A STUDY OF THE PRIMARY BIODEGRADATION OF SELECTED ANIONIC SURFACTANTS BY PSYCHROPHILIC SEWAGE LAGOON BACTERIA UNDER ENVIRONMENTAL STRESS

BY

CHANG JOO LIAN

A Thesis

submitted to

the Faculty of Graduate Studies and Research

University of Manitoba

In partial fulfilment

of the requirements for the degree of

Master of Science

October, 1976

"A STUDY OF THE PRIMARY BIODEGRADATION OF SELECTED ANIONIC SURFACTANTS BY PSYCHROPHILIC SEWAGE LAGOON BACTERIA UNDER ENVIRONMENTAL STRESS"

by

CHANG JOO LIAN

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

The author wishes to express his deep gratitude to Dr. H. Halvorson, Professor of the Department of Microbiology, for the opportunity to study Microbial Ecology. His friendship and advice during the course of this research and assistance in the preparation of this manuscript are truly appreciated.

Thanks are extended to Mr. V. Chacko of the Environmental Protection Service, Environment Canada, Winnipeg, for providing water samples from various sewage treatment plants in Manitoba.

Appreciation is extended also to the City of Winnipeg, Waterworks, Waste and Disposal Division for access to the Charleswood Sewage Lagoon.

The comradeship and discussions of fellow students in the Microbial Ecology Laboratories are also truly valued.

Financial assistance was generously provided by the Northern Studies Research Grants Committee, University of Manitoba and the National Research Council of Canada.

TO MY FATHER

AND

IN MEMORY OF MY LATE MOTHER

"We often think that when we have completed our study of 'one' we know all about 'two' because 'two' is 'one and one'. We forget that we still have to make a study of 'and'".

-Eddington-

ABSTRACT

The primary biodegradation of some selected anionic surfactants, commonly used in household detergent formulations, by psychrophilic sewage lagoon bacteria under environmental stress was studied. These surfactants: $C_{11.3}$ LAS (linear alkylbenzene sulfonate with average carbon chain length 11.3), $C_{11.8}$ LAS, $C_{13.3}$ LAS, C_{14} LAS, tallow alcohol sulfate, tallow alcohol ethoxylate sulfate and coconut alcohol ethoxylate sulfate, as measured by the 'methylene blue' method, were shown to be biodegradable under a cold condition of $5^{o}C$. At this low temperature, the low dissolved oxygen concentration of 0.25 ppm was not rate-limiting for the breakdown of $C_{11.3}$ LAS, tallow alcohol sulfate and tallow alcohol ethoxylate sulfate; rates of utilization of these surfactants were comparable to those under full oxygen level ($\simeq$ 11.8 ppm DO). For coconut alcohol ethoxylate sulfate, a DO level of 0.50 ppm was not rate-limiting. None of these anionic surfactants were degraded under anaerobic conditions. In batch cultures, yeast extract was capable of retarding the removal of LAS. Similarly the easily utilizable organics in artificial sewage delayed the breakdown of LAS. However, under steady-state conditions, the organics in the artificial sewage in fact enhanced the utilization of LAS.

The fate of anionic surfactants, measured as 'methylene blue active substances' (MBAS), in natural waters was monitored in the Charleswood Sewage Lagoon over a span of 12 months. MBAS level in the Lagoon showed that surfactants accumulated during the winter but not in summer. Effluent from this Lagoon was shown to be nutritionally adequate to support the growth of indigenous psychrophiles capable of anionic surfactant removal. Analysis of water samples from sewage treatment plants in various parts of Manitoba revealed that some of the winter effluents draining into the natural environment contained levels of MBAS higher than are normally considered to be environmentally sound. But the laboratory results indicate that these anionic surfactants are biodegradable under environmental stress and anionic surfactants released into natural waters will be unlikely to create environmental hazards.

TABLE OF CONTENTS

Table of Contents Continued

Table of Contents Continued

LIST OF FIGURES

List of Figures Continued

LIST OF TABLES

INTRODUCTION

INTRODUCTION

Surface active agents or surfactants constitute 10-30% of the common household detergent formulations. About 75% of the surfactants used today are of the anionic type. An immense volume of literature is available to support the environmental acceptability of these surfactants especially those of the linear alkylbenzene sulfonate (LAS) type. However, the standard tests used in determining the biodegradability of these surfactants are performed under what might be considered as rather favourable conditions for surfactant removal. These tests are conducted at room temperature ranging from 18°C to 30°C (74,97) and under full aeration. In nature, especially in the Canadian North and the Prairies, these conditions rarely manifest themselves throughout the year. Ice-cover formation in aqueous environment could remain as long as 9 months in some parts of Northern Canada and is responsible for low dissolved oxygen levels in this environment. Since LAS accumulation is known to result in environmental hazards (24, 105), it is therefore essential to explore its biodegradability in environments most likely encountered as a result of the Canadian climate. The present study was undertaken to determine whether LAS and other so-called 'biodegradable' anionic surfactants commonly used in household detergent

formulations could undergo degradation by microorganisms living under environmental stress.

