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Biodegradation of and comparison of Adaptability to Detergents

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미생물에 의한 계면활성제의 분해능과 적응력의 비교

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ABSTRACT

Microorganisms utilizing anionic detergent as their carbon and sulfur sources were isolated from soils and sewages. Alkyl benzene sulfonate (Hiti) and sodium dodecyl sulfonate (SDS) were the detergent compound tested.

Three of these isolated microorganisms were identified as *Pseudomonas* spp. and the others as *Klebsiella*, *Enterobacter* and *Acinetobacter*.

Biodegradation rate of the detergents and growth rate of *Acinetobacter* strain Ⅱ-8, *Pseudomonas* strain H-3-1 and 554 among six isolated microorganisms were investigated with colorimetric, warburg manometric, and ultraviolet absorption analyses.

By performance of 4 serial successive tranfer to new culture broth for the purpose of adaptation method, ABS and SDS could be degraded to far more than 40%~60% and 70%~75%, respectively. However the employment of nonadaptation method, ABS and SDS were degraded to 30%~45% and 45%~65%, respectively.

In another words, detergents degradation ability was increased to a certain extent by successive transfer to the new minimal media.

We would conclude that the development of adaptation was effective in the removal of recalcitrant compounds.

INTRODUCTION

Alkyl benzene sulfonates constitute the major class of detergent in use for household purposes, therefore play the greatest part in water pollution problems.

The ABS which has been in use for the past decade or more, is derived from tetrapropylene, a product of the petroleum industry and is a mixture of several hundred isomers and homologs with highly branched alkyl groups ranging from ten to fifteen carbon atoms with an average of twelve (Swisher, 1964).

For more than 20 years many reports were

published about the biodegradation of alkyl benzene sulfonate and sodium dodecyl benzene sulfonate with many analytical methods.

The general analytical methods of detergent biodegradation were methylene blue-detergent complex method (Hayashi, 1975, Hsh, 1963) and barium chloranilate method (Hsu, 1963).

Recently Huddleston and Nielsen (Huddleeston et al, 1979) showed that the biodegradation pathway consisted of many steps and the loss of surface activity ocurred early in the sequence (Huddleston et al, 1963). The degree of linear alkyl bezene sulfonate breakdown necessary for the loss of surface activity was found to approximately coincide with loss of reactivity to the dye, methylene blue, and was later termed primary biodegradation.

In this reports, the biodegradation ability and growth rate of *Pseudomonas* strain 554 and H-3-1 and *Acinetobacter* Ⅱ-8 among the six isolates were investigated with colorimetric, manometric, ultraviolet absorbance analytical method.

MATERIALS AND METHODS

1. Isolation and Identification bacterial strains

Alkyl benzene sulfonate (ABS) utilizing microorganisms were isolated from various sewages and soils by repetition of plating alternately on minimal salt medium (Ohwada, 1975). It's compositions are as follows; $(NH_4)_2SO_4$ 2.0g, KH_2PO_4 2.0g, Na_2HPO_4 3.0g, $MgSO_4$ 0.01g, $FeCl_3$ 0.01g, distilled water 1,000m*l* and pH was adjusted to 7.2.

General characteristics of six isolated microorganisms were examined according to the method in "Biochemical test for identification of medical bacteria" (S.T. Cowan) and "Methods in Microbiology" (J.R. Norris, D.W. Ribbons, and C. Booth) and other papers.

2. Preparation of inoculum

The flask containing 100m*l* of nutrient medium was inoculated with 0.1m*l* of precultured strains and incubated at 35°C for 15 hours aerobically in rotatory shaker.

3. Measurement of metabolic activity

Each side arm contained 0.3m*l* of a suspension of three times washed cells (1mg dry weight/m*l*) in 0.1mol phosphate buffer, pH 7.0. The main flask contained 3m*l* of minimal salt medium and 100ppm of ABS were proided in it. The center cells each contained 0.4m*l* of 30% NaOH. After these microflask were incubated at 35°C after mixing strain with medium.

4. Quantitative analysis of detergents

Alkyl benzene sulfonate was obtained from Lucky company products (Hiti) and sodium dodecyl sulfonate from sigma chemicals. Methylene blue and chloroform was the product of E. Merck, Darmastadt. Quantitative analysis of detergents followed Hayashi's Method. (Hayashi, 1975)

5. Growth in minimal media

Growth in minimal salt medium containing detergent as carbon source was determined by turbidity. Turbidity was measured at 420 nm with CE 272 linear readout ultraviolet spectropho tometer.

