Biodegradation and Aquatic Toxicity of Alkyl Polyglycoside

Yasuhiko TOSHIMA, Toyomi KOIKE*, Naohiro NISHIYAMA, and Takashi TSUGUKUNI

Biological Science Laboratories, Kao Corporation
(2606, Akabane, Ichikai-machi, Haga-gun, Tochigi-ken, 〒321-34)
* Material Research Laboratories, Kao Corporation
(1334, Minato, Wakayama-shi, Wakayama-ken, 〒640)

Values for the ultimate biodegradability of alkyl polyglycoside (APGs) were determined using three different microorganisms. Acute toxicity tests were performed on aquatic organisms in fresh and sea water.

The biodegradability of APG based on biochemical oxygen demand (BOD) and dissolved organic carbon (DOC) was 73~89 % and 74~>99 %, respectively. High performance liquid chromatographic (HPLC) and gel permeation chromatographic (GPC) analyses indicated the disappearance of nearly all test substances. Branched alcohols and differences in alcohol chain length in the APG chemical structure are considered to be without effect on biodegradability. APGs were shown to rapidly and ultimately undergo biodegradation in an aquatic environment.

The 96 h LC50 values of APG were 96~115 mg/L for fresh water red killifish (Oryzias latipes), 50 mg/L for sea water adapted red killifish and 15 mg/L for mysid shrimp (Mysidopsis bahia), indicating relatively weak toxicity among surfactants.

It follows from the present results that ecotoxicological risk of APGs is likely quite low and APGs may have good environmental compatibility in the aquatic ecosystem.

1 Introduction

Investigations on both biodegradability and toxicity to aquatic organisms of surfactants have been conducted extensively since the hard type surfactant, branched alkylbenzenesulfonate was converted to the soft type. This was conducted far before the current heightened concern for environment problems. These results have been published in certain books1),2). Also, more extensive studies on environmental compatibility for new surfactant, including the study on metabolic products resulting from biodegradation, have been published 3),4). Therefore, one of the necessary requirements when developing a new surfactant, environmental compatibility is tested for in the early stages. However, concerning such newly developed structures or new applications of a surfactant, there are only very few cases which adopt and announce the concept of environmental compatibility from the beginning of the development.

The fundamental characteristics of alkyl polyglycoside (APG) were reported by Hughes and Lew5) in 1970. APG has been applied to personal care products and household detergents6)-8). In Japan APG was first used as a major component of dishwashing detergent9). It has been generally accepted that APG biodegrades well, although specific reports on APG’s biodegradability have been limited7),10),11), because of its relative short history as a surfactant. In fact, there is no detailed report on the ultimate...
biodegradability or toxicity to aquatic organisms.

Therefore, from new points of view given below, we conducted studies of APG for the ultimate biodegradability and for the acute toxicity in aquatic organisms to investigate the environmental compatibility of this compound.

1) In order to discover the ultimate biodegradability, culture medium were analyzed by means of dissolved organic carbon (DOC) analysis, high performance liquid chromatography (HPLC) and gel permeation chromatography (GPC), and at the same time, gas chromatography (GC) was performed for the analysis of branched alcohol.

2) Although high test substance concentration is used in the typical biodegradation screening test, low concentration, 10 or 5 mg/L was used for this biodegradation study.

3) Toxicity studies were performed in marine Crustacea, mysid shrimp (Mysisdopsis bahia) and fresh water fish, red killifish (Oryzias latipes) to indicate toxicity range of APG on aquatic organisms.

2 Experimental

2.1 Test substances

Three kinds of APGs with different alcohol chain length were synthesized and tested. Table–1 shows the carbon number of alcohol, degree of polymerization and abbreviated names. APG$_{9-11}$ and APG$_{9-13}$ were synthesized by the conventional method$^{(5),12),13}$ with mainly methyl branched alcohol at the 2 position which were produced by oxo-process. APG$_{8-18}$ was synthesized with coconut alcohol.

2.2 Biodegradation tests

2.2.1 Equipments used for biodegradation test

A closed system oxygen consumption measuring apparatus (Coulometer, Ohkura Electric Co. Ltd., Japan) was employed for the testing$^{14)$. After adding test substance to dilution water containing activated sludge or river water, biochemical oxygen demand (BOD) was measured continuously at 25±1°C.

2.2.2 Sources of microorganisms

Activated sludge for Modified MITI test (I)$^{15)$, the return sludge from a municipal sewage treatment plant or river water was used for the sources of microorganisms. The activated sludge for the MITI test was purchased from Chemical Inspection and Testing Institute, Japan, and maintained in synthetic sewage at 25±2°C containing 0.1 % each of glucose, peptone and potassium dihydrogen phosphate (pH, 7.0±1.0) before use.

