## EFFECTS OF DISPERSANTS ON MICROBIAL GROWTH AND BIODEGRADATION OF CRUDE OILS

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## ABSTRACT

Four oil spill dispersants when used alone (0.1% V/V) or in combination with Saudi Arabian Crude (0.5% V/V) were non-toxic to Arthrobacter simplex and Candida tropicalis. At a higher concentration of 0.6% (V/V) only  $D_2$  was found to be toxic to both the organisms. Dispersant treatment did not increase the bio-degradation of crude oils except  $D_3$  in the presence of Bombay High crude oil.

Key-words: Dispersants, biodegradation, crude oils, toxicity.

Many chemical dispersants affect microbial and biological activity when used to remove oil spills from the marine environment (Fought and Westlake, 1982; Griffiths, Mc Namara, Caldwell and Morita, 1981; Rogerson and Berger, 1981). Therefore, factors such as toxicity of dispersants to marine life, their ability to form stable emulsion and effects on the biodegradation of crude oils need to be evaluated before dispersants are actually used for clean-up purpose.

The present paper deals with the emulsifying capacity of four dispersants and their effect on hydrocarbon utilizing micro-organisms and biodegradation of crude oils.

Dispersants ONGC-1, ONGC-2, and ONGC-4 were supplied by Oil and Natural Gas Commission (ONGC), Bombay and refered hereafter, as  $D_1$ ,  $D_2$ ,  $D_3$  and  $D_4$  respectively. The emulsifying capacity of these dispersants was studied following the method of Canavari (1969). Based on the emulsifying capacity, a 1:5 dispersant (0.1% V/V) to Bombay High or Saudi Arabian Crude (0.5% V/V) was used to study the effect on the growth of Arthrobacter simplex and Candida tropicalis.

Growth media, isolation, identification and routine maintainance of these two cultures were reported earlier (Bhosle and Mavinkurve, 1980). Effect of either Saudi Arabian Crude (0.5% V/V) or dispersants (0.1 or 0.6% V/V) and dispersant oil mixture on the growth of these two organisms was studied using 100 ml of sterile basal salt solution (Bhosle, 1981), inoculated with either of the cultures and incubated on a rotary shaker at room temperature (28-30°C). Increase in growth was monitored by surface spreading on nutrient agar plate (Bhosle, 1981). A basal salt solution containing no added

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carbon source was used as control. The degradation of Saudi Arabian and Bombay High crude oils in the presence and absence of dispersants was studied as described earlier (Bhosle, 1981).

Turbidimetric measurements of dispersants added to oil in sea water indicates the emulsifying capacity of dispersants (Fig. 1). A good dispersant form a stable emulsion of oil in water. Most stable dispersants would transmit less light and hence a low transmittance. Amongst the four dispersants tested  $D_4$  gave the most stable emulsion of crude oil (Fig. 1). Of the three combination tested, 1:5, dispersant to oil gave a most stable emulsion. The order of emulsifying capacity of the four dispersants was  $-D_3 \angle D_1 \angle D_2 \angle D_4$ 

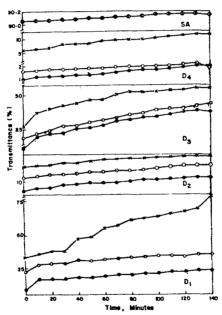


Fig. 1 Effectiveness of oil-split dispersant to form emulsions in sea water with Saudi Arabian crude oil (SA),

• SA • (1:5:01:10:×J:50

When used as a sole source of carbon and energy at either 0.1 or 0.6%; the response of both the organisms varied with the dispersant (Figs. 2 & 3). This is possible because the dispersants vary in their chemical composition and also the organisms in their metabolic capacity (Robichaux and Myrick, 1972). At 0.6% both the organisms showed fairly good growth with  $D_1$ ,  $D_3$  and  $D_4$ . With  $D_2$ , however, after an initial increase, the viable cell counts of both the organisms decreased rapidly and this effect was more prominent on A. simplex than on C. tropicalis (Fig. 2). At 0.1% concentration, none of the dispersants was toxic to A. simplex or C. tropicalis (Fig. 3).

