersperse 1583, even though Enersperse produced higher maximum oil droplet concentration than Finasol OSR-51 (Table 1). The addition of dispersants therefore stimulated the growth of hydrocarbon-degraders by supplying extra nutrients, as well as by increasing the surface area available for microbial attack. Furthermore, the tested dispersants all supported the growth of indigenous seawater bacteria, confirming that bacteria could utilise the nutrients available within the dispersants even at the low concentrations used in these experiments. Thus, at the low nutrient concentrations found in many UK estuarine and coastal waters, dispersants stimulated the growth of indigenous hydrocarbon-degrading bacteria and maintained a higher maximum hydrocarbon-degrading population than was noted in the absence of dispersants (Table 1).

The effect of higher levels of inorganic nutrients (in the "Positive Controls") on the hydrocarbon-degrading bacteria was to promote a higher rate of growth and to maintain higher microbial populations, as expected (Table 1). However, the effect of nutrients on the stimulation of the growth of the microbial population was less profound in the presence of dispersants than in their absence. This observation suggests that the hydrocarbon-degrading micro-organisms were obtaining some of their nutritional requirements from the added dispersants.

The pattern of the growth of bacteria was slightly different depending on the dispersant. Corexit and Finasol encouraged a normal sigmoidal growth curve, as indicated by the maximal growth rate occurring shortly after the start of the experiment (Table 1). In contrast, for Enersperse and Slickgone a diauxic (biphasic) growth pattern was seen, with an initial phase of growth followed by a temporary slow down and then a second phase of more rapid growth. As such the maximum growth rate was recorded later in the experiment (Table 1). Diauxic growth often reflects the biodegradation of different subsrates by bacteria, and in this context, may reflect the decomposition of a component or components of the dispersant, which then facilitates additional growth.

The oil droplets in the water phase of the biologically-active microcosms treated with dispersant showed evidence of biodegradation in comparison to the "Killed Control" after 28 days (Figure 5).

