MICROBIAL DEGRADATION OF ANIONIC DETERGENT IN NATURAL WATER

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ABSTRACT

Two kinds of anionic detergents, i.e. hard (branched chain alkyl benzene sulphonate, ABS) and soft (linear alkyl benzene sulphonate, LAS), were studied regarding their degradation by microorganisms in Chao Phraya river water. The standard Shake-flask test and methylene blue method, respectively, were used for determining the biodegradability of the surfactant and for assessing the amount of anionic surfactant. ABS was found to be partially degraded (17.9%), but LAS was almost completely degraded (96.8%).

INTRODUCTION

Detergents are chemical derivatives produced in great quantities around the world and detergent consumption is increasing every year due to increasing population. In Thailand, branched chain alkyl benzene sulphonate (ABS) detergents have been widely used in the past. ABS detergents are regarded as non-biodegradable and cause environmental problems. In order to avoid the problems caused by these recalcitrant molecules, linear-chain alkyl benzene sulphonates (LAS), which are more readily biodegradable, have been used in place of ABS since March 1984. In Thailand, where domestic wastewater treatment is not widely practised, very little research has been conducted on the biodegradation of local detergents by microorganisms. Currently we do not have adequate information to assess the environmental effects of detergents in Thailand, and it is necessary to gain more knowledge on their microbial degradation by using mixed culture systems obtained from the local environment.

The objectives of this study were to determine the degradation rates of biodegradable and non-biodegradable detergents (LAS and ABS) in Thailand, and to identify some groups of bacteria that could degrade them. The results obtained would give information that could be applied to predict environmental biodegradation rates and to formulate guidelines for appropriate microbiological techniques in water treatment plants, for development of water pollution control and for water monitoring programmes.

MATERIALS AND METHODS

The detergents used were packaged household synthetic detergents of Pao Boojin brand, of both ABS and LAS types. These were kindly provided by Lion (Thailand) Co., Ltd.

The method for determining the biodegradability of alkyl benzene sulphonate in this study was the Shake-flask Test[1] which is suitable for sulphonate detergents in general use. The method can be summarized as follows : microorganisms were inoculated into a flask that contained a chemically-defined microbial growth medium (basal medium) and the surfactant to be tested. Aeration was accomplished by continuous shaking of the flask. Following two adaptive transfers, biodegradation was determined by measuring the reduction in surfactant content during the test period.

The basal medium consisted of 3.0 g NH_4Cl, 1.0 g K_2HPO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.25 g KCl, 0.002 g $FeSO_4 \cdot 8H_2O$ and 0.3 g yeast extract, dissolved in one litre of distilled water. For quantitative measurements of bacteria, Tryptone Glucose Extract agar (TGA, Difco) was used. The microbial inoculum was obtained from surface water of the Chao Phraya River. The chosen sampling location was at the Phra Buddha Yotfa Bridge, which was expected to give a relatively high residual undegraded fraction of ABS and a relatively high concentration of bacteria.

Biodegradation tests of each surfactant were performed by inoculating 5 ml of inoculum (the number of bacteria in inoculum are undiluted river water, inoculum was 4.7×10^4 cfu/ml) to each of three 1-l Erlenmeyer flasks, each containing 500 ml of basal medium. One flask with only basal medium served as a blank. The remaining two were a test flask and a control flask. The test flask received 30 mg/l of surfactant while the control flask received 30 mg/l of standard dodecene-l derived LAS (SDS). Three repetitions were made for each test. All flasks were incubated at room temperature (25°-30°C) on a shaker. The dissolved oxygen concentration of all flasks was approximately 7 mg/l. Prior to the beginning of the biodegradation test, two 72 h acclimation transfers were made for each test flask for adaptation. Figure 1 describes the sequence of steps of inoculation, incubation and adaptation.

The loss of methylene blue active substance (MBAS) was checked by the standard methylene blue method.[2] Samples were removed from the shake flasks for analysis at zero time and at the end of every 24 h period until the eighth day.

Bacterial growth was measured by plate counts. A suitably diluted 1.0 ml sample was spread on duplicate TGA plates which were incubated at $35° \pm 0.5°C$ for 24-48 h. Assays were expressed as viable cells per millilitre. Pure cultures were transported to the Laboratory for Bacteriology, Division of Clinical Pathology, Department of Medical Sciences for identification of bacteria.

