Biodegradation of Alkylpolyglucosides

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Alkylpolyglucosides의 생분해 연구
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Abstract: The biodegradability of alkylpolyglucosides (APGs) was determined by shake culture tests, semicontinuous activated sludge test, and continuous activated sludge test mandated for testing the biodegradability of surfactants in OECD, ASTM, JIS and KS. APGs showed 100% foam loss in all tests. This means that APGs are degraded well not only in sewage treatment plant but in surface water conditions. In closed bottle test for determining the ultimate biodegradability, APGs exceeded the ready biodegradability limit (60%) of OECD with 75% BOD₅/COD. APGs may therefore be considered as readily biodegradable under actual environmental conditions.

요약: KS, JIS, ASTM 및 OECD의 개발환경적 생분해 중형시험법, 연속식활성오너법, 연속식활성오너법을 이용해 APG의 생분해성을 조사하였다. Foam loss로 측정한 APG의 일차생분해도는 모든 시험법에서 100%인 것으로 나타나, APG는 하수처리장과 표층수의 조건에서 쉽게 분해될 수 있었다. 또한 OECD의 화학물질 생분해 평가법인 closed bottle test법으로 측정한 APG의 최종생분해도는 75% BOD₅/COD로, OECD에서 제시한 ready biodegradability limit인 60%를 초과하여, APG는 자연계에서 쉽게 분해되는 "readily biodegradable"한 물질인지 것으로 확인되었다.

1. Introduction

Alkylpolyglucosides(APGs) are becoming important oleochemical surfactants as an ingredient of detergent formulations: they are made from renewable natural raw material and exhibit good washing properties[1, 2]. Because of this, it is now very important to evaluate their environmental compatibility.

Evaluation of environmental compatibility of the organic compounds is generally based on two important criteria: biodegradability and ecotoxicity[3]. Biodegradability has been defined in many different contexts, the definition depending primarily upon the parameter being utilized for measuring biodegradability. Swisher has indicated definitions for two degrees of biodegradation: (a) “Primary degradation occurs when the molecule has been oxi-
dized, or altered by bacterial action to such an extent that its characteristic properties are no longer evident or when it no longer responds to analytical procedure more or less specific for detecting the original surfactants; and (b) ultimate degradation is defined as the complete conversion of the surfactant molecule to carbon dioxide, water, inorganic salts, and products associated with the normal metabolic processes of the bacteria"[4, 5].

Several tests are in use to determine the primary biodegradability of surfactants. Shake culture tests, semicontinuous activated sludge(SCAS) test and continuous activated sludge (CAS) test are specified by detergent legislation in many countries, e.g., the United States, Japan, Korea and Organization for Economic Cooperation and Development(OECD) [6, 7, 8]. Shake culture test corresponds to surface water condition, and SCAS and CAS tests simulate the biodegradation that occurs in the communal sewage treatment plant.

A number of ultimate biodegradability tests have been used for estimation of environmental compatibility of organic compounds. But the principles of these tests are quite similar. The degradation of organic compound is measured using non-specific parameters such as BOD/COD, carbon dioxide evolution and carbon removal. Closed bottle test, modified OECD screening test, modified AFNOR test, modified STURM test, and modified MITI test are specified in OECD[9].

This paper describes the primary and ultimate biodegradability of APGs synthesized in our research institute for estimating their environmental compatibility using four primary biodegradability tests, e.g., shake culture A and B, SCAS and CAS tests, and using a ultimate biodegradation test, closed bottle test.

2. Materials and Methods

2.1. APGs

APGs are prepared in our R & D center with glucose from corn starch and fatty alcohol from palm and/or coconut oil.