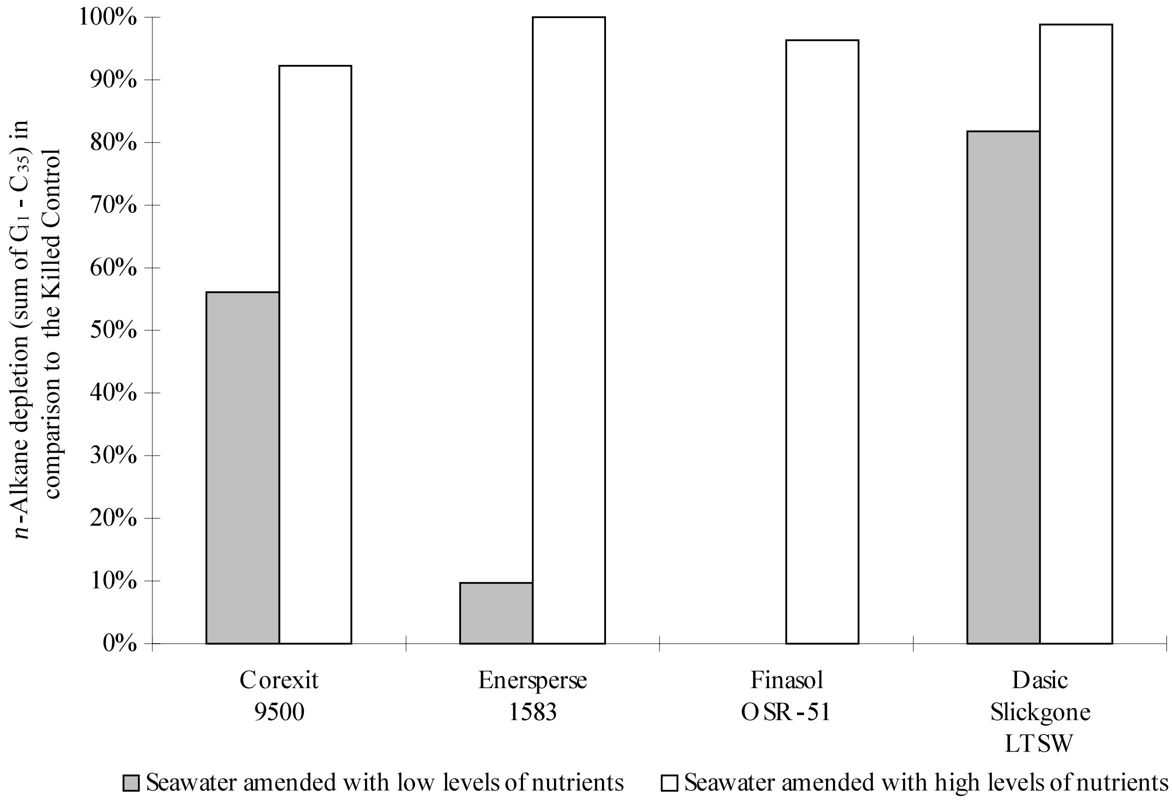


Figure 5. Degradation of *n*-alkanes in the biologically-active microcosms treated with dispersants after 28 days. NM= not measured as ample was contaminated.

For example with Corexit 9500, the *n*-alkanes (Figure 6(A)) and selected aromatics (Figure 7(A)) in the water phase were substantially depleted. In the sedimented material, which consisted of clusters of oil droplets and bacteria that had sunk over time, the oil content was more depleted than in the water phase \

