Biodegradability and Aquatic Toxicity of Glycoside Surfactants and a Nonionic Alcohol Ethoxylate

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ABSTRACT: The environmental properties of three glycoside surfactants and one alcohol ethoxylate were examined by standardized laboratory methods. All of the surfactants biodegraded extensively in aerobic screening tests and may be assumed to approach 100% removal in aerobic wastewater treatment plants, except in cases of high loadings or otherwise exceptional conditions. Anaerobic biodegradability tests showed that an ethyl glycoside monoester (EGE) and a linear alkyl polyglycoside (APG) were both mineralized (>70%) under methanogenic conditions. In contrast, a branched APG resisted anaerobic degradation, while the alcohol ethoxylate was partially mineralized by anaerobic bacteria. The EGE surfactant was most rapidly mineralized in aerobic and anaerobic biodegradability tests. None of the surfactants inhibited respiration in activated sludge at the highest concentration tested (200 mg/L). Tests with aquatic organisms showed increasing toxicity in the following order: branched APG < EGE < linear APG < alcohol ethoxylate. Negligible aquatic toxicity was observed for the branched APG, while the alcohol ethoxylate was highly toxic to examined organisms. This evaluation demonstrates that considerable variation in biodegradability and toxicity responses can be seen within structurally related glucose-based surfactants.
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KEY WORDS: Aerobic and anaerobic biodegradability, alcohol ethoxylates, aquatic toxicity, glycoside surfactants, nonionic surfactants.

Considerable amounts of surfactants are used in detergents for household and industrial purposes. The total volume of detergents used within the European Community in 1992 has been estimated at 3.575 million tons (1). Surfactants enter the environment in significant amounts *via* wastewater treatment plants (WWTP) or by direct release into aquatic recipients. Monitoring of anionic and nonionic surfactants, dispersed in the aquatic environment, has indicated that surfactants may be present at concentrations that are known to cause acute or chronic effects on sensitive species (2–5).

Besides the actual exposure, the potential environmental impact depends on the biodegradability and toxicity of the surfactants. Potential mineralization under aerobic and anoxic conditions is important for a hazard assessment of surfactants because degradation often reduces the toxicity of these chemicals (6,7). If environmental properties are considered, surfactants that biodegrade completely within the normal retention time in WWTP and have a low aquatic toxicity are normally preferred in the development of new detergents. For example, linear alcohol ethoxylates (AE) have replaced recalcitrant alkylphenol ethoxylates in many products because alcohol ethoxylates are often completely degraded, under aerobic (8) as well as anoxic conditions (9,10).

Glycoside surfactants constitute a new type of nonionic compound that have been developed for various household products. Being a new class of surfactants, few studies have addressed the environmental properties of these chemicals (11,12). It was, however, recently demonstrated that a glucose amide (polyhydroxy fatty acid amide) was readily biodegradable and less toxic than typical synthetic surfactants (11). The purpose of the present study was to compare environmental properties of three glucose-based surfactants with a classic alcohol ethoxylate. The evaluation included standardized methods normally used for environmental classification of chemicals, i.e., tests for ready biodegradability and for toxicity to algae, crustaceans, and fish. Because of the tendency of surfactants to sorb to particulate organic matter, they may accumulate in anaerobic sludges and sediments. The potential mineralization under methanogenic conditions was therefore examined by use of a gas-production screening test (13,14).

EXPERIMENTAL PROCEDURES

Surfactants. The surfactants used were a branched alkyl polyglycoside (APG), C_8 APG[b] [number of C atoms in the alkyl chain R = 8; average degree of polymerization (DP) = 1.6]; a linear APG, C_{12-14} APG (R = 12–14, DP = 1.4); an ethyl glycoside fatty acid 6-O monoester (EGE), C_{12} EGE (R = 12); and a linear primary AE, C_{12-15} AE-7 (R = 12–15, with 7 moles of ethylene oxide). Samples of C_8 APG[b] and C_{12-15} AE-7 were obtained from Akzo Nobel AB (Stenungsund, Sweden). The C_{12-14} APG was received from Henkel KGaA (Düsseldorf, Germany), and the C_{12} EGE was provided by Novo Nordisk A/S (Bagsvaerd, Denmark). Surfactant contents of the samples were 100% for C_{12} EGE and C_{12-15} AE-7, 65% for C_8 APG[b], and 50% for C_{12-14} APG. Carbon contents of the surfactants were determined by using a non-

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volatile organic carbon analyzer (Dohrmann DC-190; Rosemount Analytical Inc., Santa Clara, CA). All test concentrations are expressed on the basis of active ingredient.

