EFFECTS OF OIL SPILL DISPERSANTS ON THE GROWTH OF BRACKISH WATER HETEROTROPHIC BACTERIA

Uzoigwe, C. I. and G. C. Okpokwasili
Department of Microbiology
University of Port Harcourt
P.M.B. 5323 Port Harcourt.
Nigeria.

ABSTRACT
Five heterotrophic bacteria isolated from brackish water collected from Nembe waterside, Port Harcourt, Rivers State were cultured aerobically in the presence of varying concentrations (0, 25, 50, 75 and 100mg/ml) of oil spill dispersants (Gold Crew and Camol) to determine the effects of the toxicants on their growth. The isolates were identified as Staphylococcus, Enterobacter, Bacillus, Pseudomonas and Citrobacter. The lag phases of growth of these isolates were affected differently with increasing concentrations of dispersants. Gold Crew increased the lag phases of the bacterial isolates with increasing concentrations while Camol depressed their lag phases at all concentrations. There was a depressive effect on the growth rates of isolates in the presence of Gold Crew but their growth rates at 25mg/ml was slightly depressed compared to higher concentrations of the dispersant. Camol elicited toxic effects on the isolates since their exponential phases of growth were inhibited at all concentrations of the dispersants. The results of this study indicated that the test dispersants have toxic effects on the brackish water heterotrophic organisms and hence support the view that ecotoxicological studies should be carried out on dispersants before field application.

Keywords: Dispersants; Brackish water; Heterotrophic bacteria

INTRODUCTION
Oil pollution impacts the soil, freshwater brackish water and marine water environment (Okpokwasili and Odokuma, 1997). The microbial degradation of oil is one of the most important biological events which takes place as a result of the introduction of oil into the ecosystem and may contribute significantly to the ultimate removal of oil from the environment (Nnubia and Okpokwasili, 1998). One major option for stimulating biodegradation of spilled oil is to encourage natural dispersion by adding chemical dispersant to the oil slick (Lessard and Demarco, 2000). Dispersants are chemical agents that reduce interfacial tension between oil and water in order to enhance the natural process of dispersion by generating larger numbers of small droplets of oil that are entrained into the water column (Fingas, 1996). The important issue when discussing dispersants is toxicity, both of the dispersant itself and of the dispersed oil droplets (i.e. the oil/disperant mixtures).

Toxicity became an important issue in the late 1960s and early 1970s when application of toxic products resulted in substantial loss of sea life. For
example, the use of dispersants during the Torrey Canyon episode in Great Britain in 1968 caused massive damage to intertidal and sub-tidal life (Fingas, 2000). Since that time, dispersants have been formulated with lesser aquatic toxicity. Dispersants available today are much less toxic (often one hundredth as toxic) than earlier products. A key factor contributing to the toxicity of dispersants meant for environmental release is their degradability (Odokuma and Okpokwasili, 1997). Chemical dispersant biodegradability or a measure of amount of oxygen required to breakdown the chemical added to the oil contaminated water is another major environmental concern when using dispersants. Dispersant themselves exhibit a high demand for oxygen hence their use on spills in polluted coastal bays or inland waters with limited circulation, could deplete or lower the dissolved oxygen resources therefore causing damage to biological community in such waters (Dewling and McCarthy, 1980). Oil dispersants have lethal effects on various aquatic organisms at concentration of 1-1000ppm and their effects on the fertilization and consequent early growth of various aquatic organisms are more acute (Tsutsumi et al., 2000).

According to Okpokwasili and Nnubia (1995), heterotrophic microbial processes by marine bacteria were negatively affected by oil dispersants. In another study, Okpokwasili and Odokuma (1997) reported that nitrite utilization by Nitrobacter sp was decreased by some dispersants with increasing concentration and exposure time to the toxicants. The results from the study showed that the test oil spill dispersants inhibited the nitrification processes in the ecosystem and elicited mortality of Nitrobacter sp (Okpokwasili and Odokuma, 1997). This study was designed to determine the effects of oil spill dispersants on the growth phases of heterotrophic bacteria isolated from brackish water.

**Materials and Methods**

**Source/Collection of Samples**

Brackish water samples were collected from Nembe waterside, Rivers State, Nigeria. The water samples were collected from four sections of the river into sterile plastic bottles and were sent immediately to the laboratory for bacteriological analysis. Camol dispersant was obtained from Thermosteel Nigeria Limited, Warri, Delta State while Gold Crew dispersant was obtained courtesy of C. Nnubia of Clean Nigeria Associates, Port Harcourt, Rivers State.

