Biodegradation of Non-Ionic Dispersants in Sea-Water

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Summary. In this paper, we describe the aerobic biodegradation of some non-ionic dispersants of the Span, Tween, and Corexit series in sea-water, where they are now more frequently found as a result of their application to the removal of oil spills. First, the extent to which dispersants are biodegraded, as an indication of their suitability for use on a large scale, is discussed. Biodegradation may be carried out by means of monocultures or mixed cultures of marine bacteria of the genera *Aeromonas*, *Pseudomonas*, and *Flavobacterium*. Analytical techniques based on absorbance measurements were used to follow the process. On the other hand, by determining the kinetics of the biodegradation process a more complete analysis is obtained. The kinetic coefficients controlling the process are deduced and it is shown that for some dispersants the experimental results are in close agreement with the proposed scheme. Where observed values are explained on the basis of ethylene oxides content of the dispersants, estimations of the amount of dispersant present in the sea at a given time can be made, if the amount of the dispersant first used is known.

Introduction

The continuous impoverishment in oxygen both in waste and marine waters was soon related to industrial progress and the birth of detergents. The presence of detergents in the sea has been greatly increased due to their use in the treatment of oil spills. In order to carry out this treatment on a large scale with dispersants it is necessary to find out previously how they will affect the medium. Thus it is quite usual to carry out a study on the biodegradation of dispersants, which will allow a prediction of their level in the sea at any time.

Garret (1967) has pointed out the presence in the sea of surfactants and their residual products; the latter result, possibly, from the metabolization of the hydrophobic part of these compounds (Fischer and Gerike 1975).

Non-ionic dispersants have replaced ionic in certain fields, because of advantages such as their compatibility with many substances that react with and inactivate anionic and cationic agents (Cruickshank 1968; Bourrel et al. 1979), although they have not been greatly applied to the treatment of oil spills. Among the non-ionic dispersants an important group is the polyoxyethylene fatty acid esters, which result from the reaction of fatty acids C₁₂ to C₁₈ with sorbitol and a later addition of ethylene oxides. Due to the wide structural variety shown by this group of substances their study is very complex and although some authors (Fincher and Payne 1962) have related their biodegradation to the content of ethylene oxides, others disagree (Schick 1967).

In order to follow the biodegradation process with a certain facility compounds with oleic acid, thus having double bonds C=C, were first chosen. The theoretical degradation scheme of these compounds was discussed in a previous paper of this department (Bao and Bergueiro 1977). Our study was later extended to other surface active agents of the same or equivalent series.

The micro-organisms used were selected on the basis of reports by many authors who have carried out degradation of dispersants with cultures of *Pseudomonas* and *Flavobacterium* genera (Goodnow and Harrison 1972; Hsu 1965; Atlas and Bartha 1972). It is well known that micro-organisms adapt themselves to the medium, so that by isolating them in polluted areas, satisfactory results can be obtained. Thus for this investigation *Flavobacterium* and *Pseudomonas* together with *Aeromonas* genera were isolated from...
Aeromonas sp., Micro-organisms. Flavobacterium pictorum, Pseudomonas sp., collected throughout this study. The first two belong to genera which easily degrade dispersants and petroleum in sea-water will be carried out.

Materials and Methods

Micro-organisms. Flavobacterium pictorum, Pseudomonas sp., and Aeromonas sp., isolated from Galicia Estuaries, were used throughout this study. The first two belong to genera which easily degrade dispersants and the third one has been chosen because of its great abundance in the place where the samples were collected.

Substrate. The following non-ionic dispersants were used in sea-water in the following concentrations:

- Span 65 = Sorbitan trioleate – 1 g/l.
- Span 80 = Sorbitan monooleate – 5 g/l.
- Tween 20 = Polyoxyethylen (20) sorbitan monolaurate – 5 g/l.
- Tween 80 = Polyoxyethylen (20) sorbitan monooleate – 2 g/l.
- Tween 85 = Polyoxyethylen (20) sorbitan trioleate – 1 g/l.
- Corexit 7664 = Polyoxyethylen (13.5) glycerol trioleate – 2 g/l.