Aerobic biodegradability. Aerobic ultimate biodegradability was tested by internationally standardized procedures as prescribed in the Organization for Economic Cooperation and Development (OECD) test guidelines (15):

(i) CO_2 evolution test, OECD 301B (15). Activated sludge was collected at a municipal WWTP and inoculated into test flasks to achieve a concentration of approximately 30 mg suspended solids (d.w.) per liter in a final liquid volume of 1.8 L. Empirically determined carbon contents of the surfactants were used for calculating theoretical CO_2 evolution ($ThCO_2$) for complete mineralization. Surfactants were added at initial concentrations that corresponded to 10 mg of dissolved organic carbon (DOC) per liter, i.e., 16 to 20 mg/L. The CO_2 produced was trapped in absorbers containing 0.0125 M $Ba(OH)_2$, and the amounts were determined by titration with 0.050 M HCl.

(ii) Closed bottle test OECD 301D (15). Secondary effluent from the WWTP described above was applied at 0.5 mL per liter of mineral medium in closed respirometric bottles (approx. 290 mL). Chemical oxygen demand (COD) was determined by oxidation with potassium dichromate. Additions of the surfactants corresponded to COD values between 4.1 and 5.1 mg O_2 per liter, i.e., 1.9 to 3.1 mg/L. Biochemical oxygen demand (BOD) was measured by means of an oxygen electrode (Microprocessor Oximeter, OXI 2000; Wissenschaftlich-Techische Werkstätten G.M.B.H., Weilheim, Germany).

Anaerobic biodegradability. Potential mineralization of the surfactants in absence of molecular oxygen was examined by a modification (14) of a methanogenic gas production test (13). Anaerobically digested sewage sludge was collected from another WWTP, which received primarily domestic wastewater. The sludge was filtered through two layers of cloth and preincubated for 5 d without addition of chemicals. Closed 117-mL serum bottles were inoculated to achieve a concentration of sludge solids of 0.15 g/L (d.w.) in a final liquid volume of 106 mL. Surfactants were added at a concentration corresponding to 20 mg DOC per liter, i.e., 30 to 40 mg/L. Total gas production was measured in the serum bottle headspace by using a pressure transducer equipped with a digital readout meter (Institute of Grassland and Environmental Research, Aberystwyth, United Kingdom). Mineralization of the test chemicals was calculated from the net gas production (NGP; total gas production minus gas production in controls without test chemical), by assuming that the gas evolved from the anaerobic processes consisted of the terminal products CO_2 and CH_4. Methane formation was determined at the end of the incubation by analysis of headspace samples (10–100 µL) with an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) with flame-ionization detection and a capillary column. Dissolved CO_2 was quantitated at the end of the test by measuring the increase of headspace pressures after acidification of the liquid to pH 1–2 by addition of concentrated H_2SO_4 (9).

Inhibition of activated sludge respiration. Activated sludge was added to wide-necked 500-mL bottles at a concentration of approximately 2 g of suspended solids (d.w.) per liter. Inhibition of respiration in the sludge relative to unexposed controls was determined by measuring O_2 concentrations in the liquid after 30 min and 3 h (15).

Aquatic toxicity. Chronic and acute toxicity tests with aquatic organisms were performed according to standardized OECD (15) and ISO procedures (16,17). The freshwater microalga *Kirchneria subcapitata* (new name for *Raphidocelis subcapitata,* which was formerly also known as *Selenastrum capricornutum*) was selected for chronic static toxicity tests by using the growth rate as the parameter for inhibition. Exponentially growing cultures of *K. subcapitata* were acclimatized to the test conditions for at least 24 h. Algae were then exposed to the surfactants under continuous shaking at 23 ± 2°C and with constant illumination. Cell density was adjusted to $3 \cdot 10^3$ cells/mL at the start of the test and was measured as fluorescence after 24, 48, and 72 h (Sequoia-Turner Model 450 digital fluorometer; Unipath, Mountain View, CA).