RESULTS AND DISCUSSION

The standard test method for assessing the biodegradability of alkyl benzene sulphonate was designed to determine the extent to which tested sulphonates would be removed by the usual methods of single treatment and discharge to effluents. If the surfactant

reduction in the presumptive test was equal to or exceeded 90%, it was classified as "adequately biodegradable" without further testing. The material was subjected to a confirmatory test only if surfactant reduction in the presumptive test was between 80% and 90%. Below 80%, the material was considered "inadequately biodegradable". In the presumptive tests, all the results for ABS were below 80% ; for LAS they were above 90%. Therefore, it was not necessary to run confirmatory tests.

The chemical composition of any laundry detergent[3] is as follows : LAS or ABS as surfactants, organic and inorganic compounds as builders, sodium sulphate as electrolytic filler, sodium perborate as bleach, and fluorescent dyes as brighteners. Many other constituents (enzymes, corrosion inhibitors, perfume etc.) are also added to provide additional desirable properties. Detergents usually contain only 10-30% surfactant,[4] 30-50% builder, 6% electrolytic filler, and 20% bleaching ingredient. In this experiment, the approximate surfactant percentages in the two different commercial detergents were measured using the methylene blue method[2] and were found to be 28.2 and 26.5 for the Pao Boonjin ABS type and LAS type, respectively. The characteristics of the Chao Phraya River water used as the microbial inoculum are shown in Table 1.

TABLE 1 Analytical results of the water samples.

Parameters	Units	Concentrations	Methods
MBAS	mg/l	0.06	Methylene blue method[2]
Chloride as Cl^-	mg/l	21.40	Mercuric nitrate method[2]
Salinity	g/l	0.0	S-C-T meter (Y-S-I model 33)
Hardness as $CaCO_3$	mg/l	97.17	Titrimetric method[2]
Alkalinity as $CaCo_3$	mg/l	96.00	Titration method[2]
Dissolved Oxygen	mg/l	1.60	Azide modification method[2]
Biochemical oxygen demand	mg/l	3.39	Azide modification method[2]
Chemical oxygen demand	mg/l	13.96	Dichromatic reflux method[2]
Electrical conductivity	uMHOS/cm	410.00	S-C-T meter (Y-S-I model 33)
pH Value		7.5	pH meter
Water temperature	°C	30.00	S-C-T meter (Y-S-I model 33)
Total Bacteria	cfu/ml	4.7×10^4	Standard plate count[2]

It was clearly shown that the bacterial population could metabolize both ABS and LAS surfactants after inoculation (Figure 2). However, the rate and extent of biological destruction of LAS were significantly higher than those of ABS ($p < 0.01$ Wilcoxson matched pairs signed ranks test). The ABS biodegradability at 17.9% was in the range of that of hard surfactants, i.e. 0-35% as classified by Swisher.[5] The LAS biodegradability at 96.8% was considered in the range of "soft" adequately biodegradable surfactants, according to the American Society for Testing and Materials (ASTM).[6]

It has been mentioned elsewhere[7,8,9,10] that the biodegradation rates of surfactants depend not only on their structures but also on many other variables. These include surfactant concentration, initial bacterial concentration, nutrients, oxygen and temperature of the environment. The tests for biodegradation rates of surfactants in Thai aquatic environment were conducted using different amounts of inoculum, before the enrichment cycle, i.e. no dilution or river water inoculum (D_0, 4.7×10^4 cfu/ml), 1:2 dilution (D_1, 2.4×10^4 cfu/ml) and 1:10 dilution (D_2, 4.7×10^3 cfu/ml). However, after the subsequent enrichment phase, the inoculum for the test flasks was approximately 10^9 cells per ml, yielding an initial concentration of approximately 2×10^6 cells per ml. Results of biodegradation tests are shown in Figures 3 and 4. It was found that bacterial counts in all tests were equivalent but that the extent of degradation depended upon the initial dilution of river water before enrichment. With no dilution and with 1:2 dilution, there was no difference in amount of degradation for the two types of detergents. At 1:10 dilution there was no significant degradation. A similar effect was reported by Wayman and Robertson.[8] These results probably indicate that the degradative bacteria were either diluted out or reduced below competitive levels at the start of the enrichment process. It also indicates that they are not the dominant flora in the Chao Phraya river water.