RESULTS AND DISCUSSIONS

1. Identification of isolated strains

Six isolated strains were identified according to Bergey's Manual of Determinative Bacteriology (eight edition). The morphological and physiological characteristics of

six isolated strains were described in Table 1. The utilization of various carbon sources was shown in Table 2.

All of the strains were Gram-negative, rodform, aerobic, catalase-positive, starch hydrolysis-negative. Four strains among them were motile with single or bipolar flagella. As shown in Table 1 and 2, three strains were identified to be Genus *Pseudomonas* and others Genus *Klebsiella*, *Enterobacter* and *Acinetobacter* (Table 3).

Table 1. Characteristics of Isolates

	H-3-1	Ⅱ-1	554	Ⅱ-3	Ⅱ-8	552
Gram Stain	−	−	−	−	−	−
Form	rod	rod	rod	rod	rod	rod
No. of Flagella	1	0	1	1~2	0	1
Growth in Air	+	+	+	+	+	+
Growtg Anaerobically	−	+	−	+	−	−
Oxidation/Fermentation	O	O/F	O	O	O	O
Growth at 41°C	−	+	−	+	+	−
Motility	+	−	+	+	−	+

Table 1. Characteristics of Isolates (Ⅱ)

	H-3-1	Ⅱ-1	554	Ⅱ-3	Ⅱ-8	552
Oxidase	−	−	+	−	−	+
Catalase	+	+	+	+	+	+
Starch Hydrolysis	−	−	−	−	−	−
Decarboxylase Tests						
Lysine	+	+	−	+	−	−
Ornithine	−	−	−	−	−	−
Arginine Dehydrolase	−	−	−	−	−	−
Phenylalanine Deaminase	−	−	−	−	−	−
Indole	−	+	+	+	+	−
MR	−	−	+	−	−	−
VP	+	+	−	+	+	−
Cimon's Citrate	+	+	+	+	−	−
Nitrate Reduction	−	+	+	−	+	−
Urea	+	+	+	+	+	−
KCN	+	+	+	+	+	−
H_2S	−	−	−	−	−	−

Table 2. Utilization of Carbon Sources

Isolants	H-3-1	Ⅱ-1	554	Ⅱ-3	Ⅱ-8	552
Carbon Sources						
Malonate	+	+	−	+	+	−
Glucose/Gas	+/+	+/+	+/−	−/+	+/+	+/−
Lactose	+	+	+	+	+	−
Sucrose	+	+	+	+	+	+
Mannose	+	+	+	+	+	−
Dulcitol	−	−	−	+	+	−
Salicin	+	+	+	+	+	−
Adonitol	+	+	+	+	+	+
Inositol	+	+	−	+	+	−
Sorbitol	+	+	−	+	+	−
Arabinose	+	+	+	+	+	+
Raffinose	+	+	+	+	+	−
Ramnose	+	+	−	+	+	−
Fructose	+	+	+	+	+	+
Galactose	−	+	+	+	+	+
Xylose	+	+	+	+	+	+
ONPG	+	+	−	+	+	+

Table 3. Identification of 6 Isolated Strains

Strains	Identification
H-3-1	*Pseudomonas syringae*
Ⅱ-1	*Klebsiella pneumonia*
554	*Pseudomonas flava*
Ⅱ-3	*Entrobacter aerogenes*
Ⅱ-8	*Acinetobacter calcoaceticus*
552	*Pseudomonas cichorii*

2. Degradation of detergents

The metabolic activity was measured by O_2 uptakes of six isolated strains with manometric method (Cain et al, 1968. Heyman et al, 1968. Huddleston et al, 1979.). Figure 1 shows that the oxidation of *Acinetobacter* strain Ⅱ-8, *Pseudomonas* strain 554 and H-3-1 was more rapidly occured. These three strains were selected in the further experiments.