Acute static toxicity tests were conducted with the freshwater crustacean *Daphnia magna.* Young animals (<24 h of age) were collected from a laboratory strain of *D. magna.* Tests were run at 20 ± 1°C with a daily light/dark period of 12:12 h. The number of immobile animals, out of 20, was recorded after 24 and 48 h.

Acute semi-static toxicity tests were performed with zebra fish (*Brachydanio rerio*) which were acclimatized to the assay conditions for 12 d. Feeding was withheld from 24 h before the start of the tests and throughout the test period. Tests were performed at 23 ± 1°C with a daily light/dark period of 12:12 h. The synthetic medium containing the surfactants was renewed every 24 h, and mortality in groups of ten fish was recorded after 2, 24, 48, 72, and 96 h.

RESULTS AND DISCUSSION

Aerobic biodegradability. According to guidelines for tests used in this study, the primary criterion for characterizing a chemical as "readily biodegradable" is that the CO_2 evolution or O_2 uptake achieved at least 60% of the $ThCO_2$ or COD during the 28-d test period (15). In the CO_2 evolution test, the surfactants biodegraded to approximately the same extent. Total CO_2 production corresponded to a mineralization of 78% for C_8 APG[b], 81% for C_{12-14} APG, 78% for C_{12} EGE, and 82% for C_{12-15} AE-7 (Fig. 1). Besides the 60% pass level, a rate criterion is also included in the OECD definition of "ready biodegradability". Test guidelines (15) prescribe that mineralization of a readily biodegradable chemical must reach the pass level within 10 d after exceeding 10% (the so-called 10-d window). The results of our tests show that the surfactants, with the exception of C_{12} EGE, had difficulties in passing the 10-d window criterion in the CO_2 evolution test (Fig. 1). In a study of another glycoside surfactant, a glucose amide (polyhydroxy fatty acid amide), the accumulated CO_2 production from C_{12-14} glucose amide corresponded to 89%

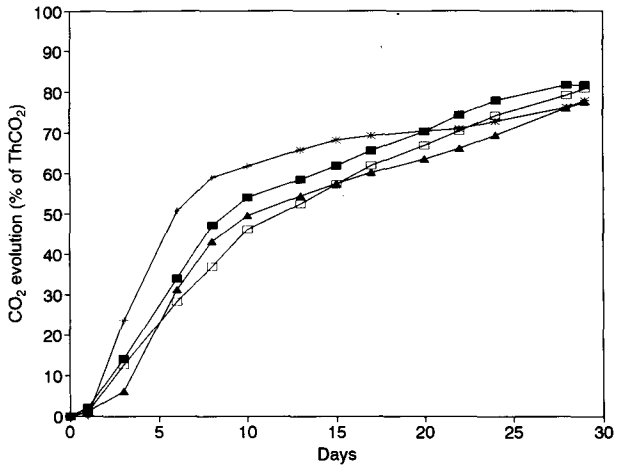

FIG. 1. Aerobic mineralization of C_8 alkyl polyglycoside (APG)[b] (▲), C_{12-14} APG (□), C_{12} ethyl glycoside monoester (*), and C_{12-15} alcohol ethoxylate-7 (■) in the CO_2 evolution test; $ThCO_2$, theoretical CO_2.

of $ThCO_2$ but, also here, the 60% level was not attained within the limits of the 10-d window (11). Previous observations have indicated that 11 of 22 chemicals examined in the CO_2 evolution test failed the 10-d window criterion, although they passed the 60% $ThCO_2$ level (18). To a large extent, the apparently slow "rate" of CO_2 evolution in this test can be explained by delayed CO_2 release from the liquid at neutral pH (18).