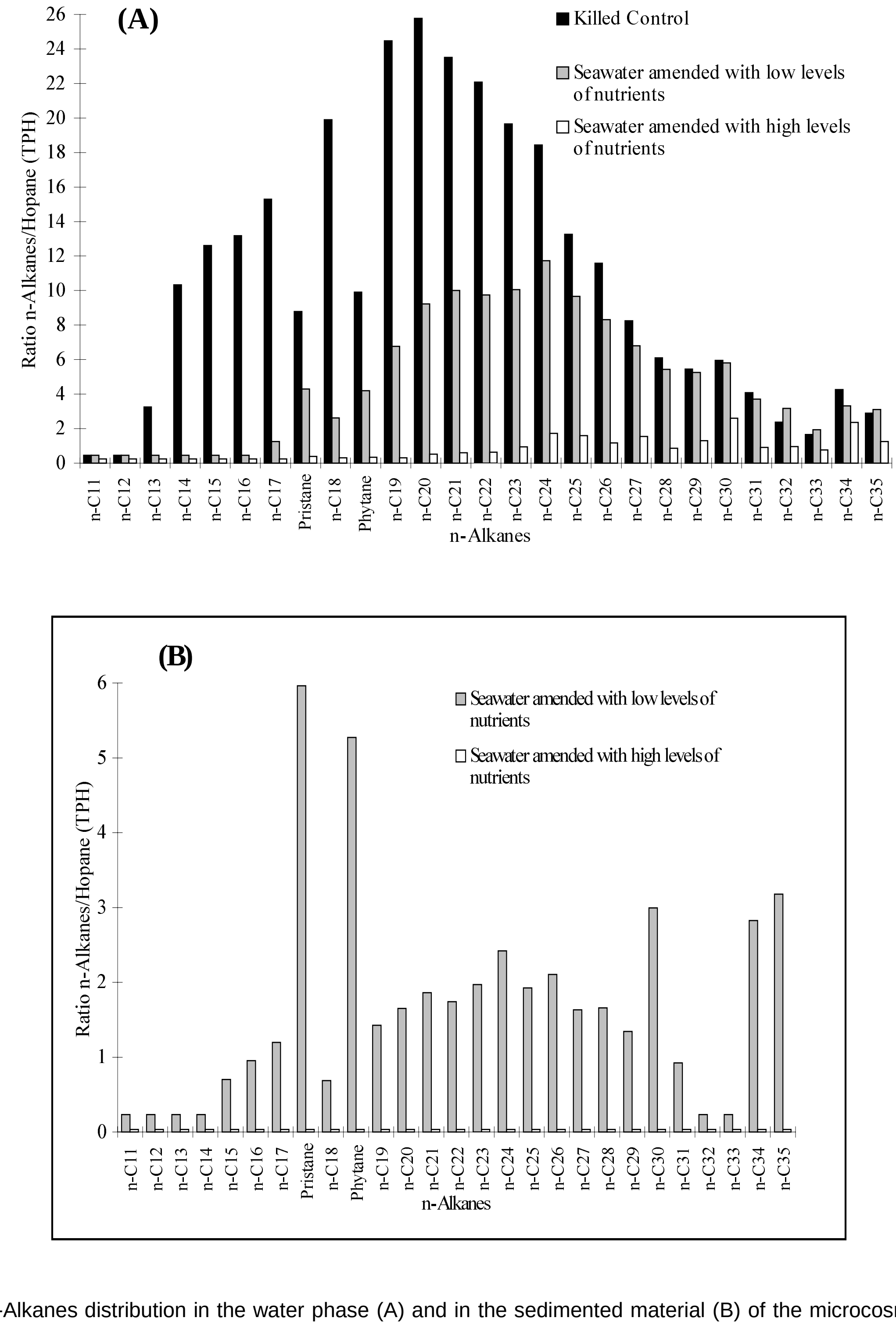


Figure 6.*n*-Alkanes distribution in the water phase (A) and in the sedimented material (B) of the microcosms treated with Corexit 9500.