The return sewage from the Kawada municipal sewage treatment plant in Tochigi prefecture was used on the day of testing. The Kawada municipal sewage treatment plant uses a standard activated sludge method and mainly treats domestic sewage. Its treatment capacity is 130,000 m$^3$/d of sewage from a population of 250,000.

The river water collected near the Azuma bridge of the Ta river flows through Utsunomiya city, Tochigi prefecture. The Ta river is a tributary of the Kinu river and the area where the water was collected is designated as C type in the environmental quality standards. The analytical results of water qualities at the time of sampling are shown in Table–2.
2.2.3 Dilution water
Dilution water specified in Japanese Industrial standard (JIS K 0102)\(^{16}\) was used, except when the river water was used for the source of microorganisms.

2.2.4 Incubation conditions
Concentrations of test substances and the inocula, and abbreviated names for each incubation condition are shown in Table-3. In this study, both high concentration (100 or 30 mg/L) and low concentration (10 or 5 mg/L) of the test substance were tested. In the low concentration tests, 1000 mL culture bottles were specially made and used in place of the usual 300 mL culture bottles to obtain sufficient BOD. TPAS\(_{10/10}\) and RW\(_5\) were carried out in duplicate and all other tests were carried out in triplicate. Each data was represented by the mean value.

2.2.5 Analytical methods
The BOD biodegradability was calculated by subtracting the BOD of inoculum without test substance from the BOD of test substance and divided it by the theoretical oxygen demand of test substance.

After measuring the BOD, the culture medium was centrifuged at 1000 x g, for 10 min and supernatant was analyzed for dissolved organic carbon (DOC) with a total organic carbon analyzer (TOC-500 or TOC-5000, Shimadzu Corp., Japan). The DOC was determined by subtracting the concentration of inorganic carbon from that of total carbon. All concentrations were calculated from calibration curves previously made using standard solutions defined in JIS K 0102\(^{17}\). The rate of DOC decrease during the incubation period was represented as the DOC biodegradability.

In the test using APG\(_9\)–\(_{13}\), the test substance was extracted from the freeze dried supernatant of the culture medium with methanol/water (9/1) and injected into high performance liquid chromatograph (HPLC). The APG\(_8\)–\(_{18}\) test substance was extracted from the culture medium with diethyl ether and subjected to gel permeation chromatography (GPC). The rate of test substance decrease during the incubation period was represented as the HPLC or GPC biodegradability.

Also, the APG\(_9\)–\(_{13}\) extract was dried, hydrolyzed with 0.5 M sulfuric acid, neutralized, extracted with n-hexane and injected into gas chromatograph (GC) to analyze for alcohol. The conditions for analyses with HPLC, GPC and GC are shown in Table-4.

### Table-3 Incubation conditions.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Incubation volume (mL)</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test substance</td>
<td>Inoculum(^a)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>30(^b)</td>
<td>MTAS(_{100/30})</td>
</tr>
<tr>
<td>100</td>
<td>100(^b)</td>
<td>MTAS(_{100/100})</td>
</tr>
<tr>
<td>100</td>
<td>10(^c)</td>
<td>TPAS(_{100/30})</td>
</tr>
<tr>
<td>10</td>
<td>10(^c)</td>
<td>TPAS(_{10/10})</td>
</tr>
<tr>
<td>5</td>
<td>–(^d)</td>
<td>RW(_5)</td>
</tr>
</tbody>
</table>

\(^a\)The value represents Mixed Liquor Suspended Solid (MLSS).
\(^b\)Activated sludge for the MITI test.
\(^c\)Activated sludge from the municipal sewage treatment plant.
\(^d\)River water.
2.3 Acute toxicity tests to aquatic organisms

Test fish and shrimp were exposed to test substance at various concentrations to obtain acute toxicity value (LC₅₀) from the number of death at each concentration using Probit method or graphic interpolation method.

The fresh water tests were performed in accordance with JIS K 010218) using red killifish (Oryzias latipes). The total length and body weight of the fish were 2.80 ± 0.24 cm (mean ± SD) and 0.19 ± 0.05 g respectively. The test fish were exposed to test substance in dechlorinated tap water (concentration of residual chlorine, <0.01 mg/L; water hardness, 57 mg/L as CaCO₃) at 25±2°C for 96 h. Fish were not fed for 24 h before the start of the test and during the test period. The number of fish per group for the acute toxicity test was 10 per 3 L.