Also in the closed bottle test, C_{12} EGE biodegraded more rapidly than the other surfactants (Fig. 2). The O_2 consumption with C_{12} EGE corresponded to 65% mineralization after 5 d (BOD_5) and 80% at the end of the test (BOD_{28}). For comparison, O_2 uptake from sodium benzoate indicated 71 and 86% mineralization after 5 and 28 d respectively. The extent of degradation at day 5 (BOD_5) and at the end of incubation (BOD_{28} or maximum O_2 uptake) was 49 and 68% for C_8 APG[b], 51 and 67% for C_{12-14} APG, and 30 and 62% for C_{12-15} AE-7. The C_{12-15} AE-7 biodegraded relatively slowly in the closed-bottle test, and the O_2 consumption rate was too low to accomplish the 10-d window requirement. Mineralization of C_{12-15} AE-7 complies with a 14-d window criterion, which has been proposed for the closed-bottle test for technical reasons (18). Oxygen consumption for C_{12-15} AE-7 was >10% of ThOD at day 5, and extrapolation between data points (Fig. 2) indicates that the BOD passed 60% ThOD after 18–19 d.

Anaerobic biodegradability. The anaerobic biodegradability tests showed that the surfactants had different potentials for mineralization in the absence of molecular oxygen (Fig. 3). The C_{12} EGE and C_{12-14} APG were mineralized, and NGP reached 82 and 72% of theoretical gas production (ThGP) for a complete conversion of substrates to CO_2 and CH_4. Following a lag phase of one week, NGP rapidly increased with C_{12} EGE, while C_{12-14} APG inhibited the sludge inoculum for 3 to 4 wk before significant gas production was observed. Experiments in our laboratory have indicated that the effective concentrations that inhibited anaerobic gas production by 20 and 50% were 9 and 67 mg/L, respectively, for C_{12-14} APG (data not shown). C_{12-15} AE-7 was inhibitory to sludge bacteria during the first 3 wk. After 5 wk of incubation, NGP from C_{12-15} AE-7 reached 38% of ThGP, indicating that the compound was partially mineralized under methanogenic conditions. In contrast to the two other glucose-based surfactants, C_8 APG[b] was poorly degraded or resistant to anaerobic mineralization because NGP was only 22% of ThGP at the end of the test. Accumulated CH_4 at the termination of the tests confirmed theoretical mineralization of the surfactants, which had been calculated on the basis of NGP. Net CH_4 formation (corrected for CH_4 produced in unamended controls) from C_{12} EGE and C_{12-14} APG corresponded to 9.9–10.8 mg C/L, i.e., 50% of the added carbon. Net CH_4 formation from C_{12-15} AE-7 corresponded to 4.2 mg C/L while accumulated CH_4 from C_8 APG[b] was not significantly higher than that in the controls.

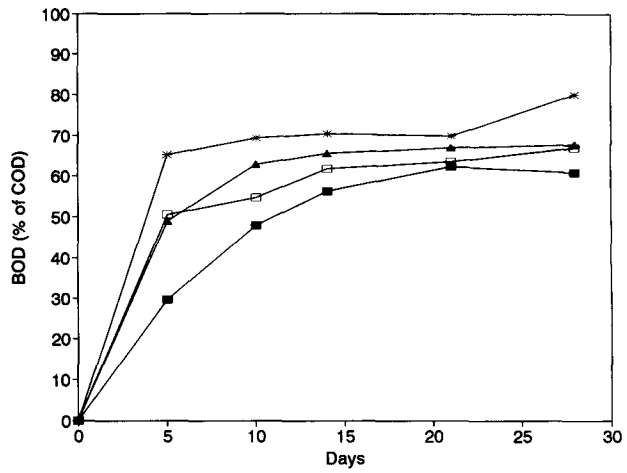

FIG. 2. Oxygen consumption from degradation of C_8 APG[b] (▲), C_{12-14} APG (□), C_{12} ethyl glycoside monoester (*), and C_{12-15} AE-7 (■) in the closed bottle test; BOD, biochemical oxygen demand; COD, chemical oxygen demand. See Figure 1 for other abbreviations.