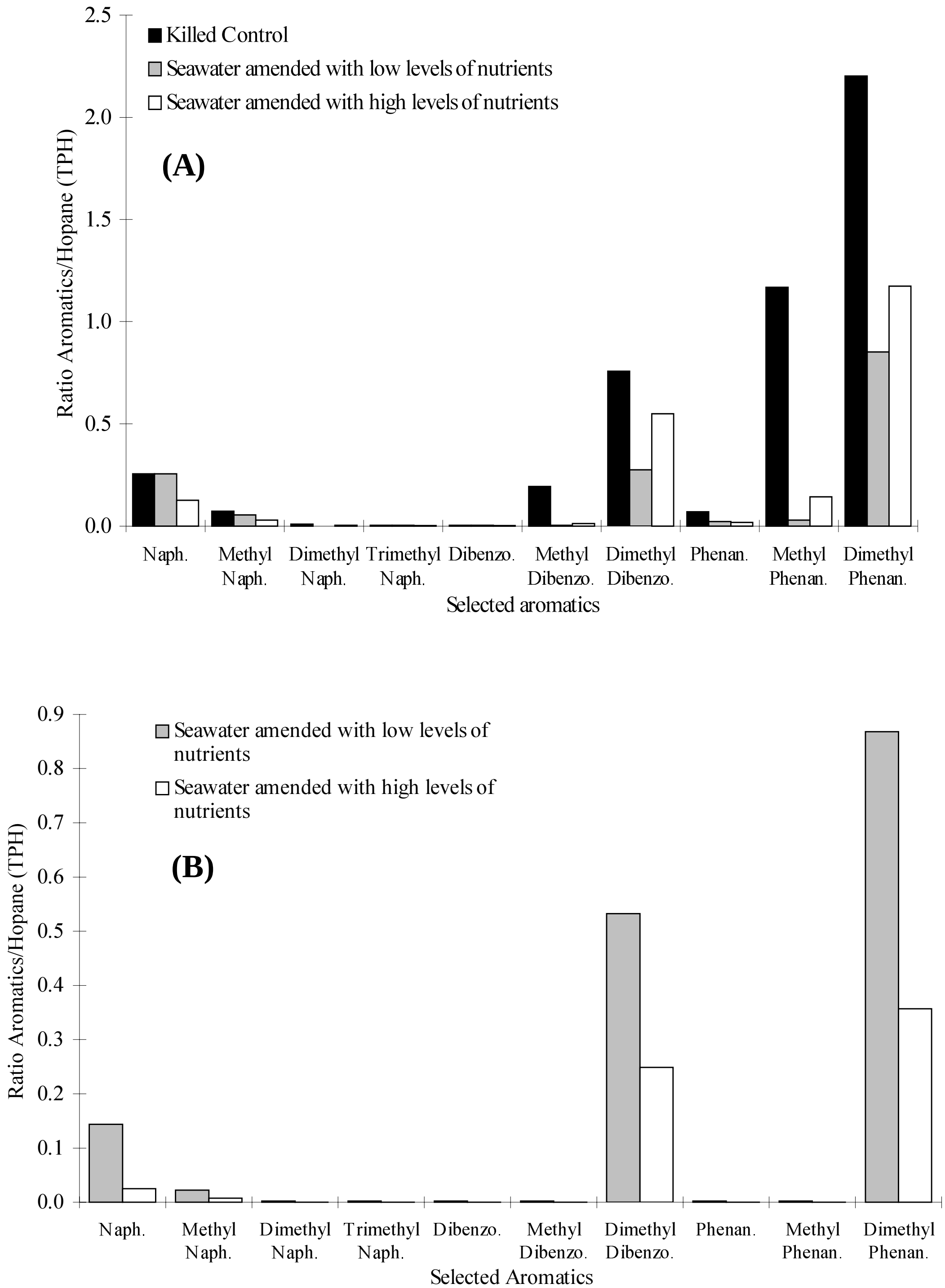


Figure 7.Selected distribution of aromatics in the water phase (A) and in the sedimented material (B) of the microcosms treated with Corexit 9500.

The oil residue analysis data suggested that increased levels of inorganic nutrients induced a more extensive biodegradation of the oil (Figure 5) present in clusters or as single droplets in the water phase (as expected, see Leahy and Colwell, 1990; Prince, 1993; Swannell *et al.*, 1996). In previous work we have also shown that non-dispersed oil was barely degraded emphasising the importance of dispersion in hydrocarbon biodegradation at sea (Swannell *et al.*, 1997).

Conclusions

The application of the four tested dispersants to an oil slick in mesocosms stimulated oil dispersion and oil biodegradation even at the low nutrient concentrations characteristic of UK estuarine and coastal waters. However, there were some differences in the success of the dispersant in terms of, for example, its ability to promote the growth of indigenous hydrocarbon-degrading micro-organisms. Other workers who have found that dispersants support microbial growth (Lee *et al.*, 1985; Mulkins-Phillips and Stewart, 1974). Moreover, Varadaraj *et al.* (1995) noted that oil biodegradation was stimulated by sorbitan surfactants which supplied nutrients to the micro-organisms. Therefore, as suggested by Varadaraj *et al.* (1995), dispersant application may have the dual environmental benefit of promoting oil dispersion and oil biodegradation within the marine water column even at low nutrient concentrations. This latter benefit is of particular importance as most seas and oceans normally have a low nutrient content. The positive effect of the tested dispersants on oil biodegradation seems to be related to their ability to promote the growth of indigenous hydrocarbon-degrading bacteria, as well as their ability to promote the formation of small droplets. The naturally-occurring micro-organisms colonised the dispersed droplets and caused many to agglomerate into neutrally-buoyant clusters, which became small micro-habitats where bacteria were biodegrading oil and where they were decomposed by protozoal and nematode predators. These clusters apparently remained suspended in the water column of the microcosms until much of the biodegradable residues of oil had been decomposed (and therefore the buoyancy reduced), before sinking to the bottom of the microcosms. Such findings have important implications for the understanding of the fate of oil in the marine environment, and to devising responses to oil spill incidents which result in a net environmental benefit. Should these findings be verified in the field, it seems that the application of certain dispersants may encourage the dispersion and the biodegradation of oil at sea. Microbial colonisation of dispersed oil droplets and agglomeration into clusters may also help reduce the mass of oil impacting the sea bed.

Biography

Richard Swannell is a Senior Environmental Microbiologist who has been studying the biodegradation of oil in marine environments for the last 5 years. He has responded to oil spills in the UK and the Middle East and acted as a Technical Consultant to the shoreline clean up conducted after the *Sea Empress* incident.

