Biodegradation Mechanism of Linear Alkylbenzenesulfonate- ^{14}C

Tadayoshi KUBODERA, Toshio MUTO, and
Tatsuo YAMAMOTO

Tokyo Metropolitan Isotope Research Center, (2-11-1,
Fukazawa, Setagaya-ku, Tokyo)

The biodegradation of linear alkylbenzenesulfonate- ^{14}C (LAS- ^{14}C) tagged with ^{14}C at the linear side chain was studied on activated sludge by tracer method in addition to the methylene blue method which is widely employed in the biodegradation of LAS. It was found that there were three periods of rapid adsorption period, acclimation period, and degradation process. The radiolysis of dodecylbenzenesulfonate was studied on irradiating by 5000 Ci ^{60}Co source.

The decomposition products were identified by GLC and GC-MS spectrometry after desulfonation. 1-Tetralone, 1-indanone, 4-methyl-1-tetralone, naphthalene *et al.* were found in them.

1 Introduction

In view of environmental problems, a waste water treatment is becoming more pressing problem as population densities increase. Now synthetic detergents (syndets) are used widely in housewashing and in industry, therefore, amounts of syndets running into sewage treatment plants are increasing more and more every year. The fate of syndets in sewage treatment is interest from the standpoint of their effect on an operation of sewage treatment plants and of possible contamination of receiving waters. Although a detergent may be partially inactivated by adsorption on or by reaction with the soil which is removed during washing, almost all the syndets used in households enter into sewers and are carried into sewage treatment plants. So it may be significant to study the behavior of syndets in river and the sea. Many reports have been published on the biodegradation mechanism of linear alkylbenzenesulfonates (LAS).^{1)~7)}

Previously the authors⁸⁾ synthesized the linear alkylbenzenesulfonate labelled with ^{35}S and studied its biodegradation using activated sludge. It was found that LAS- ^{35}S was predominately adsorbed by activated sludge in

the initial step and it was gradually decomposed. However, since a sulfonic group is linked to a benzene ring, the aspect on decomposition of the alkyl side chain was not apparent in this experiment of the LAS tagged with ^{35}S . Therefore, dodecylbenzenesulfonate ^{14}C (DBS- ^{14}C) labelled with ^{14}C at the α position of its alkyl side chain was prepared in order to study the decomposition of the alkyl side chain. This report concerns with a preliminary experiment of the biodegradation of DBS- ^{14}C labelled at α carbon atom of its alkyl side chain.

2 Experimental procedures

2.1 Preparation of DBS- ^{14}C

Dodecyl alcohol- ^{14}C obtained by Grignard reaction with para formaldehyde- ^{14}C and undecyl chloride, was chlorinated with thionyl chloride as usual. The dodecyl chloride- ^{14}C was condensed with benzene by the Friedel-Crafts method, then the alkylbenzene was sulfonated with fume or conc. H_2SO_4 , and neutralized with NaOH . Purified commercial undecyl chloride by distillation was used as a starting material. The purity of dodecyl alcohol- ^{14}C , dodecyl chloride- ^{14}C and dodecylbenzene- ^{14}C was identified by gas chromatography.

graphy and radiogaschromatography. The purity of DBS- ^{14}C was determined by IR spectroscopy and paper chromatography and its specific radioactivity was 24 mCi/mol. The details of the preparation were published by the authors⁹⁾.

2.2 Biodegradation of DBS- ^{14}C

The culture solution of DBS- ^{14}C was prepared as shown in Table-1.

Table-1 Composition of medium used in shake-flask studies.

Component	mg/l
KH_2PO_4	8.5
K_2HPO_4	21.75
Na_2HPO_4 2 aq	33.4
NH_4Cl	1.7
MgSO_4 7 aq	22.5
CaCl_2	27.5
FeCl_3 6 aq	0.25

Distilled water

DBS- ^{14}C 76.0 ppm

Total volume of the solution: 2000 ml

Two liters of this solution were added into a Sakaguchi flask and 30 ml of activated sludge (suspended solid concentration was 0.6%) obtained from Ochiai waste water treatment plant was added, then the mixed liquor was incubated at 24°C in a reciprocating shaker. The concentration of DBS was determined by the methylene blue method (Fig.-1).

2.3 Measurement of radioactivities

Radioactivities of the cultivated solutions were determined to obtain the information for decomposition and adsorption of DBS on activated sludge. After the cultivated solution was filtered through a milipore filter (0.8 μm pore size) and a part of the filtrate was extracted with the same volume of hexane, radioactivities of its water layer and hexane layer were determined by use of a Packard Model 3320 liquid scintillation counter (Fig.-2).

