

## Effect of Dispersants on the Growth of Indigenous Bacterial Population & Biodegradation of Crude Oil

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Received 15 October 1982; revised received 17 March 1983

Oil dispersants (5 from Castrol Ltd. Bombay and 2 from British Petroleum, London) were studied individually and in combination with Saudi Arabian crude oil for their effect on the growth of indigenous bacteria and on the biodegradation of oil. None of the dispersants were toxic, except IB 2:80, to the bacterial population. All of the dispersants, when used alone, or in combination with the crude oil, supported good growth of bacteria. The Saudi Arabian crude oil by itself was toxic to the bacteria, and when mixed with the dispersants reduced their rates of biodegradation. Maximum degradation (46.4%) was observed with the mixture of crude oil and dispersant IB 1:80.

Chemical dispersants are used to remove oil and petroleum products from the contaminated environment. However, the use of such dispersants during Torrey Canyon episode caused more harm than oil to marine life<sup>1</sup>. Since then number of reports have been published describing the effects of dispersants on marine organisms<sup>2-10</sup>. Despite these, little is known about the effect of dispersant and oil-dispersant mixture on the marine organisms and effect of dispersant on the biodegradation of crude oil<sup>10</sup>.

In the present study, effect of 5 dispersants manufactured in India and 2 others obtained from British Petroleum, on the growth of indigenous bacterial population and the biodegradation of Saudi Arabian crude oil was studied.

Microbial population isolated from sediment samples collected from Gaspar Dias beach (Goa) by adding 10 g of sediment to 100 ml of sterilized salt solution (NaCl, 30 g; KCl, 0.75 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 7 g; NH<sub>4</sub>NO<sub>3</sub>, 1 g; 1000 ml distilled water, pH 7) was used as inoculum.

Erlenmeyer flasks containing 100 ml of salt solution were sterilized at 120°C for 15 min. To this 0.3 ml of KH<sub>2</sub>PO<sub>4</sub> and 0.7 ml of K<sub>2</sub>HPO<sub>4</sub> (both 10% solutions, sterilized separately) were added. Chemical dispersants (1% v/v), viz. IB 2:80; IB 1:80; IB 11:80; IB 12:80; IB 13:80 from Castrol Ltd. India and BP 1100 WD, BP 1100 × from British Petroleum, London numbered serially D<sub>1</sub> to D<sub>7</sub> were added. Saudi Arabian crude was added where indicated at the same concentration (1% v/v). Control flasks were identical to test flasks but contained no added carbon source (dispersant or oil).

The flasks were inoculated with 5% indigenous bacterial population prepared as above and were incubated at room temperature (28-30°C) on rotary shaker (120 strokes min<sup>-1</sup>). Samples were withdrawn every day. Increase in bacterial number was monitored by spread plate method on nutrient agar plate<sup>11</sup> and

the colonies were counted after 24 h incubation at room temperature (28-30°C).

In order to study the biodegradation of crude oil and dispersant individually and the effect of dispersant on biodegradation of crude oil, flasks with oil, dispersant and oil-dispersant mixture were inoculated and incubated at 28-30°C on rotary shaker for 8 d. Undegraded portions of oil, dispersant and oil-dispersant mixture were extracted thrice with 10 ml of analytical grade carbon tetrachloride each time. The combined extracts were evaporated on a water bath at 80°C under reduced pressure and the residues weighed in Mettler mono-pan balance.

All dispersants, except D<sub>1</sub>, supported growth of the indigenous bacterial population (Fig. 1A). The growth curve obtained did not indicate any lag phase. Stationary phases of microbial populations varied with respect to dispersants.

Growth of microbes on oil-dispersant mixtures was nearly the same as with the dispersant alone (Fig. 1B). There was no lag phase for microbes when dispersants were mixed with oil. Here also, dispersant D<sub>1</sub> when mixed with oil did not remove the growth inhibitory effect.

Indigenous bacterial population used in the present study did not grow on crude oil (Fig. 1B). All the dispersants were degraded by the indigenous bacteria, however, the rate of biodegradation varied between individual dispersants. Dispersant D<sub>2</sub> was the most degradable with 99.6% degradation. Dispersants D<sub>3</sub> and D<sub>7</sub> gave the same percentage of degradation and D<sub>5</sub> was least biodegradable (52.5%; Table 1).