References

1. Atlas, R.M. & Bartha, R. (1972) Degradation and mineralization of petroleum in seawater: Limitation by nitrogen and phosphours. *Biotechnology and Bioengineering* No.14, pp. 309–318.
2. Bartha, R. and R. M. Atlas, 1987. Transport and transformation of Petroleum : Biological Processes. *Long-term Environmental Effects of Offshore Oil and Gas Development*, Elsevier Applied Science Publishers Ltd, London, pp. 287–341.
3. Brown, E.J. and J.F. Braddock, 1990. Sheen screen: a miniaturised most-probable-number method for the enumeration of oil-degrading micro-organisms. *Applied and Environmental Microbiology*, No. 56, pp. 3895–3896.
4. Croft, B.C., R. P. J. Swannell, A.L. Grant and K. Lee, 1995. The Effect of Bioremediation Agents on Oil Biodegradation in Medium-Fine Sand. *Applied Bioremediation of Hydrocarbons*, Battelle Press Columbus, Ohio, pp. 423–434.
5. Delvigne, G. A. L. and C. E. Sweeney, 1988. Natural Dispersion of Oil. *Oil and Chemical Pollution*, Vol. 4, pp. 281–310.
6. Floodgate, G.D., 1984. The Fate of Petroleum in Marine Ecosystems. *Petroleum Microbiology*, ed. RM Atlas, Macmillan, New York, pp. 355–98.
7. Greenberg, A.E., L.S. Clesceri and A.D. Eaton, 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, American Public Health Association, Washington DC, USA, pp. 4–85 to 4–87
8. Krigsvoll, S., K. Josefsen and I. Storrø, 1991. Microbial emulsification of crude oil. *5th European Congress on Biotechnology,* Copenhagen, Denmark, Vol. 1, pp.221–224.
9. Leahy, J.G. and R.R. Colwell, 1990. Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews*, 54, pp.305–315.
10. Lee, K., C.S. Wong, W.J. Cretney, F.A. Whitney, T.R. Parsons, C.M. Lalli and J. Wu, 1985. Microbial response to crude oil and Corexit 9527: SEAFLUXES enclosure study. *Microbial Ecology*, 11, pp.337–351.
11. Lunel, T., 1993. Dispersion: Oil Droplet Size Measurements at Sea.*16th Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, Environment Canada, Vol. 2, pp. 1023–1056.
12. Mackay, D. and F. Szeto, 1980. Effectiveness of Oil Spill Dispersants - Development of a Laboratory Method and Results from Selected Commercial Products. Institute of Environmental Studies, University of Toronto, Environment Canada, Publication Number EE-16.
13. MPCU, 1996. The *Sea Empress* incident. *A Report by The Marine Pollution Control Unit*. Published by The Coastguard Agency, Southampton, UK, ISBN-1-901518-00-0.
14. Mulkins-Phillips, G.J. and J.E. Stewart, 1974. Effect of four dispersants on biodegradation and growth of bacteria on crude oil. *Applied Microbiology*, Vol. 28, No. 4, pp.547–552.
15. Nagata, S., 1983. Influence of salinity and temperature on the aliphatic hydrocarbon degradation by marine bacteria. *Bulletin of the Japanese Society of Scientific Fisheries*, 49(2), pp.269–271.

16. Prince, R.C., 1993. Petroleum spill bioremediation in marine environments. *Crit. Rev. Microbiol.*, 19, pp.217–242.
17. Russell, A.D., W.B. Hugo and G.A.J. Ayliffe, 1992. Principles and Practice of Disinfection. *Preservation and Sterilisation*, Blackwell Scientific Publications, 2nd Edition.
18. Swannell, R.P.J., K. Lee and M. McDonagh, 1996. Field evaluation of marine oil spill bioremediation. *Microbiological Reviews*, June 1996, pp.342–365.
19. Swannell, Richard P.J., Fabien Daniel, Barry C. Croft, Mark A. Engelhardt, Samantha Wilson, David J. Mitchell and Tim Lunel, 1997. Influence of Physical and Chemical Dispersion on the Biodegradation of Oil under Simulated Marine Conditions. *20th Arctic and Marine Program Technical Seminar (AMOP)*, Vancouver, Vol. 1, pp.617–641. Environment Canada, Canada.
20. Varadaraj, R., M. L. Robbins, J. Bock, S. Pace and D. MacDonald, 1995. Dispersion and Biodegradation of Oil Spills on Water. *1995 Oil Spill Conference*, API, USA, pp.101–106.
21. Venosa, A.D., M. Kadkhodayan, D.W. King, B.A. Wrenn, J.R. Haines, T. Herrington, K. Strohmeier, and M.T. Suidan, 1993. Testing the Efficacy of Oil Spill Bioremediation Products. *1993 International Oil Spill Conference*, API, USA, pp. 487–493.
22. Walker, M.I., D.J. Albone, M. McDonagh, A.D. Wilkinson, G.R. Baron, and S.J.W. Grigson, 1992. Final Report on the Joint Programme of Research on Source Identification, Behaviour and Modelling of North Sea Crudes at Sea. *WSL Report CR 3698*.

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