We have used a range of high concentrations because we think that there may be similar cases to those found in Galicia Estuaries (where the samples of water and micro-organisms have been taken) in which the concentrations of dispersants after an oil spill are higher than average. This can be explained as a result of the slow renewal and movement of water in the absence of naturally generated turbulence.

The dispersants of Span and Tween series, developed by Atlas Powder Co., were supplied by Comercial Química Massó (Barcelona, Spain). The Corexit was supplied by Esso Research and Engineering Co.

Sea-water from Galicia Estuaries was autoclaved (at 121°C for 15 min) before use.

Process Conditions. The pH is maintained between 7.5–7.8, a quite normal value in Galicia Estuaries. The bacteria are cultivated in a relatively poor PYM medium (1 g Peptone, 0.5 g yeast extract, 800 cm³ sea-water, 200 cm³ distilled water) keeping the flasks in a rotary shaker at 30°C until high growth occurred (30 h), and the viable cell count had risen to 10⁹/ml. This culture was used to inoculate (in a quantity of 0.1 ml) the solutions of dispersants in sea-water; test tubes of 2.5 cm diameter filled up to one third of their capacity (30 ml) were used. Three test tubes were prepared for each measurement of absorbance: one with dispersant and inocule (experimental), other without dispersant and with inocule (blank) and a third one with dispersant and sterile medium (standard). The set of test tubes was incubated at 15°C and 150 rpm for a maximum of 25 days, during which measurements of absorbance are taken in a psychrometer incubator (New Brunswick Sci. Co). The samples were centrifugated at 5,000 rpm for 30 min and their degradation was determined by analytical techniques.

Analytical Methods: The APHA (American Public Health Association) method was used to determine the chemical oxygen demand (COD) whereas the API (American Petroleum Institute) method modified (Núñez 1982) was used to control the biochemical oxygen demand (BOD).

To follow biodegradation two methods were utilized:

Method 1. This method is based on the absorbance shown by the C=C group at 235 nm. The determined concentration corresponds to compounds with double bonds present in the dispersant and in its biodegradation products. In our case it is only valid for dispersants containing oleic acid.

Method 2. This method proposed by Clanet and Viller (1976) is based on the formation of a coloured complex with the salt of a heteropolyacid, such as ammonium cobaltothiocyanate, which is extracted from benzene. Its absorbance is measured at 520 nm (Pye Unicam 1700 Spectrophotometer).

This method is limited because the loss of the dispersant property and not necessarily the full degradation is measured. Our interest is, however, precisely the biodegradation which leads to the loss of that dispersant property, and thus the method is suitable.

Results and Discussion

A first group of dispersants has been chosen on the basis of their content in oleic acid, each one of which contains a different amount of ethylene oxides: Span 80 (0 mol), Tween 80 (20 mol), and Corexit 7664 (13.5 mol).

On the other hand, to select a second group of dispersants, the BOD and COD of a large number of compounds of the Span and Tween series were determined and we chose Span 65, Tween 20, and Tween 85 because they showed the lowest oxygen demand.

The selected micro-organisms were first tested in pure and mixed cultures on Tween 80, the Aeromonas sp. being found to be the most effective (Fig. 1). Thus it was used in the experiments with the other dispersants. The Aeromonas genus, although not usually studied as a degradator of surface-active agents, was found in large amounts in the place where the samples were collected and thus its high degrading capacity did not surprise us.

The results are summarized in Figs. 1–5. Figures 1–3 were obtained by following the biodegradation process using the analytical Method 1 applicable only to surface active agents that contain oleic acid. Figures 4 and 5 were obtained by applying the analytical Method 2 to all the tested dispersants. It can be assumed that all final degradation values (which are the same for each compounds, irrespective of the analytical method used) indicating that the degradation is in direct relation to the number of ethylene oxides of the molecule.

It must be pointed out that all figures are similar in showing a close resemblance to graphs of bacterial growth, indicating that this and biodegradation take place in a parallel way (Van der Kooij et al. 1982). In each figure a minimum degradation value is observed which arises at different times according to the genus of the bacterium; in some cases biodegradation