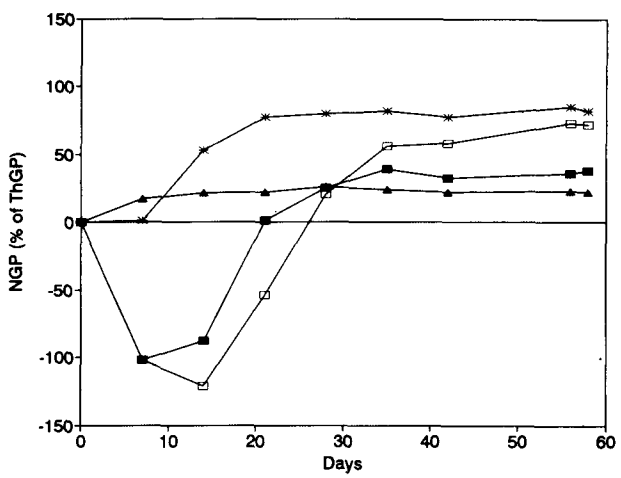

FIG. 3. Anaerobic mineralization of C_8 APG[b] (▲), C_{12-14} APG (□), C_{12} ethyl glycoside monoester (*), and C_{12-15} AE-7 (■) in a methanogenic gas production test; NGP, net gas production; ThGP, theoretical gas production. See Figure 1 for other abbreviations.

Another ethyl glycoside fatty acid monoester, analogous to C_{12} EGE in structure but with a C_{10} alkyl chain, showed an NGP corresponding to 96% of ThGP in the anaerobic biodegradability test (19). The patterns of NGP for both C_{10} and C_{12} EGE were similar to gas production seen for easily degradable substrates, such as benzoate and phenol (data not shown). This demonstrates that surfactants of the EGE type are readily utilized by anaerobic bacteria. Inhibition of gas production caused by C_{12-14} APG illustrates the more toxic properties of this surfactant compared to those of C_{12} EGE. Bacterial adaptation to C_{12-14} APG was possibly coupled to a reduction of toxicity by primary transformation of the compound. However, the antibacterial effects of C_{12-14} APG may be markedly reduced by sorption and the presence of a higher biomass density in anaerobic digesters. Results of the test indicate that C_{12} EGE may be expected to biodegrade completely in anaerobic digesters operated at temperatures around 35°C. This is probably also true for C_{12-14} APG, although it cannot be excluded that high concentrations may impede detoxification of the APG as well as microbial adaptation.

For C_8 APG[b], total gas production was only 22% above that in the controls. The recalcitrant nature of the short-chained APG is probably related to alkyl chain branching. Branched nonionic surfactants have previously been shown to be more resistant to aerobic biodegradation than nonionics with a linear alkyl chain (20). In our study, branching of C_8 APG[b] may have precluded initial attack by bacteria in the anaerobic sludge.

Partial anaerobic mineralization of C_{12-15} AE-7 may be due to toxic effects on one or more bacterial groups required for complete mineralization of the compound. Linear AE have been shown to be mineralized under anoxic conditions (9,10,14, 21). Incomplete degradation of C_{12-15} AE-7 in the present study does not mean that the compound will not biodegrade more efficiently in the environment. There is little doubt that the concentration of chemicals applied in the biodegradability test (30–40 mg/L) is rarely encountered in aquatic recipients (4,5) or sludge-amended soils (22). However, surfactants may accumulate to concentrations above this level in anaerobic sludges (22,23). The widely used linear alkylbenzene sulfonates (LAS) have been found at concentrations ranging from 5.9 to 14.3 g/kg solids (d.w.) in anaerobically digested sludges (22). Compared to LAS, AE are more efficiently sorbed by sludge solids (24) and, although degraded anaerobically, current use of these surfactants might lead to elevated concentrations in anaerobic sludge.

Inhibition of activated sludge. None of the surfactants inhibited respiration in activated sludge at the highest concentration tested (200 mg/L). Concentrations above this level could not be examined because of foaming.

Aquatic toxicity. Comparison of the effective concentrations shows increasing toxicity in the order C_8 APG[b], C_{12} EGE, C_{12-14} APG, and C_{12-15} AE-7, independent of tested species (Table 1). Branched C_8 APG[b] was clearly the least toxic of the surfactants, with EC/LC_{50} values above 500 mg/L (EC_{50} and LC_{50}: effective concentration and lethal concentration, respectively, causing 50% growth inhibition, immobilization, or mortality). The low toxicity of C_8 APG[b] may be due to the branched structure of this surfactant, as branched AE have been shown to be less acutely and chronically toxic than linear alcohols (20). For C_{12} EGE and C_{12-14} APG, the most sensitive of the organisms was *B. rerio*. The C_{12-14} APG was more toxic than the two other glycoside surfactants, particularly to *B. rerio*. Low EC/LC_{50} values were seen with C_{12-15} AE-7, as previously reported for AE of similar alkyl chainlength and ethoxylation level (3).