Since the alkyl benzene sulfonate and sodium dodecyl sulfonate are toxic to microorganisms above some criteria, the optimal

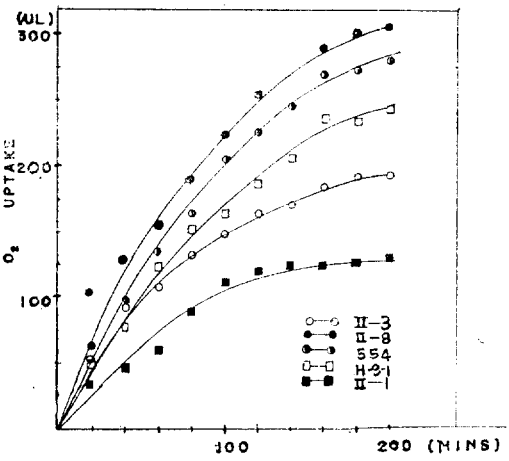

Fig. 1. O_2 uptakes of six isolated strains on the minimal salt medium containing 100ppm ABS were measured by Yanaco Warburg manometry.

concentration of these detergents is important (Huddleston et al, 1979). As shown in figure 2 and 3, when $3\text{-}4\times10^{10}$ cells per m*l* were inoculated, *Pseudomonas* strain H-3-1, and 554, and *Acinetobacter* strain Ⅱ-8 degraded ABS optimally in the media containing 250 ppm ABS. But *Pseudomonus* strain H-3-1 and 554 and *Acinetobacter* strain Ⅱ-8 degraded SDS optimally in the media containing 250 ppm, 250ppm and 500ppm SDS, respectively. ABS and SDS in the media containing more than 1,000ppm are toxic to microorganisms.

When 7×10^9 cells of *Acinetobacter* strain Ⅱ-8 per m*l* was inoculated, the extent of biodegra dation against time of two structurally different anionic detergents was shown in figure 4. SDS was degraded more rapidly than ABS (Allred et al, 1964). The results were obtained by methylene blue colorimetric analysis.

The results in figure 5 shows the effects of glucose and yeast extract on the degradation of ABS and SDS. In this experiment 0.03% yeast extract and 1.5g/l glucose were

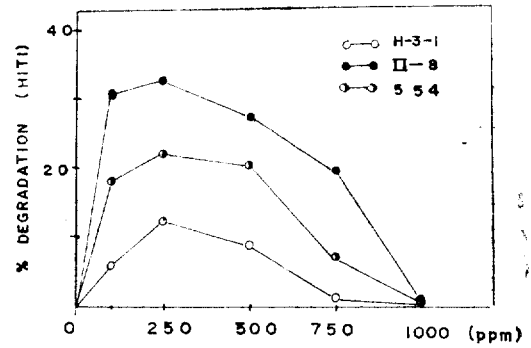

Fig. 2 Effects of varying concentration of ABS on the quantity of substrate utilized ay strain Ⅱ-8, 554 and H-3-1. % cegradation=utilized ABS/ initial ABS ×100; culture time was 24 hours.

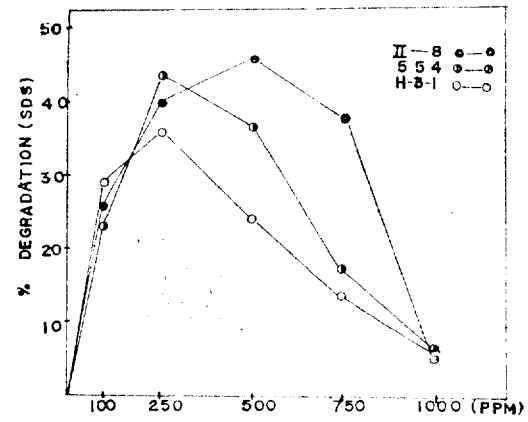

Fig. 3 Effects of varying concentration of SDS as a carbon source utilized by strain Ⅱ-8, 554 and H-3-1. Culture time was 24 hours.

added in the minimal salt detergent media. ABS and SDS were degraded more rapidly in the media containing yeast extract than glucose. Addition of yeast extract was more effective on the degradation of detergent than that of glucose.

These results suggest that the metabolism of ABS and SDS could be carried out by microorganisms isolated from nature. If an energy source other than the detergent molecule itself was supplied, the degradation of

detergent was occured more rapidly than without energy source(Bernarde et al, 1965). We would conclude that the permeability effect of detergent is dependent on the metabolic state of cells, such as energized or nonenergized cells (Komor et al, 1979).