**Isolation of brackish water heterotrophic bacteria**

The brackish water heterotrophic isolates were isolated using the spread plate method. A ten-fold serial dilution of the water sample was prepared and 0.1ml aliquots of $10^{-2}$ and $10^{-3}$ dilutions was plated out on sterile dried nutrient agar plates. The plating was done in triplicates and all plates were incubated at $35^\circ C$ for 24h. Discrete colonies were isolated and sub-cultured, then stored on nutrient agar slants for further identification.

**Microbial Growth Studies**

**Determination of standard growth curves for the isolates**

The standard growth curve of the isolates was determined using the viable count technique. A loopful of each test isolate was inoculated aseptically into conical flasks containing 9ml of sterile nutrient broth. The nutrient cultures were incubated on a rotary shaker at $30^\circ C$. The population size of each test isolate
at any point in the growth cycle was quantified by making a ten-fold serial dilution from each flask and plating out 0.1ml into triplicate plates of nutrient agar. This procedure was repeated at 1-hour intervals for 7 hours and then at 2-hour intervals for 15 hours. The plates were incubated at 35°C for 24 hours. The mean counts on the triplicate plates were used to calculate the colony forming counts (cfu/ml) of the original broth culture. The growth curve of each isolate was determined by plotting a graph of the number of viable cells expressed as a logarithm to base 10 against time.

**Effects of oil spill dispersants on the growth of brackish water isolates.**

The test dispersants were used at four different concentrations (25, 50, 75 and 100mg/ml). Each isolate was diluted appropriately and 1ml portion of this dilution was inoculated into the test flasks containing the four concentrations of the dispersants (Gold Crew and Camol). The flasks were incubated at room temperature (30°C) on a rotary shaker set at 120rpm. The population size of each isolate at any given concentration of the test dispersants was determined by making a ten-fold serial dilution from each stock culture flask and plating out 0.1ml into triplicate plates of nutrient agar. This procedure was repeated at 1-hour interval for 7 hours, then at 2-hour intervals until 15 hours. The mean counts from the triplicate plates were used to calculate the colony forming unit (cfu/ml). The growth curve of each isolate at a given concentration was determined by plotting a graph of the number of viable cells expressed as a logarithm to base 10 against time.

**Identification of isolates**

The isolates were identified by their cultural, morphological and biochemical characteristics using the method described by Cheesbrough (1984).

**Results**

The effects of varying concentrations of Gold Crew and Camol dispersants on the three bacterial isolates in batch cultures are shown in Fig 1-10. These results showed that Gold Crew had no effect on the lag phase of *Staphylococcus* sp at 25mg/ml but increased the lag phase of the organism to 1.5h at 50mg/ml and to 2.8h at 75 and 100mg/ml compared to the control. The lag phase of *Bacillus* sp was increased to 2h at all concentrations of Gold Crew. The results also showed that Gold Crew dispersant increased the lag phase of *Pseudomonas* to 2 hours at 25, 50 and 75mg/ml compared to the control but extended the lag phase to 2.5 hours at 100mg/ml. The lag phase of *Enterobacter* sp was also increased to 1.5 hours at 25 and 50mg/ml by Gold Crew but extended to 2hours at 75 and 100mg/ml. The lag phase of *Citrobacter* was increased to 2hours at all concentrations of Gold Crew dispersants. The exponential phases of the five isolates were slightly depressed at all concentrations of gold crew dispersant compared to the control. Gold Crew supported the exponential phase of *Staphylococcus* sp to a maximum time of 5.4h at all concentrations after which it entered the stationary phase and then death after 7h. The exponential phase of *Bacillus* sp reached a maximum time of 7.5h at 25mg/ml compared to the control but was depressed to 4.5h at higher concentrations of Gold Crew and entered the death phase after 7h. The growth of *Enterobacter* sp reached 7.5h at 25mg/ml but was slightly depressed to 6hrs at higher concentrations. The growth of *Pseudomonas* sp reached a
maximum time of 4.4h at all concentrations, followed by stationary phase and entered the death phase after 7h. The exponential growth phase of *Citrobacter* sp reached a maximum time of 5.5h at 25 and 50mg/ml concentrations of the Gold Crew dispersants but the time was reduced to 5h at higher concentrations.

Camol dispersant extended the lag phases of *Staphylococcus* and *Bacillus* sp to 2h compared to the control at 25 and 50mg/ml but depressed the lag phases of *Pseudomonas, Enterobacter* and *Citrobacter* from 0h at all concentration followed by death of the isolates. Camol dispersant inhibited the exponential growth phases of the five isolates at all concentrations.
Figure 2: Effect of Gold Crew dispersant on the growth of Enterobacter sp

Figure 3: Effect of Gold Crew dispersant on the growth of Bacillus sp
Figure 4: Effect of Gold Crew dispersant on the growth of *Pseudomonas* sp.