The C_{12-14} glucose amide, examined by Stalmans *et al.* (11), was less toxic than C_{12-14} APG and more toxic than C_{12} EGE. Toxicity of the glucose amide to *K. subcapitata* and *D. magna* increased with increasing chainlength. Stalmans *et al.* (11) reported 96-h EC_{50} values to be 3.9, 12.6, and 56.8 mg/L for C_{14}, C_{12-14}, and C_{12}, respectively, in a growth inhibition test with *K. subcapitata*. Similarly, the 48-h-EC_{50} for *D. magna* was 5.0 mg/L for C_{14}, 18.0 mg/l for C_{12-14}, and 44.3 mg/L for C_{12}.

TABLE 1
Aquatic Toxicity of C_8 APG[b], C_{12-14} APG, C_{12} EGE, and C_{12-15} AE-7[a]

	Effect concentrations (mg/L)			
	C_8 APG[b]	C_{12-14} APG	C_{12} EGE	C_{12-15} AE-7
Kirchneria subcapitata				
EC_{50} (72 h)	1543 (1474–1621)	11 (10–13)	38 (37–38)	0.85 (0.84–0.86)
NOEC[b]	100	3.1	11	0.50
Daphnia magna				
EC_{50} (48 h)	557 (465–717)	12 (10–14)	23 (21–25)	1.0–2.0
Brachydanio rerio				
LC_{50} (96 h)	558 (447–718)	2.5–5.0	11–17	1.0–2.0

[a]Parentheses indicate 95% confidence intervals; APG, alkyl polyglycoside; EGE, ethyl glycoside monoester; EC_{50} and LC_{50}, effective concentration and lethal concentration, respectively, causing 50% growth inhibition, immobilization, or mortality.
[b]NOEC, no-observed-effect concentration.

Chemical structure and environmental properties. Glycoside surfactants have glucose unit(s) in common, but their biodegradation and aquatic toxicity may be quite different. The C_{12} EGE was, first of all, characterized by a rapid mineralization under aerobic as well as anoxic conditions. This might be due to the monoglycoside nature of EGE and to an immediate enzymatic attack on the ester bond linking the ethyl and glucose units. The linear C_{12-14} APG was degraded both aerobically and under methanogenic conditions, but anaerobic mineralization required a period of acclimatization or detoxification. Adverse effects of APG on aquatic organisms might be related to the alkyl chain length as observed for glucose amides (11). The C_{12-14} APG was more toxic than the other glycoside surfactants, while the short-chained C_8 APG[b] showed low toxicity with all examined species. The branched hydrophobe of C_8 APG[b] may also explain the low toxicity of this surfactant. Compared to the linear alkyl chain of C_{12-14} APG, the branched structure of C_8 APG[b] was apparently more persistent in the absence of molecular oxygen.