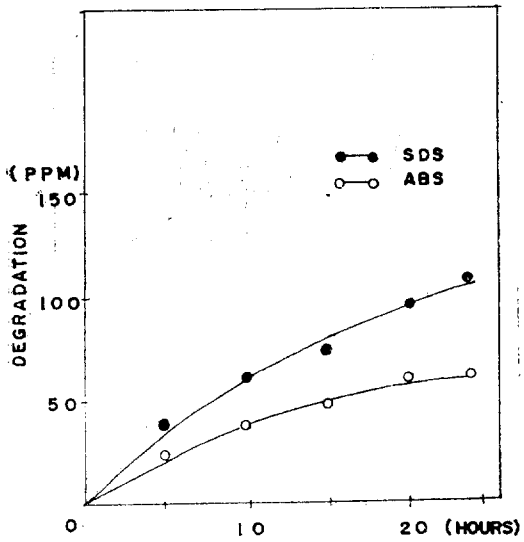

Fig. 4 Biodegrdaaion of sodium dodecyl sulfonate and alkyl benzene sulfonate; the concentration of SDS and ABS were 250 ppm.

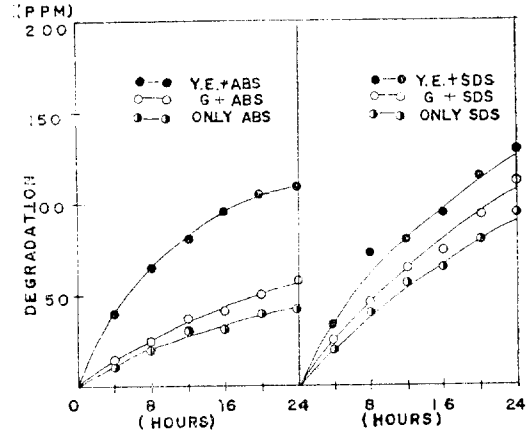

Fig. 5 The effects of glucose and yeast extract on the degradation of ABS and SDS. Viable cell number of isolated strain Ⅱ-8 was 3.4×10^9 cells/*ml* and 2.7×10^8 cells/*ml* in the media containing ABS and SDS, respectively.

Many reports of enzymatic studies on the microbial degradation of Alkyl benzene sulfonates with short alkylchain length were carried out (Voss, 1964. Willetts et al, 1970. Willetts et al, 1972. Willetts, 1974). Microorganisms oxidised the alkyl side chain by a β-oxidation or by a combination of an initial w-oxidation plus subsequent β-oxidation(Willetts, 1974).

Degradation aromatic compounds are carried out through the β-ketoadipate pathway, especially through the ortho cleavage or meta cleavage.

In this report, enzyme activity was not detected, however, ring cleavage was detected by ultraviolet absorbance (Swisher, 1968) and increase of respiration rate (Payne et al, 1963).

The validity of the ultraviolet analytical method has been discussed in detail elsewhere (Swisher, 1968). The benzene ring has two very intense and characteristic absorption bands which in the case of ABS appear at 193nm and 223nm (Fig. 6). So the disappearance of the bands should be good evidence for destruction of the rings.

Precultured *Acinetobacter* strain Ⅱ-8 cells were washed three times with 0.1M phosphate buffer (pH 7) after harvesting 3.4×10^9 cells per m*l* were transfered into the minimal salt media containing ABS and 0.03% yeast extract and incubated 35C with sufficient aeration and agitation. In each regular interval 10m*l* of culture broth were centrifuged at 10,000rpm for 10 minutes. The supernatant was diluted to one tenth in 0.1M phosphate buffer (pH 7.2) and ultraviolet spectrum was measured. Spectrum measurements were made in 1cm silica cells and give a light path equivalent to 1mm and the referance was 0.1M phosphate buffer (pH 7.2).

The spectra in Fig. 7 indicates that subs-

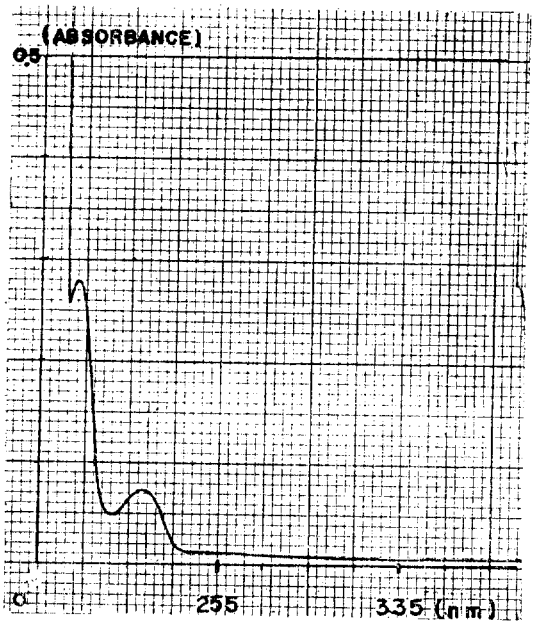

Fig. 6 UV spectrum of ABS (25mg/*l*). Solutions were diluted with 0.1 M phosphate buffer (pH 7.0), measured against 0.1M phosphate buffer (pH 7.0) as reference in 1cm cells.