2.4 Analysis of biodegradation products

The decomposition products in the residual solution were identified by GLC after desulfonation: The desulfonation was practised by the Knight method¹¹⁾ as follows. Four hundred milliliters of the residual solution were placed into a flask and were evaporated until 2~4 ml, and then phosphoric acid and phosphorous

pentoxide were added. The solution was heated at 200°C for 15~16 h and extracted with each 100 ml of hexane three times after cooling. The hexane extract was concentrated to about 2 ml and was determined by GLC (FID) and GC-MS spectrometry.

GLC (FID) column: 0.3 cm $\times$ 1.7 m, stationary phase: PEG-20 M, temp.: 200°C.

The biodegradation products were analyzed at three stages of degradation, including the initial acclimation period (at 24 h), degradation process (at 46~48 h) and the initial period of complete degradation (at 68~70 h and 93~120 h).

2.5 Radiolysis

The DBS solution of 530 ppm was irradiated by 5000 Ci ^{60}Co source at dose rate of 1.3×10^5 R/h. The concentration of DBS decreased to 208 ppm at irradiation of 18 h. After desulfonation, components in the irradiated DBS solution were analyzed by GLC and GC-MS spectrometry.

3 Results and Discussion

3.1 The change in concentration of methylene blue active substances (MBAS)

The result obtained by methylene blue method was shown in Fig.-1.

Fig.-1 Biodegradation of DBS- ^{14}C followed by MB method.

In the course of 10 min after addition of activated sludge to the cultivated solution, the concentration of DBS rapidly decreased from 76.0 ppm to 65.6 ppm and continued to decrease until 10 h. It seems that the rapid decrease in the concentration of DBS is based upon the adsorption of DBS on activated sludge. The

curve shows an induction period (from 10 h to 35 h) during which there is little change in surfactant concentration. This is the period of acclimation during which appropriate enzymes of bacteria become adapted to oxidize DBS. From 40~50 h, a rapid degradation of DBS ensued and MBAS concentration became less than 0.6 ppm at 90 h. This phenomenon shows characteristics of a readily degradable DBS.

3.2 Radioactivity method

As shown in **Fig.-2**, the radioactivity, as well as the decrease in MBAS concentration, continued to decrease rapidly until 10 h and reached to the minimum value at 25 h. The phenomenon in this period (range A in **Fig.-2**) occurred by adsorption of DBS on activated sludge.

Fig.-2 Biodegradation of DBS- ^{14}C followed by tracer method.

The radioactivity increased in a period from 25 h to 70 h (range B in **Fig.-2**) and the increase of radioactivity reached to maximum at 70 h. It can be considered that, in this range B, DBS- ^{14}C adsorbed by activated sludge was biodegraded by bacteria and that water soluble biodegradation products eluted into water. Formation of water soluble biodegradation products would be shown by increase of activity at 40 h~70 h (in **Fig.-2**) and by decrease of MBAS (in **Fig.-1**). The increase of the radioactivity corresponds to the decrease of MBAS. After that, the radioactivity dropped gradually and was kept constant.

3.3 Analysis of biodegradation products

Swisher⁵⁾ studied the biodegradation of LAS in river water and identified 1-indanone, 1-tetralone and etc. as the interim biodegradation

products. So the authors carried out a similar experiment using high concentration of DBS and activated sludge. As shown in **Fig.-3**, gas chromatogram of the biodegradation products after desulfonation indicated three major peaks (a, b, c). The mass spectrum of the peak (a) in **Fig.-3** corresponds to the spectrum of 1-indanone (**Fig.-4**).

Fig.-3 Gas chromatogram of biodegradation products after desulfonation.

Fig.-4 Mass spectrum of peak (a) (1-indanone).

3-Phenylpropionic acid produced by biodegradation would undergo cyclodehydration during desulfonation to give 1-indanone. The peak (b) in **Fig.-3** corresponds to 1-tetralone. 4-Phenylbutyric acid would undergo cyclodehydration to give 1-tetralone as in the case of 1-indanone. The peak (c) was identified as dodecylbenzene. **Fig.-5** and **Fig.-6** show bio-

Fig.-5 Biodegradation of DBS. (initial concentration : 28 ppm)

Fig. 6 Biodegradation of DBS. (initial concentration : 78 ppm)

degradation curves of different initial concentrations of DBS (28 ppm and 78 ppm) obtained by MB method. The compositions of the biodegradation products obtained at each stage and then desulfonated were shown in **Table-2** and **Table-3**.