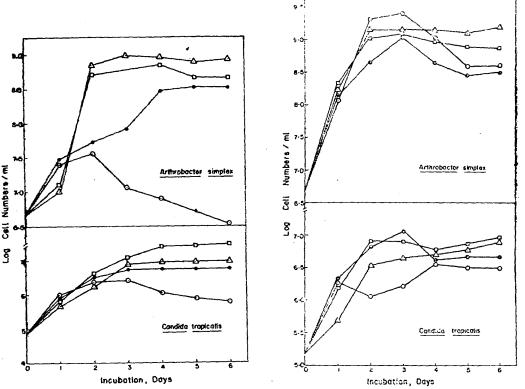


Fig. 2 Growth pattern of the organisms in basal medium with dispersant (0.6 % V/V), ●, D₁; ○, D₂; △,D₂; □,D₄.

Fig. 3. Crowth pottern of the organisms in basel medium with dispersent (Θ-1% Vv.), e,D<sub>1</sub>, O,D<sub>21</sub>, Δ<sub>1</sub>D<sub>31</sub>, D,D<sub>4</sub>

Some of the dispersants are known to stimulate microbial growth (Liu and Townsley, 1970; Marty, Bianchi and Gatellier, 1979; Mulkin-Phillips and Stewart, 1974), however, the growth stimulatory effect depends upon the concentration used (Gunkel, 1968), as observed in the case of D<sub>2</sub>. Some of the toxic effects of the dispersants at higher concentrations are their damaging effect on cell membrane, cell permeability and irreversible denaturation of the enzymes (Gesamp, 1977).

It was observed that the dispersant (0.1% V/V) and oil (0.5% V/V) mixture was non toxic and increased the growth rate of both the organisms, though growth yield remained constant (Fig. 4). Faster growth of the micro-organisms is expected when dispersant forms a simple carbon source (as compared with the crude oil) and is utilised preferentially (Robichaux and Myrick, 1972). Alternatively, due to dispersant treatment, crude oil breaks into fine oil droplets, providing more surface area for microbial utilization and which ultimately

leads to the faster growth of the organisms (Robichaux and Myrick, 1972). If the increase is due to the latter phenomenon, increase in biodegradation of crude oil is expected and this was evaluated by studying the biodegradation of Saudi Arabian and Bombay high crude oils by these organisms in the presence and absence of dispersants.

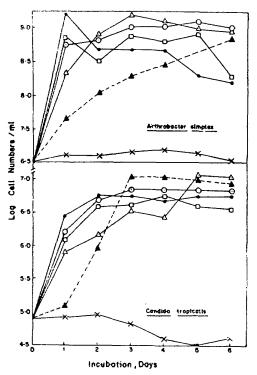
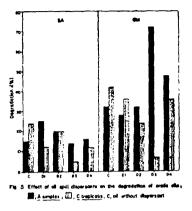


Fig. 4 Growth pattern of the organisms in basal medium, X Control; A,OH; OH + dispersant - e, DI; O; D2; △,D3; □, D4.

The effect of the four oil-spill dispersants on biodegradation of Saudi Arabian and Bombay High crude oil was variable (Fig. 5). For example, addition of  $D_1$  and  $D_2$  increased the degradation of Saudi Arabian crude cil by A, simplex but not of Bombay High crude oil. In contrast to this, the addition of these dispersants reduced the extent of degradation of both the crude oils by C, tropicalis (Fig. 5). Such reduced degradation of crude oils in the presence of dispersants is possible when the dispersants are preferentially utilised by micro-organisms (Atlas and Bartha, 1973; Mulkin-Phillips and Stewart, 1974). However, in the presence of  $D_3$ , biodegradation of Bombay



High crude oil by A. simplex increased. This observation confirms the earlier findings of Robichaux and Myrick (1972); Liu and Townsley (1970) and Mulkin-Phillips and Stewart. (1974), that some dispersants enhance the biodegradation of crude oils.

The data presented here shows that dispersants when used alone or in combination with crude oil at lower concentration, stimulate microbial growth, however the effect of dispersant on the biodegradation of crude oils, depends on the type of dispersant, crude oil and the micro-organisms used.

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