The sea water tests were performed in accordance with the Environment Agency’s Test Guidelines for Untested Liquid Substances19). Sea water adapted red killifish (Mysidopsis bahia) and mysid shrimp (Mysidopsis bahia) were used for the tests. Red killifish originally kept in fresh water were adapted to 36±0.1 salt concentration of natural sea water, by adding 1 volume of sea water to 3 volumes of original water and acclimating the fish at this concentration for 3~4 d and by repeating the same procedures. The test volume using natural sea water was 5 L per group and the testing conditions were the same as the fresh water tests except that the test solution was replaced daily. The total length and body weight of the test fish were 3.22±0.10 cm and 0.29±0.03 g respectively. To test the mysid shrimp, natural sea water was diluted to about 20 % to sea solution. The number of shrimp per group was 20 per 400 mL of test solution and the test solution was replaced daily. The shrimps were fed larvae of artemia at the test solution replacement. Otherwise, the test conditions were the same as those of sea water adapted red killifish. The analysis of total organic carbon concentration of test solution indicated that the concentration of test substance before the replacement was maintained at 82~98 % (mysid shrimp) and 78~100 % (sea water adapted red killifish).

3 Results and Discussion

3.1 Results of biodegradation tests

Results of the biodegradation tests for APG₉₋₁₃ and APG₈₋₁₈ are shown in Table 5. The BOD biodegradability, which represents the degree of oxidative degradation of test substance by microorganisms, was 73~89 %. The DOC biodegradability, which represents the rate of disappearance of dissolved organic matter, was 74~>99 %. The HPLC and GPC analyses indicated that almost all of the test substance disappeared. OECD guidelines20) defines a substance is readily biodegradable when the BOD biodegradability is above 60 % and the DOC biodegradability is above 70 % by Modified MITI test (I) or Manometric Respirometry test21) using 100 mg/L of test substance and

| Table 4 Conditions of HPLC, GPC and GC analyses. |
|-----------------|-----------------|-----------------|
| Apparatus       | HPLC            | GPC             | GC               |
| Shimadzu LC-6 AD| HITACHI 655     | Shimadzu GC-7 AG|
| Column           | Inarisol OD-S,  | TSK-gel G 2000 HXL, | 10 % SE-30 on    |
| 4.6 mm φ×250 mm | 7.8 mm φ×300 mm ×2| Chromosorb W AW-DMCS |
| Room temp.       | 40°C            | Initial 100°C   |
| Mobile phase     | Methanol/H₂O (8/2) | Rate 6°C/min    |
| Flow rate        | 1 mL/min        | Final 300°C     |
| Detector         | RI, SHODEX RI SE-51 | He              |
|                  |                 | 30 mL/min       |

3.2 Experimental procedures

The test fish and shrimp were exposed to test substance at various concentrations to obtain acute toxicity value (LC₅₀) from the number of death at each concentration using Probit method or graphic interpolation method.

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30 mg/L of activated sludge. Hence APG can be considered to be readily biodegradable. The test results agree well with past reports performed in accordance with the official method\(^7\),\(^10\),\(^11\).

In the MTAS\(_{100/30}\) for APG\(_{9-13}\), a small amount of DOC components were detected. The origin of the organic compounds found may have derived from the test substance or growing microorganisms. As described above, the biodegradability monitored with HPLC indicated that almost all of the test substance disappeared. Therefore we investigated its biodegradation intermediate as possible candidate. The linear and branched type alcohols in APG\(_{9-13}\) were analyzed by GC and the peak area ratios were determined. The linear/branched ratios were 84/16 before incubation and 90/10 after incubation (Table 6). This result indicates similar biodegradabilities of the two alcohol types and suggests that a certain amount of DOC component remaining in the culture medium was of microorganisms origin which increased as the test substance was utilized as the energy source. Similarly a small amount of DOC components were detected in the TPAS\(_{100/30}\) for APG\(_{9-13}\). APG as discussed above does not contain any structure resistant to microorganisms and therefore it can be speculated that the test substance will ultimately biodegrade.

Table 5  Biodegradability of APG.

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Conditions(^a)</th>
<th>Biodegradability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BOD</td>
</tr>
<tr>
<td>APG(_{9-13})</td>
<td>MTAS(_{100/30}) (28 d)</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>TPAS(_{100/30}) (14 d)</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>TPAS(_{10/10}) (14 d)</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>RW(_5) (14 d)</td>
<td>88</td>
</tr>
<tr>
<td>APG(_{8-18})</td>
<td>MTAS(_{30/100}) (14 d)</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>TPAS(_{100/30}) (28 d)</td>
<td>78</td>
</tr>
</tbody>
</table>

\(^a\) Numbers in parentheses represent incubation time (d).

\(^b\) – , not tested.

Table 6  Alcohol analysis of APG\(_{9-13}\) in MTAS\(_{100/30}\).