Figure 5: Effect of Gold Crew dispersant on the growth of *Citrobacter* sp.
Figure 6: Effect of Camol dispersant on the growth of *Staphylococcus* sp.

Figure 8: Effect of Camol dispersant on the growth of *Bacillus* sp.
Computation at the exponential phases of growth of the five isolates showed that their growth rates in the presence of Gold Crew were slightly depressed at 25mg/ml but there was a higher depressive effect with increasing concentrations. The generation time of each isolate was also increased with increasing concentrations of Gold Crew dispersants.
Table 1: Growth rates of the five isolates at different concentrations of Goldcrew dispersants.

<table>
<thead>
<tr>
<th>Test Chemical Gold crew Dispersant</th>
<th>Test Isolates</th>
<th>Control</th>
<th>25mg/ml</th>
<th>50mg/ml</th>
<th>75mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>0.36</td>
<td>0.32</td>
<td>0.31</td>
<td>0.25</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Bacillus</td>
<td>0.35</td>
<td>0.34</td>
<td>0.22</td>
<td>0.22</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Enterobacter</td>
<td>0.35</td>
<td>0.30</td>
<td>0.28</td>
<td>0.28</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0.35</td>
<td>0.34</td>
<td>0.30</td>
<td>0.24</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Citrobacter</td>
<td>0.36</td>
<td>0.34</td>
<td>0.31</td>
<td>0.22</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The Generation time per hour of the five isolates at different concentrations of Goldcrew dispersants.

<table>
<thead>
<tr>
<th>Test Chemical Gold crew Dispersant</th>
<th>Test Isolates</th>
<th>Control</th>
<th>25mg/ml</th>
<th>50mg/ml</th>
<th>75mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td>5.88</td>
<td>8.33</td>
<td>8.33</td>
<td>10.00</td>
<td>12.50</td>
<td></td>
</tr>
<tr>
<td>Bacillus</td>
<td>5.26</td>
<td>6.25</td>
<td>10.00</td>
<td>9.09</td>
<td>10.00</td>
<td></td>
</tr>
<tr>
<td>Enterobacter</td>
<td>0.35</td>
<td>0.30</td>
<td>0.28</td>
<td>0.28</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0.35</td>
<td>0.34</td>
<td>0.30</td>
<td>0.24</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Citrobacter</td>
<td>0.36</td>
<td>0.34</td>
<td>0.31</td>
<td>0.22</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>
Discussion
The examination of effect of oil spill dispersants on five brackish water heterotrophic isolates showed that increasing concentrations of Gold Crew dispersant depressed the growth of the isolates while Camol dispersant was bactericidal to the isolates with increasing concentration. The isolates identified in this study were Staphylococcus, Bacillus, Enterobacter Pseudomonas, and Citrobacter.
This observation is in agreement with the work of Walker et al. (1974) who reported the inhibition of bacterial growth by petroleum products. The inhibitive effect on the growth of the isolates was noticed although to a little extent during the lag periods of growth which increased at higher concentrations of Gold Crew dispersant but the lag phases of the isolates were highly depressed at all concentrations of Camol dispersant. These results suggest that the tested oil spill dispersants either increased or depressed the lag phase of growth of the isolates. The pattern of increase and decrease of lag phase is in agreement with the work of Calder and Ladar (1976).
Growth rate analysis showed that the growth of the isolates were slightly depressed at 25mg/ml while there was a higher depressive effect on their growth rates with increasing concentrations of Gold Crew. Camol did not support the exponential phase of growth of the isolates. The generation time of the isolates in the presence of increasing concentrations of Gold Crew was greatly increased as a result of the toxic effect of the dispersant on the isolates. Camol showed a higher toxic effect that did not support the doubling of the isolates at any concentration, hence this experiment demonstrated the differential toxic effect of the oil dispersants on the heterotrophic isolates. This is in agreement with the report by Okpokwasili and Nnubia (1995) which showed that oil dispersants supported mild stimulation and inhibition of the growth of specific marine heterotrophic bacteria. The high depressive effect of the dispersants on the isolates may be related to the high specific gravities of the chemical. The specific gravities of the test dispersants (Gold Crew and Camol) in this study indicated that these chemicals were heavy compounds. The dispersants had specific gravities of 1.02 and 1.06g/ml respectively which is greater than that of pure water. Statistical analysis showed that there was a significant difference (at p<0.001) in the growth of the isolates at various concentrations of the test oil dispersants at the different time intervals.
In conclusion this work shows that Gold Crew and Camol dispersants have toxic effects on test bacteria. These effects may be attributed to the different ingredients used in formulating the dispersants; therefore, studies aimed at elucidating the effects of the individual ingredients in the products are recommended for future research.

REFERENCES