The disappearance of surfactant in the absence of surfactant-degrading bacteria can occur by bacterial adsorption.[5] This result is in accordance with Fuhrmann *et al.*[7] This process may account for the low level of apparent degradation in the 1:10 dilution tests. Since the degree of biodegradation depends upon the bacterial species present, bacteria which grew in the test cultures were identified. The results are presented in Table 2. It was found that *Pseudomonas* spp. were predominant in the LAS test cultures, while only a few were found in the ABS test cultures. In the control flask, *Pseudomonas* spp. were rare. The present results agree with those of other scientists[4,11,12] who report that *Pseudomonas* spp. occupy a prominent place among detergent degrading bacterial groups.

As stated before, the biodegradation rate of surfactants also depends upon oxygen and temperature. Wayman and Robertson[8] found that the degradation of surfactant was better under aerobic conditions than under anaerobic conditions. ABS-type surfactants cannot be degraded at all under anaerobic conditions.[9] Many researchers[8,9,11,13] have shown that the biodegradation of ABS and LAS surfactants is markedlly poorer at low temperatures than at high temperatures. Halvorson and Ishaque[9] indicated that a temperature change from 25°C to 10°C, would increase the time required for the biodegradation of a fixed amount of surfactant. At 0°C, no biodegradation of ABS-type detergents occurred. Hence, in the Thai aquatic environment (Table 1) where the water temperature is 30°C and oxygen concentration is 1.6 mg/l, the biodegradation rate of both LAS-and ABS-type detergents should be quite good. Pretresa[10] used the bacteria from the natural environment in Thailand at 10^4 cfu/ml for testing the

biodegradability of surfactants under European environmental conditions and found that 90% LAS and around 30% ABS were degraded after 14 days. However, in our experiments these respective percentages of degradation for ABS and LAS surfactants were achieved after only 8 days.

TABLE 2 Groups of bacteria isolated from the test cultures.

Groups of bacteria	Surfactant degraded or supporting growth
1. Facultatively anaerobic gram negative rods	
Aeromonas caviae	Control, ABS, LAS
Enterobacter cloacae	ABS, LAS
Klebsiella ozaenae	Control, ABS, LAS
Pasteurella multocida	LAS
Serratia liquefaciens	LAS
2. Gram negative aerobic rods, non-fermented groups	
Acinobacter calcoaceticus	Control
Alcaligenes denitrificans	Control
Moraxella osloensis	Control
Pseudomonas aeruginosa	Control, ABS, LAS
Pseudomonas putida	Control, LAS
Pseudomonas sp. (va-l gr)	Control
Pseudomonas stutzeri	ABS
3. Gram-positive aerobic cocci, non-fermented groups	
Corynebacterium bovis	Control
Micrococcus luteus	Control, ABS
Micrococcus varians	Control, ABS
Staphylococcus epidermidis	ABS
Staphylococcus sp.	Control

Note : *Pseudomonas aeruginosa* was the predominant microbe in LAS culture. There were few cells of this species in ABS cultures and very few in control cultures.

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บทคัดย่อ

การศึกษาการย่อยสลายตัวของสารลดแรงตึงผิวอนุมูลประจุลบในผงซักฟอกทั้งชนิด hard detergent (ABS) และ soft detergent (LAS) โดยจุลินทรีย์ที่มีอยู่ในแม่น้ำ (เจ้าพระยา) การทดลองหาการย่อยสลายตัวใช้วิธี Shake-flask Test และการหาปริมาณของสารอนุมูลประจุลบด้วยวิธี methylene blue พบว่าการสลายตัวของสารลดแรงตึงผิวอนุมูลประจุลบชนิด ABS ย่อยสลายได้เพียงบางส่วน (17.9%) ในขณะที่ LAS ย่อยสลายได้เกือบทั้งหมด (96.8%) อัตราการย่อยสลายของ ABS และ LAS ไม่มีความสัมพันธ์กับปริมาณแบคทีเรีย