Indigenous bacterial population did not show the capacity to utilize crude oil (Table 1, Fig. 1B). Moreover, in the presence of this crude oil the extent of degradation of all the dispersants was decreased (Table 1). Although crude oil was toxic to microbial population, dispersant D<sub>2</sub> when mixed with crude oil

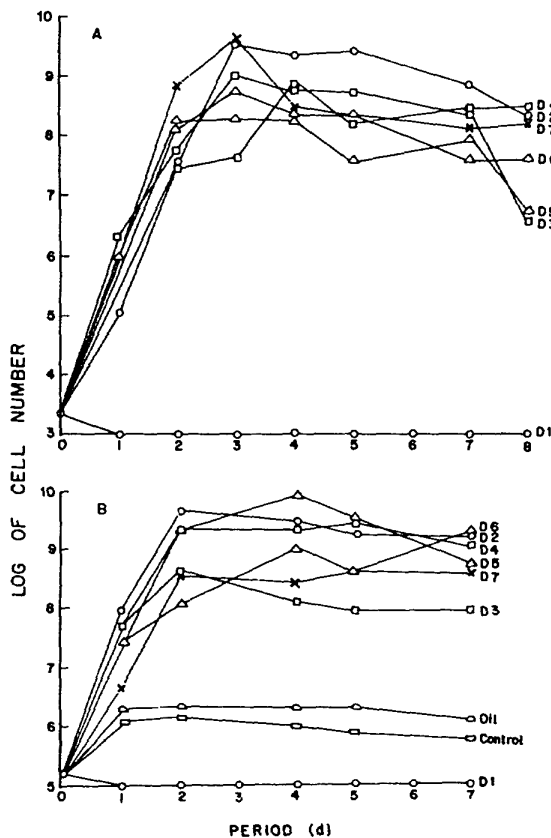


Fig. 1—Effect of dispersant (A) and oil-dispersant mixture (B) on the growth of indigenous bacteria

underwent maximum degradation (46.4%) as compared with other dispersant oil mixture (Table 1).

Many of the chemical dispersants and oil-dispersant mixtures are known to stimulate microbial growth<sup>5,8,12</sup>. Not all the dispersants support bacterial growth as observed in the case of dispersant D<sub>1</sub>. At 1% concentration dispersant D<sub>1</sub> exhibited toxic effect and hence did not support growth of indigenous microorganisms. Toxic effect of some dispersants and oil-dispersant mixture on microbial and biological activity is well known<sup>9,12,13</sup>.

The Saudi Arabian crude oil used in the present investigation did not support the microbial growth (Fig. 1B) which might be due to toxicity of some hydrocarbon components present in crude oil. Toxicity of crude oil components such as hexane, benzene, xylene, toluene and ethyl benzene to microorganisms is documented<sup>11,14</sup>.

Addition of dispersant to crude oil did not improve its degradation probably because of dispersant treatment resulting in increased mobilization of some hydrocarbons toxic to microorganisms<sup>18</sup>. Less or no degradation of crude oil in the presence of dispersants might be because of preferential utilization of dispersant as compared with a complex substrate such as crude oil<sup>7</sup>. This is in contrast with the observation that dispersant treatment increased the extent of crude oil degradation<sup>3</sup>.

Although Saudi Arabian crude oil exhibited toxic effect on microorganisms used, amongst the dispersant-oil combination tested, dispersant D<sub>2</sub> and Saudi Arabian crude oil showed maximum degradation.

Table 1—Biodegradation of Dispersant and Oil-Dispersant Mixture by Indigenous Bacteria

Sl.No.	Dispersant	Wt in control flask (g)	Wt after 8 days incubation (g)	Amount utilised (g)	Biodegradation (%)
Dispersant					
D <sub>1</sub>	IB 2/80	0.9677	---	---	---
D <sub>2</sub>	IB 1/80	0.8134	0.0027	0.8107	99.6
D <sub>3</sub>	IB 11/80	0.8374	0.3687	0.4687	55.9
D <sub>4</sub>	IB 12/80	0.8242	0.2740	0.5502	66.7
D <sub>5</sub>	IB 13/80	0.8120	0.3852	0.4268	52.5
D <sub>6</sub>	BP 1100 WD	0.8594	0.3069	0.5525	64.2
D <sub>7</sub>	BP 1100 X	0.7818	0.3475	0.4343	55.5
Oil-dispersant					
D <sub>1</sub>	IB 2/80	1.1107	1.1100	---	---
D <sub>2</sub>	IB 1/80	1.0406	0.5576	0.483	46.4
D <sub>3</sub>	IB 11/80	1.2804	1.0294	0.251	19.6
D <sub>4</sub>	IB 12/80	1.2497	0.9120	0.337	27.0
D <sub>5</sub>	IB 13/80	1.1483	0.8674	0.260	22.6
D <sub>6</sub>	BP 1100 WD	1.4710	0.9411	0.529	35.9
D <sub>7</sub>	BP 1100 X	1.2460	0.9847	0.261	20.9
—	Saudi Arabian crude oil	0.4825	0.4807	0.0018	0.37

Authors thank Dr V V R Varadachari, Director for his keen interest and encouragement. Authors also acknowledge Dr S Z Qasim, Secretary, Department of Ocean Development and Dr R Sen Gupta for their valuable comments on the manuscript.

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