Table-2 The components of biodegradation product in each solution after desulfonation. (initial concentration : 28 ppm)

	A%	B%	C%	D%
1-Indanone	—	—	1.8	1.5
1-Tetralone	0.6	11.9	90.3	93.5
Dodecylbenzene	91.1	63.3	0.8	—
Unknown product	—	—	7.1	5.0
	8.3	24.8	—	—

Table-3 The components of biodegradation product in each solution after desulfonation. (initial concentration : 78 ppm)

	A%	B%	C%	D%
Naphthalene	—	—	—	34.6
Unknown product	3.7	4.1	—	—
1-Indanone	—	—	0.5	1.5
1-Tetralone	—	—	97.5	62.3
Dodecylbenzene	92.1	92.5	0.7	0.7
4-Methyl-1-tetralone	—	—	1.3	0.9
Unknown product	4.2	3.4	—	—

In **Table-2** solution A (sampled at 24 h) mainly contained dodecylbenzene. It shows that biodegradation of DBS proceeded scarcely. In solution B (at 48 h), ratio of dodecylbenzene decreased and those of 4-phenylbutyric acid and unknown products increased. And in solu-

tion C (at 68 h) and D (at 120 h), the composition ratio of dodecylbenzene was very little (0.8% and trace), while that of 1-tetralone much increased (90.3% and 93.5%). This phenomenon shows that DBS was decomposed to 4-phenylbutyric acid by biodegradation. A small amount of an unknown product and 1-indanone were observed. In **Fig.-6** and **Table-3**, solution B (27% degradation at 46 h) mainly consisted of dodecylbenzene, so DBS seemed to be little degraded. The main component of solution C (at 71 h) was 1-tetralone (formed from 4-phenylbutyric acid by desulfonation) and small amounts of 1-indanone (from 3-phenylpropionic acid), dodecylbenzene and 4-methyl-1-tetralone were confirmed. Also it was found that DBS decomposed completely to yield 1-tetralone (62.3%) and naphthalene (34.6%) from the composition of solution D (at 94 h). The origin of naphthalene was not known yet. This fact shows that 1-tetralone was formed by decomposition of dodecylbenzene and was further degraded to an unknown product. From this result, it seems that DBS was rapidly biodegraded to 4-(α -sulfophenyl) butyric acid, since the presence of the intermediates from DBS to 4-phenylbutyric acid, were not confirmed. Biodegradation of DBS begins with the ω -oxidation of a terminal methyl group of a side chain, following gradual β -oxidation of the resulted carboxylic acid. Although it is said that biodegradation of DBS occurs by β -oxidation on

Fig.-7 Gas chromatogram of DBS in radiolysis after desulfonation.

the straight side chain and DBS loses two carbon atoms successively, the above mentioned intermediates which have the carbon atoms from 5 to 11 in a side chain, were not detected in this experiment.

The gas chromatogram of products in radiolysis, desulfonated by 100% H_3PO_4 after radiolysis, was shown in **Fig.-7**. The peak B was identified to be dodecylbenzene from DBS but the peak A was not identified yet.

4 Conclusion

The following fact was shown in biodegradation of DBS- ^{14}C by the tracer method. At first, DBS was adsorbed on activated sludge and then syndet adsorbed was biodegraded on activated sludge and changed into water soluble compounds. Activity of ^{14}C at 10 h **Fig.-3**) was less than activity at 70 h in water layer because substances- ^{14}C were removed with activated sludge by filtration or escaped from the system as decomposed gases. But escaping of activity as decomposed gases is not considered in a short time (10 min~60 min). Therefore, the rapid decrease of MBAS (13.7% decrease) for 10 min and the decrease of activity (in **Fig.-2**) seems to be occurred by adsorption of DBS on activated sludge. 1-Tetralone (from 4-phenylbutyric acid) and 1-indanone (from 3-phenylpropionic acid) were also identified in the biodegradation products as mentioned in Swishers study on the biodegradation of LAS in river water. It seems that DBS may yield directly to 4-phenylbutyric acid and 3-phenylpropionic acid or may decompose very rapidly to those acids by the successive elimination of two carbon atoms from the terminal carbon.

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直鎖アルキルベンゼンスルホン酸- ^{14}C の生分解

窪寺忠良・武藤利雄・山本龍夫

東京都アイソトープ総合研究所

直鎖を ^{14}C で標識した DBS- ^{14}C を用いてその生分解性をトレーサー法と、それに合わせて LAS の分析法として広く使用されているメチレンブルー法で実験を行った。活性汚泥を用いて、DBS 溶液を $24^{\circ}C$ にて生分解を行わせた。その分解過程には吸着、じゅん化、分解の3期間があると考えられる。5000 キュリーのコバルト 60 で DBS の放射線分解を行った。

分解生成物は GLC と GC-MS スペクトルで確認した。それらの中に 1-テトラロン、1-インダノン、4-メチル-1-テトラロン、ナフタレン等が見いだされた。