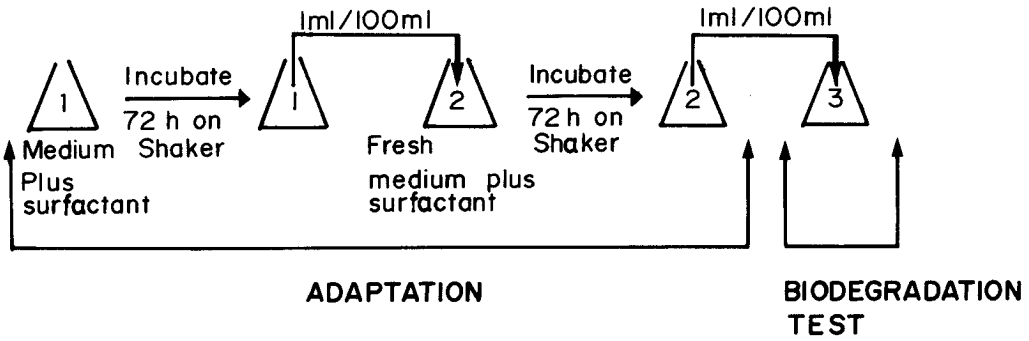

Fig. 1 Steps of inoculation, incubation and adaptation for the Shake-flask Test.

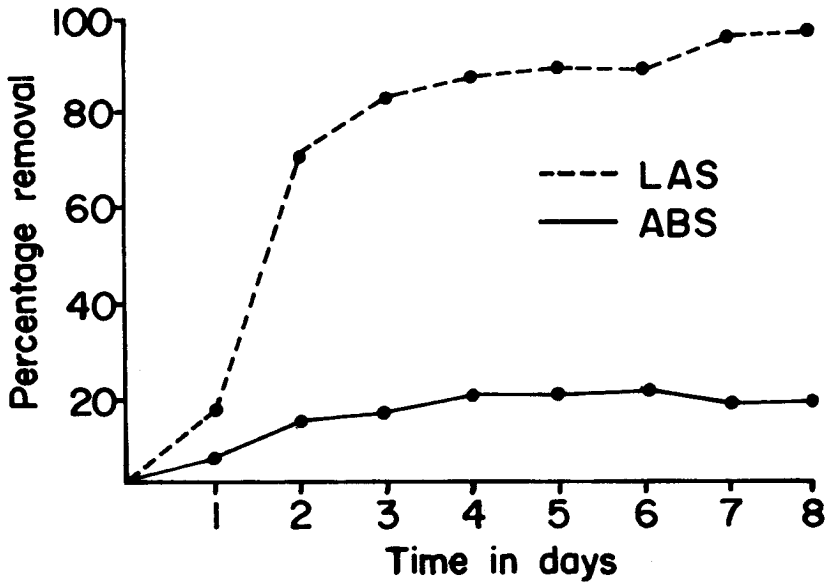

Fig. 2. The biodegradation of branched - and straight-chain alkyl benzene sulphonates in the Shake-flask Test.