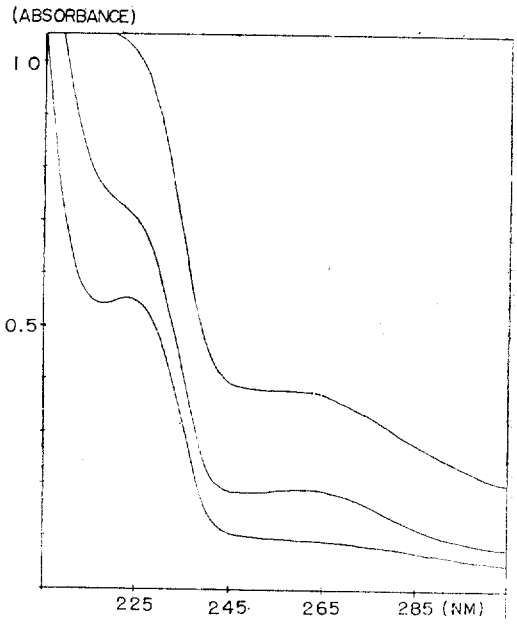

Fig. 7 Ring cleavage of alkyl benzene sulfonate

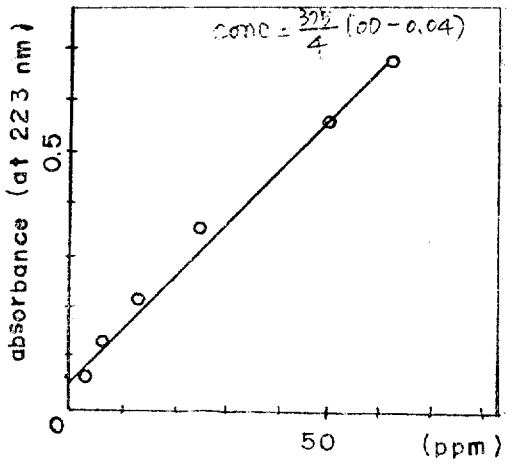

Fig. 8 Relationship between UV absorbance and ABS concentration.

tantially ring degradation in ABS would be accoumplished in above media.

According to the Fig. 7 and 8, in 15 hours, 15% of ultraviolet absorbance at 223nm were disappeared and in 24 hours 36.25% of that were disappeared (Cain et al, 1968. Willetts et al, 1979). The absorbance at near 260nm is due to the 300mg/*l* of yeast extract.

Adaptation (Fredricks, 1966) means that 1 m*l* of precultured culture broth were transfered serially into the minimal media containing minimal salts and 250ppm detergent and 0.03% yeast extract. In this experiment four successive transfers were carried out at 35C.

When ABS was used as carbon source, the growth rate and degradation ability and benzene ring cleavage in the adapted state were 100~125 fold, 120~135 fold, 115~130 fold increased than that in the nonadapted state at the stationary phase, respectively. However, in 24 hours incubation, the growth rate and degradation ability and benzene ring cleavage were 450~800 fold, 400~600 fold, 400~1,000 fold increased, respectively (Fig. 9~11).

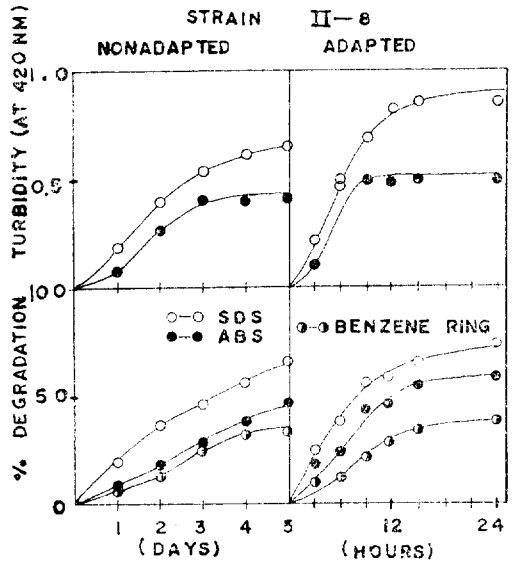

Fig. 9 The comparison of growth and degradation rate in the adapted state with that in the nonadapted one by strain II-8.

