

J. Vives-Rego, M. D. Vagué, J. Sanchez Leal and J. Parra, Barcelona/Spain

# Surfactants Biodegradation in Sea Water

Primary Biodegradation and Bacterial Population Response in Sea Water

## 1 Introduction

The increasing production and use of surfactants has prompted a great number of studies in relation to their biodegradability. An important part of these studies has employed analytical techniques which assess surfactant disappearance [4, 6, 14, 15, 25]. The determination of the fate and ultimate biodegradability has lately been studied using surfactants labelled with  $^{14}\text{C}$  or  $^3\text{H}$  at different portions of the molecules in enriched or natural waters [11, 17, 18, 19]. However, to the date, little attention has been focused on the changes in the bacterial community of natural waters after surfactant addition.

Gledhill suggested that the rates and extent of  $\text{CO}_2$  production from a surfactant depend on factors such as number and types of microorganisms capable of metabolizing the tested compound, stability of the cells after growth, endogenous respiration, ecotoxicity and others [11]. It seems evident that more information on the evolution and changes of bacterial number and activity after surfactant discharge is desirable so as to reach a better understanding of surfactant biodegradation in sea waters.

The present paper describes the primary biodegradation of sodium dodecyl sulphate (SDS), sodium alkyl benzene sulphate (LAS) and cetyl trimethyl ammonium bromide (CTAB) in sea water. Thymidine incorporation and epifluorescence counts were used to check the bacterial response to the surfactant addition.

## 2 Material and Methods

### 2.1 Surfactants

Sodium dodecyl sulphate (SDS) is a Merck analytical grade compound whose anionic active matter content was 99.2% according to the analysis performed in our laboratory.

Sodium alkyl benzene sulphonate (LAS): the raw alkylbenzene obtained from Petroquímica Española S.A. (Algeciras, Spain), was submitted to a sulphonation process in our laboratory, and afterwards neutralized with  $\text{NaOH}$ . In the final product the anionic active matter content was 65%, water content was 33-34% and that of unsulphonated matter was 1-2%.

Cetyl trimethyl ammonium bromide (CTAB) is a Fluka analytical grade compound whose cationic active matter content was 98.6% according to the analysis performed in our laboratory.

### 2.2 Biodegradation assays

Sea water for all biodegradation assays was collected from Barceloneta, the natural beach of Barcelona, a Mediterranean city of 3 million inhabitants. Sea water samples were collected in 10 l plastic containers, and immediately transported to the laboratory. Biodegradation assays were carried out in 2 l-Er-

lenmeyer flasks provided with internal edges containing 1 l of sea water; 20  $\text{mg l}^{-1}$  of surfactant was used in all experiments as active matter. Incubations were performed at 22 °C in a rotatory shaker (120 oscillations per min.) Water samples received no exogenous inoculum, so that only the indigenous bacterial community was measured. Controls without surfactants were done in each biodegradation assay.

### 2.3 Bacterial counts

Enumeration of bacteria was carried out by epifluorescence, after acridine orange staining and filtration through 0.2  $\mu\text{m}$  filters of polycarbonate (Nuclepore), following the standard procedure of Hobbie et al. [13]. Observations were done through a Nikon Optiphot equipped with an epifluorescence system EF-D, mercury lamp Osram HBO 100 W/2, B and ND<sub>28</sub> filters (Nikon).

### 2.4 Bacterial activity

Bacterial heterotrophic activity was measured by the thymidine incorporation method of Fuhrmann and Azam [8, 9]. Subsamples from biodegradation assays (10-20 ml) were incubated 30 min. at room temperature (22-23 °C) with 20 nM methyl- $^3\text{H}$  thymidine (80-90 Ci mmol $^{-1}$ , Amersham). At the end of the incubation period an equal volume of ice-cold 10% trichloroacetic acid was added and the mixture kept five minutes in an ice bath. The cold trichloroacetic acid insoluble material was collected by filtration through 25 mm acetate cellulose filters of 0.2  $\mu\text{m}$  pore size (Sartorius). Filters were dissolved in the scintillation vials with ethyl acetate (1 ml) before addition of scintillation fluid (10 ml). The radioactivity was assayed by liquid scintillation using a Tri-Carb 460 C, Packard Instruments Co. Efficiency was determined using internal  $^3\text{H}$ -n-hexadecane standard (Amersham).

### 2.5 Analytical procedures

The analysis of SDS and LAS anionic surfactants was carried out following the methylene blue spectrophotometric method according to the Longwell and Maniace method as modified by Abbot [1].

Determination of CTAB cationic surfactant was carried out by method based on the reaction of the surfactant with an anionic dyestuff (disulphine blue VN 150) followed by spectrophotometric measurement of the chloroform extract of the surfactant dyestuff complex [20].

### 2.6 Data analysis

Biodegradation of sodium dodecyl sulphate shows a first order decay which can be expressed as  $A = A_0 \times e^{-kt}$ , where  $A$  is the concentration at time  $t$ , and  $A_0$  is the initial concentra-

# Surfactant Biodegradation

**D Keegan**



## **Surfactant Biodegradation:**

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### **Table of Contents Surfactant Biodegradation**

1. Understanding the eBook Surfactant Biodegradation
  - The Rise of Digital Reading Surfactant Biodegradation
  - Advantages of eBooks Over Traditional Books
2. Identifying Surfactant Biodegradation
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Surfactant Biodegradation
  - User-Friendly Interface
4. Exploring eBook Recommendations from Surfactant Biodegradation
  - Personalized Recommendations
  - Surfactant Biodegradation User Reviews and Ratings

- Surfactant Biodegradation and Bestseller Lists
- 5. Accessing Surfactant Biodegradation Free and Paid eBooks
  - Surfactant Biodegradation Public Domain eBooks
  - Surfactant Biodegradation eBook Subscription Services
  - Surfactant Biodegradation Budget-Friendly Options
- 6. Navigating Surfactant Biodegradation eBook Formats
  - ePub, PDF, MOBI, and More
  - Surfactant Biodegradation Compatibility with Devices
  - Surfactant Biodegradation Enhanced eBook Features
- 7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Surfactant Biodegradation
  - Highlighting and Note-Taking Surfactant Biodegradation
  - Interactive Elements Surfactant Biodegradation
- 8. Staying Engaged with Surfactant Biodegradation
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Surfactant Biodegradation
- 9. Balancing eBooks and Physical Books Surfactant Biodegradation
  - Benefits of a Digital Library
  - Creating a Diverse Reading Collection Surfactant Biodegradation
- 10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
- 11. Cultivating a Reading Routine Surfactant Biodegradation
  - Setting Reading Goals Surfactant Biodegradation
  - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Surfactant Biodegradation
  - Fact-Checking eBook Content of Surfactant Biodegradation
  - Distinguishing Credible Sources

13. Promoting Lifelong Learning
  - Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
14. Embracing eBook Trends
  - Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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