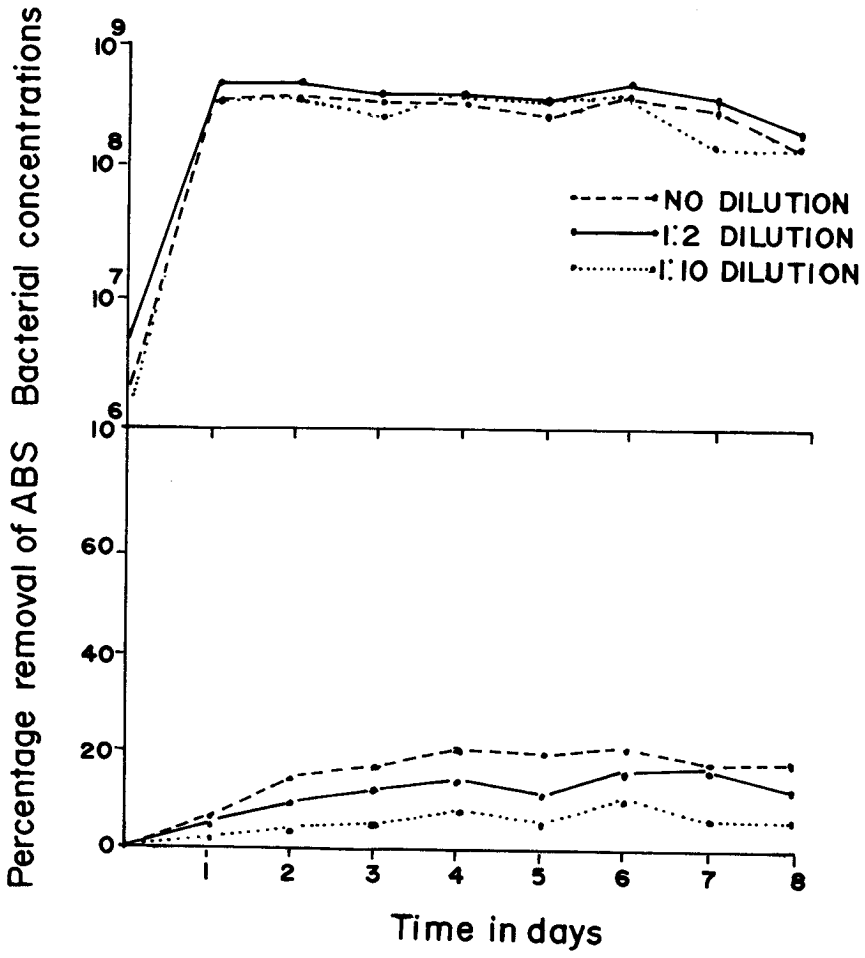

Fig. 3 Daily interaction between bacterial concentrations and biodegradation rates of branched chain ABS.

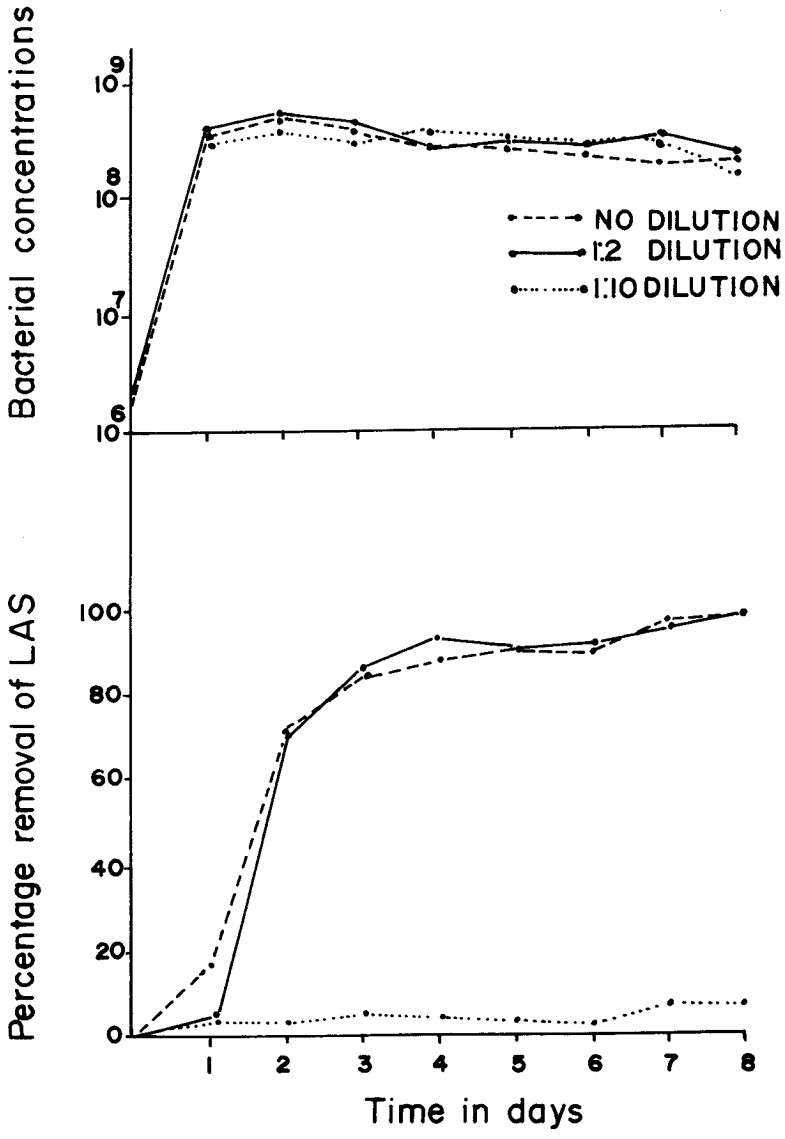

Fig. 4 Daily interaction between bacterial concentrations and biodegradation rates of straight chain LAS.