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Alkylpolyglucoside</th>
</tr>
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<tr>
<td><strong>Structure</strong></td>
<td>![Structure Diagram]</td>
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<table>
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<tr>
<th>Component of Alkyl Chain</th>
<th>C₂₅C₃₀C₃₅C₄₀ = 20:30:35:15</th>
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<tr>
<td>Active Content</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Purity</td>
<td>&gt;99.5%</td>
</tr>
</tbody>
</table>

2.2. Biodegradation Tests

2.2.1. Primary Biodegradation Tests

Shake Culture Test A

Activated sludge microorganisms are inoculated into a flask that contains a microbial growth medium and the surfactants to be tested. Following two adaptive transfer, biodegradation is determined by measuring the reduction in surfactant content during the test period (Fig. 1-a)[7].

Shake Culture Test B

A small number of polyvalent bacteria are inoculated into a flask that contains a chemically defined medium and the surfactant as a sole carbon source by simply adding a little sewage treatment plant effluent. Biodegradation is determined by measuring the reduction in surfactant content at fixed interval for up to 19 days (Fig. 1-b)[8].

Continuous Activated Sludge Test

The surfactant to be tested is added in a concentration corresponding to 20mg MBAS/liter to a synthetic sewage. This sewage is fed into the activated sludge vessel of the model treatment plant where it remains for an average of three hours. Sludge and supernatant are separated in the settling vessel and the effluent is collected. Biodegradation is determined by measuring the reduction in surfactant content(Fig. 2-a)[8].

Semicontinuous Activated Sludge(SCAS) Test

The activated sludge obtained from a sewage
Fig. 1. The illustrating drawings of shake culture test A(a) and shake culture test B(b).

Fig. 2. Test apparatus of continuous activated sludge test (a) and semicontinuous activated sludge test (b).

A : Storage Vessel  B : Dosing Device  C : Aeration Chamber(3ℓ)  D : Settling Vessel
E : Air-lift Pump  F : Collector  G : Sintered Aerator  H : Air-flow Meter

Treatment plant, the surfactant to be tested, and the synthetic sewage as an energy source for the sludge microorganisms are placed in an aeration chamber. The mixture is aerated for 23 hours, allowed to settle, and the supernatant material removed. The sludge remaining in the aeration chamber is then brought back to volume with fresh surfactant and synthetic sewage and the cycle repeated. Biodegradation is determined by the reduction in surfactant content during each cycle (Fig. 2-b)[7].

2.2.2. Ultimate Biodegradation Test

A predetermined amount of the compound is dissolved in an inorganic medium providing a concen-
tration of usually 2mg of test substance per liter. The solution is inoculated with a small number of microorganisms from a mixed population and kept in closed bottle in the dark in a constant temperature or enclosure at 20 to 21°C. The degradation is followed by oxygen analysis over 28-day period. Closed bottle test was carried out based on the OECD guidelines[9].

2.3. Microorganisms

The activated sludge and secondary effluent collected from Chungnang sewage treatment plant in Seoul were used as polyvalent bacterial inocula.

2.4. Analytical Methods

2.4.1. HPLC Equipment and Operating Conditions

The liquid chromatography (Waters Associate) consisted of a model 510 pump system, an injector (U6K) with a 1ml loop, a differential refractometer. Peak areas and retention times were obtained by using HP-3390 integrator (Hewlett Packard). The HPLC separation was achieved on a 10μm, 30cm × 4.6mm i.d. μ-Bondapak C18 column (Waters Associate). The elution of the APGs was achieved isocratically with mobile phase composition of methanol/water=7/3(v/v). The column effluent was monitored using RI detector. The flow rate was maintained at 1ml/min.

2.4.2. Others

Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were conducted in accordance with Standard Methods No. 507 and No. 508A [10]. Bubble volumetric method was carried out according to KS M 2714[6].

3. Results and Discussion

3.1. Primary Biodegradability

The primary biodegradability of APGs in shake culture test A and B, semicontinuous activated sludge test and continuous activated sludge test were shown in Fig. 3.

Most of the biodegradability tests specified in detergent legislations are designed to determine the primary biodegradability of surfactants. In KS, JIS and ASTM, surfactants used in detergents are required to show at least 90% biodegradability in shake culture test A[6, 7]. APGs easily exceeded this stipulated minimum by showing 100% foam loss after only 2 days. APGs showed 100% foam loss after 4 days in shake culture test B which is an official test of OECD and corresponds to surface water conditions[8]. APGs also showed 100% foam loss in continuous activated sludge test and semicontinuous activate sludge test, which are laboratory scale model tests of communal sewage treatment plant and widely used to estimate the biodegradability of surfactants. These results mean that APGs are degraded well not only in sewage treatment plants but in surface water conditions.