<table>
<thead>
<tr>
<th>Types of alcohol</th>
<th>Linear (%)</th>
<th>Branched (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before incubation</td>
<td>84</td>
<td>16</td>
</tr>
<tr>
<td>After incubation</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

chain length of C\(_{12,14}\), and average degree of polymerization of 1.4, on bacterial oxygen consumption is 29.4 mg/L\(^23\). In this study, the rates of the biodegradation in MTAS\(_{100/30}\) and TPAS\(_{100/30}\) for APG\(_{9-13}\) might be affected by the high initial concentration (100 mg/L). In the actual environmental water, the concentration of a chemical will be of the \(\mu g/L\) order or less and biodegradation tests usually performed at a high concentration of a test substance may underestimate its potential of biodegradation\(^24\). We have designed our study by introducing a larger culture bottle (1000 mL) and reduced the concentration of the test substance, at the same time, maintaining sufficient BOD. In the TPAS\(_{10/10}\) performed at 10 mg/L of APG\(_{9-13}\), all DOC components disappeared within 14 d despite the fact that the concentration of activated sludge was reduced from 30 to 10 mg/L (Table 5). A similar test result of complete disappearance of DOC components within 14 d was obtained in the RW\(_5\) where test substance concentration was reduced to 5 mg/L. Therefore, these results indicate that APG\(_{9-13}\) rapidly and ultimately biodegrades in the actual en-

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environmental water.

The MTAS_{30/100} for APG_{8-18} showed that almost all of DOC component disappeared within only 14 d compared with the MTAS_{100/30} for APG_{9-13}. This difference is probably due to reversal of concentration between the activated sludge and the test substance. Also, increase in DOC biodegradability for TPAS_{100/30} appeared to reflect the 2-fold increase of incubation time compared with a similar test for APG_{9-13}. The two test substances were never tested using the same condition but these results at least indicate that difference in chain length of alcohol did not affect biodegradability.

3.2 Acute toxicity tests to aquatic organisms

The results of acute toxicity tests to aquatic organisms for APG_{9-11} are shown in Table 7. Fig. 1 summarizes reported acute toxicity data including our results. The 96 h LC_{50} values in this study were 96−115 mg/L for red killifish (fresh water), 50 mg/L for sea water adapted red killifish and 15 mg/L for mysid shrimp. The APGs shown in Fig. 1 have slightly different compositions but most of the LC_{50} (EC_{50}) values of these commercially available APGs are within the range of 10−100 mg/L.

Kimerle summarized acute toxicity of 7 major surfactants and indicated that LC_{50} (EC_{50}) of these surfactants to fish and Daphnia were mainly in the 1−100 mg/L range. Hence acute toxicity of APG is relatively weak among these surfactants. The major factors determining compatibility of a chemical in aquatic environment are its toxicity to aquatic organisms and biodegradability which relates to exposure concentration and duration. Since APGs have relatively weak aquatic toxicity and rapid and ultimate biodegradability, we think that APGs are highly compatible with the aquatic environment.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red killifish (Fresh water)</td>
<td>96−115</td>
<td>96−115</td>
<td>96−115</td>
<td>96−115</td>
</tr>
<tr>
<td>Red killifish (Sea water)</td>
<td>54</td>
<td>52</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mysid shrimp</td>
<td>63</td>
<td>22</td>
<td>17</td>
<td>15</td>
</tr>
</tbody>
</table>

![Fig. 1 Reported acute toxicity values of APGs.](image-url)
4 Conclusion

Biodegradability tests and acute toxicity tests to aquatic organisms were performed for alkyl polyglycoside (APG). Results indicate that APGs rapidly and ultimately biodegrade in aquatic environment and have weak toxicity to aquatic organisms. Therefore, it can be suggested that the ecotoxicological risk of APGs is very low and APGs have good environmental compatibility in the aquatic ecosystem.

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アルキルポリグリコシドの生分解性及び水生生物毒性

都島康彦・小池豊美・西山直宏・鶴見孝司

花王株式会社生物科学研究所
（〒321-34 福岡県福岡市東区赤羽 8806）
* 花王株式会社素材研究所
（〒640 和歌山県和歌山市葵 1334）

アルキルポリグリコシド（APG）について、3 種類の植物油を用いた実験にて生分解性を確認し、淡水及び海水における水生生物に対する急性毒性試験を実施した。
生分解性試験において、APG の BOD 分解率は 73～89 %、DOC 分解率は 74～99 % であり、HPLC 及び GPC 分析では被験物質のほぼ全量が消失した。APG の構造中のアルコールは不飽和型であっても、不飽和度が異なっても生分解性に影響はないと考えられた。これらの結果から、APG が水系環境中での速やかな生分解性を有することを示している。

APG の 96 h LC50 値は、ヒメダカ（淡水）で 96～115 mg/L、海水を含むヒメダカで 50 mg/L、mysid shrimp で 15 mg/L であり、主要界面活性剤の中でも比較的弱いものであった。

以上のことがから、APG の生態毒性上のリスクは非常に低く、水系において高い環境適合性を有するものと考えられた。

（連絡者：都島康彦）