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REFERENCES

1. *Ecolabelling Criteria for Laundry Detergents*, Final Proposal, Umweltbundesamt, Berlin, 1993.
2. Schöberl, P., K.J. Bock, M. Huber, and L. Huber, Ökologisch Relevante Daten von Tensiden in Wasch- und Reinigungsmitteln, *Tenside Surfact. Det. 25*:86–98 (1988).
3. Tryland, Ø., and Ø. Haraldstad, *SFT Report No. 91:06C*, Norwegian Pollution Control Authority, Oslo, 1991.
4. Holt, M.S., G.C. Mitchell, and R.J. Watkinson, The Environmental Chemistry, Fate, and Effects of Nonionic Surfactants, in *Detergents*, edited by N.T. de Oude, Springer-Verlag, Berlin, 1992, pp. 89–144.
5. Painter, H.A., Anionic Surfactants, in *Ibid.*, pp. 1–88.
6. Kimerle, R.A., and R.D. Swisher, Reduction of Aquatic Toxicity of Linear Alkylbenzene Sulfonate (LAS) by Biodegradation, *Water Res.* 11:31–37 (1977).
7. Turner, A.H., F.S. Abram, V.M. Brown, and H.A. Painter, The Biodegradability of Two Primary Alcohol Ethoxylate Nonionic Surfactants Under Practical Conditions, and the Toxicity of the Biodegradation Products to Rainbow Trout, *Ibid. 19*:45–51 (1985).
8. Swisher, R.D., Surfactant Biodegradation, Surfactant Science Series, Vol. 18, Marcel Dekker, Inc. New York, 1987.
9. Steber, J., and P. Wierich, The Anaerobic Degradation of Detergent Range Fatty Alcohol Ethoxylates. Studies with ^{14}C-Labelled Model Surfactants, *Water Res. 21*:661–667 (1987).
10. Wagener, S., and B. Schink, Anaerobic Degradation of Nonionic and Anionic Surfactants in Enrichment Cultures and Fixed-Bed Reactors, *Ibid. 21*:615–622 (1987).
11. Stalmans, M., E. Matthijs, E. Weeg, and S. Morris, The Environmental Properties of Glucose Amide—a New Nonionic Surfactant, *SOFW J 19*:795–808 (1993).
12. Weuthen, M., R. Kawa, K. Hilland, and A. Ansmann, Long-Chain Alkyl Polyglycosides—A New Generation of Emulsifiers, *Fat. Sci. Technol. 97*:209–211 (1995).
13. Water Quality—Evaluation of the "Ultimate" Anaerobic Biodegradability of Organic Compounds in Digested Sludge—Method by Measurement of the Biogas Production, International Organization for Standardization, Geneva Draft International Standard No. ISO/DIS 11734, 1994.
14. Madsen, T., H.B. Rasmussen, and L. Nilsson, Anaerobic Biodegradation Potentials in Digested Sludge, a Freshwater Swamp and a Marine Sediment, *Chemosphere 31*:4243–4258 (1995).
15. Organization for Economic Co-operation and Development, *OECD Guidelines for Testing of Chemicals*, Organization for Economic Co-Operation and Development, Paris, 1993.
16. Water Quality—Determination of the Inhibition of the Mobility of *Daphnia magna* Straus (Cladocera, Crustacea), International Organization for Standardization, Geneva, International Standard No. ISO 6341, 1982.
17. Water Quality—Fresh Water Algal Growth Inhibition Test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*, International Organization for Standardization, Geneva, International Standard No. ISO 8692, 1989.
18. Painter, H.A., *Detailed Review Paper on Biodegradability Testing, Environment Monograph No. 98*, Organization for Economic Co-Operation and Development, Paris, 1995.
19. Madsen, T., H.B. Rasmussen, and L. Nilsson, Methods for Screening Anaerobic Biodegradability and Toxicity of Organic Chemicals, *Danish Environmental Protection Agency*, Copenhagen, 1996.
20. Kravetz, L., J.P. Salanitro, P.B. Dorn, and K.F. Guin, Influence of Hydrophobe Type and Extent of Branching on Environmental Response Factors of Nonionic Surfactants, *J. Am. Oil Chem. Soc. 68*: 610–618 (1991).
21. Federle, T.W., and B.S. Schwab, Mineralization of Surfactants in Anaerobic Sediments of a Laundromat Wastewater Pond, *Water Res. 26*:123–127 (1992).
22. Holt, M.S., E. Matthijs, and J. Waters, The Concentrations and Fate of Linear Alkylbenzene Sulphonate in Sludge Amended Soils, *Ibid. 23*:749–759 (1989).
23. Brunner, P.H., S. Capri, A. Marcomini, and W. Giger, Occurrence and Behaviour of Linear Alkylbenzenesulphonates, Nonylphenol, Nonylphenol Mono- and Nonylphenol Diethoxylates in Sewage and Sewage Sludge Treatment, *Ibid. 22*:1465–1472 (1988).
24. Urano, K., and M. Saito, Adsorption of Surfactants on Microbiologies, *Chemosphere 13*:285–293 (1984).

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