Cobalt thiocyanate active substance (CTAS) and bismuth active substance (BiAS) methods are specified for determination of nonionics in OECD, JIS, and KS[6, 7]. These colorimetric methods are based on the fact that nonionic surfactant forms a complex with cobalt ammonium thiocyanate or barium iodo bismuthate, which can be extracted and quantified photometrically. These colorimetric methods, therefore, may be used only for quantification of nonionic surfactants that contain polyoxyethylene units. In other words, alkanol fatty acid amides and APGs are not to be determined by CTAS and BiAS. Bubble volumetric method is used as quantitative method for these surfactants. KS and JIS prescribe that the determination of nonionic surfactants shall be carried out in accordance with absorptiometry, but alkanol fatty acid amide with bubble volumetric method[6]. Bubble volumetric method, however, inherently has some problems to be used as quantitative analysis of surfactants. The foaming phenomenon is a complex and transient one, deriving from the interaction of several physical properties. That depends on the concentration of the surfactant and other materials present (which may either enhance or inhibit), the means used for
generating the foam, the immediate history of the solution, temperature, humidity, and undoubtedly other factors as well[4]. These reasons made us use a new quantification method using HPLC as an alternative to make up for the drawback in bubble volumetric method.

The degradability of APGs in shake culture test A showed some what difference according to the analytical methods used(Fig. 4).

At day 1, the biodegradability of APGs was 0% by bubble volumetric method but 90% by HPLC method. This may be caused by the difference of inherent properties of analytical methods. The HPLC method was designed to detect the original chemicals themselves, while the bubble volumetric method was designed to detect the surface active properties of original chemicals. Therefore the results mean that 90% of APGs began to be broken down in a day even though it was not detected by bubble volumetric method. And it is thought that bubble volumetric method has a problem to estimate the initial stage of biodegradation of APGs.

3.2. Ultimate Biodegradability

Although APGs showed high primary biodegradability, definitive conclusions with regard to the total biodegradability of surfactants can only be reached after investigating ultimate biodegradation [3]. OECD has specified several tests for estimating the ultimate biodegradability of all "new" chemicals [9, 11]. But the principles are quite similar. They all employ the surfactant as the sole carbon source, a mineral nutrient solution, and a comparatively small inoculum, usually sewage treatment plant effluent. The degradation is measured by various means. In the closed bottle test, it is followed by the BOD whereby the BOD is expressed as a fraction of the COD (or ThOD). In the modified Sturm test, the carbon dioxide evolution during the metabolism of the test material is quantified. In the modified OECD screening test and in the modified AFNOR test, the carbon removal is followed. All these tests have their special merits. Respirometric tests such as the closed bottle test and the carbon dioxide evolution test according to modified Sturm furnish proof of true biodegradation, while carbon removal tests allow a full quantification of degradation [12]. In this study we chose the closed bottle test for estimating the ultimate biodegradability of APGs because that is the most stringent of all OECD tests [11]. APGs clearly exceeded the ready biodegradability limit (60%) with 75% BODs/COD. APGs, therefore, may be considered as "readily biodegradable" under actual environmental conditions.

4. Conclusion

APGs showed 100% foam loss in shake culture test A and B, SCAS and CAS which are mandatory tests for testing the biodegradability of surfactants in OECD, ASTM, JIS and KS. This means that APGs are degraded well not only in sewage treatment plants but in surface water conditions. In closed bottle test, APGs also exceeded the OECD ready biodegradability criterion (60%) with 75% BODs/COD. APGs, therefore, may be considered as "readily biodegradable" under actual environmental conditions.

Reference

9. OECD, "OECD Guidelines for Testing of Chemicals. Section 3: Degradation and Accumula-