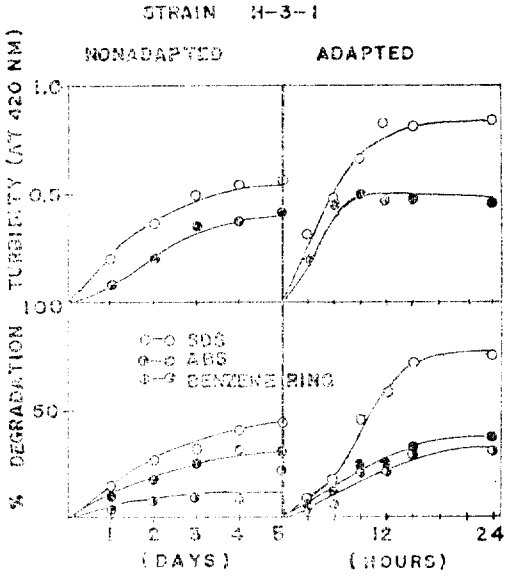

Fig. 10 The comparison of growth and degradation rate in the adapted state with that in the nonadapted one by strain H-3-1.

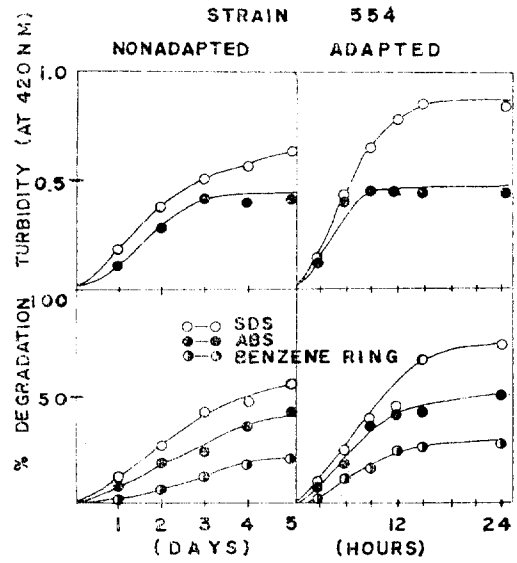

Fig. 11 The comparison of growth and degradation rate in the adapted state with that in the nonadapted one by strain 554.

When media was containing SDS as carbon source, the growth rate and degradation ability were 135~150 fold, 120~170 fold increased at the stationary phase, respectively, however, 400~450 fold and 450~550 fold increased in 24 hours incubation (Fig. 9~11).

These results suggest that the development of adaptation was effective in the removal of recalcitrant compounds.

摘 要

탄소원으로 alkyl benzene sulfonate(Hiti)와 sodium dodecyl sulfonate를 이용하는 미생물을 토양이나 폐수에서 분리하여 동정하였다.

분리한 미생물중의 세균주는 *Pseudomonas* spp로 동정되었고 다른 세균주는 *Klebsiella*, *Enterobacter*, *Acinetobacter* 로 동정되었다.

6개의 분리된 균주중에서 *Acinetobacter* 균주 II-8와 *Pseudomonas* 균주 554, H-3-1에 의한 계면활성제의 생분해능과 생장력을 압력계, 비색측정기, 자외

선 흡광도 분석기등을 사용하여 측정하였다.

적응을 시키기 위하여 계면활성제가 첨가되어 있는 새로운 배지에 계속적으로 균을 옮겨주므로서, ABS와 SDS의 분해가 각각 40%~60%, 70%~75% 이상으로 증가하였다. 그러나 그렇지 않을 때에는 30%~45%, 45%~65% 이었다. 다시 말해서 ABS나 SDS가 첨가되어 있는 최소배지에 계속적으로 옮겨주므로써, 계면활성제의 분해능은 어느정도까지 증가하였다. 따라서 적응은 자연상태에서 쉽게 분해되지 않는 오염물의 제거를 위해선 일차적으로 중요하다는 것을 알수 있다.

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