HISTORICAL

HISTORICAL

Between 1950 and 1960, many 'natural' soap products commonly used in households and industries were replaced by synthetic detergent raw materials. In the early 1950's the most important surface-active agent or surfactant base used in detergents was the petroleum-based tetrapropylene sulfonate, commonly known as TBS. Although the technical advantages offered by this petroleum-based synthetic surfactant were considerable compared to those of soaps which were made from biodegradable natural fats, there were however, many undesirable effects. The early surfactant, TBS, was relatively resistant to bacterial attack because of its branched and compact alkyl groups (99). TBS is, in fact, a mixture of several hundred isomers and homologs with highly branched alkyl groups ranging from ten to fifteen carbon atoms (101). Undegraded TBS which survived waste treatment processes was discharged into surface and ground waters and accounted for the considerable and undesirable foaming. As far back as 1948, there was concern over the possibility of synthetic surfactants surviving the sewage purification processes (18). England and Germany took the lead in setting up special committees in the 1950's to study the biodegradability of synthetic detergents, especially the TBS type. Under their then existing

sewage treatment processes, the rate of biodegradation of TBS reached a maximum of 25% as ascertained by the 'methylene blue' method (58). The level of surfactants normally encountered in public water supplies, especially those using recycled waste water, was essentially non-toxic to humans (104). However, their presence in drinking water was undesirable, especially from an aesthetic viewpoint. A concentration as low as 0.50 ppm of TBS would cause considerable foaming (97). After the recognition that TBS was recalcitrant (93, 99) there was intense research to find a replacement for this surfactant. A major step was the development of surfactants such as the linear alkylbenzene sulfonate (LAS) which, in addition to having all the superior functional and economic properties leading to the overwhelming acceptance of synthetic detergents in the first place, was also easily biodegradable under the normal existing sewage treatment conditions. Such biodegradable surfactants are called 'soft' surfactants compared to the 'hard' recalcitrant TBS.

Today synthetic detergents comprise the major alternative to 'natural' soap products in many fields of application in households and industries (58). In most modern detergents, 10-30% of their composition is usually made up of surfactant and the rest is made up of builders, mainly polyphosphate salts or nitrilotriacetic acid (NTA), and sequestrants like borax, soda and citrates (63). Depending upon the dissociation of the surfactant in aqueous

solution and the charge present on the hydrophobic part of the molecule, the surfactant may be anionic, cationic, non-ionic or amphoteric. Commercially, the anionic surfactants constitute the major fraction of the surfactants used today. Of these, about 80% is of the LAS type. The 1969 production data released by the United States Tariff Commission (63) showed the following:

Ionic class	Percent of total output	Million pounds
Anionic	70.6	2,753
Non-ionic	24.9	971
Cationic	4.3	169
Amphoteric	0.2	8.5

Public awareness of chemical pollution of the environment triggered the study of surfactant biodegradation. Stringent control for a clean environment has led to an agreement established in 1968 and enforced in 1971 by the Council of Europe (74) that restricts the use of certain anionic surfactants in washing and cleaning products. The restriction demands an 80% biodegradation for anionic surfactants, as measured by the 'methylene blue' method, in 19 days under full aeration at $25^{o}C \pm 1^{o}C$, as a minimal standard for accepting the anionic surfactants in the market. However, each country in Europe remains free to fix the exact degree of biodegradability required, 80% or over, to suit the particular conditions of that country. In the United States and Canada the manufacturers agreed that the limit be 90% (SDA, 1965) (97). Non-ionic surfact-

ants have not yet been affected by the law because of their much lesser importance and the difficulty of assessing them analytically.

The test methods used in studying surfactant biodegradation are exceedingly simple in principle. The surfactant is exposed to microorganisms in a test system, and its fate is observed by analyzing the test compound at intervals. The microorganisms chosen may be a pure strain or a mixed culture depending upon one's objective. Mixed cultures used are normally obtained from river water (30, 61, 62, 112), activated sludge (86, 102), sewage (25, 62), sewage lagoon (27), or soil (25, 83). The general microbiological make-up of mixed cultures from these sources seems to be fairly constant (65). Their performance in the laboratory could provide a realistic basis for extrapolation to field conditions. Pure cultures like Alcaligenes, Bacillus, and Pseudomonas species have been used, mainly to study the detailed mechanisms involved in biodegradation (7, 114). Ever since Degens (18) tested the biodegradability of TEEPOL, a Shell product then based on secondary alkyl sulfate, in an aquarium by analyzing the water at subsequent intervals, a variety of tests have been developed which differ significantly from each other in approach, in operational complexity, and in the extent to which they simulate actual waste treatment conditions. On the whole there are four broad categories of test methods widely used. They involve: 1) Biochemical Oxygen Demand (BOD) and Warburg respirometric tests,