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Biodegradability of Ethoxylated Fatty Amines and Amides and the Non-Toxicity of their Biodegradation Products

Using the "prolonged" closed bottle test (OECD Guideline 301 D) the biodegradability of various ethoxylated fatty amines and amides was determined. All alkylbis(2-hydroxyethyl)amines were readily biodegradable. The biodegradation curves of the fatty amines and amides with more than 2 oxyethylene groups suggested a "rapid" mineralization via an initial oxidation of the alkyl chain. The intermediates formed, viz. ethoxylated secondary amines, were biodegraded "slowly". This total mineralization of the ethoxylated fatty amines was demonstrated in "prolonged" closed bottle tests by "two phase" biodegradation curves. In the SCAS test (OECD Guideline 302 A), polyoxyethylene(15)tallow amine (hydrogenated tallow-alkyl) was immediately converted into carbon dioxide, water, biomass and a water-soluble intermediate. The intermediate, an ethoxylated secondary amine, was removed from the waste water after an adaptation period of approximately two months. This intermediate was non-toxic to the aquatic life as assessed in the algal growth inhibition test (OECD Guideline 201) and in the acute toxicity test for Daphnia (EEC Guideline 79/831).

Die biologische Abbaubarkeit einiger ausgewählter ethoxylierter Fettamine und Fettamide wurde mit Hilfe des verlängerten Geschlossenen Flaschentests (OECD Guideline 301 D) untersucht. Alle im Test eingesetzten Alkylbis-(2-hydroxyethyl) amine sind durchweg schnell (readily) biologisch abbaubar. Der Verlauf der Abbaukurven von Fettaminen und Fettamiden mit mehr als 2 Oxyethylengruppen läßt darauf schließen, daß der Abbau zunächst über eine rasche Oxidation der Alkylkette erfolgt. Die dabei gebildeten Intermediate – ethoxylierte sekundäre Amine – werden langsam abgebaut. Die vollständige Abbaubarkeit der ethoxylierten Fettamine zeigt sich im verlängerten Geschlossenen Flaschentest durch den zweiphasigen Verlauf der Abbaukurven. Im SCAS-Test (OECD Guideline 302 A) wird Polyoxyethylen(15)talgamin (hydrogeniertes Talgalkyl) direkt in CO_2, Wasser, Biomasse und ein wasserlösliches, inhärent abbaubares Intermediat umgewandelt. Das Intermediat, ein ethoxyliertes sekundäres Amin, war nach einer Adaptationszeit von zwei Monaten aus dem Abwasser entfernt. Dieses Intermediat ist für aquatische Organismen nicht toxisch, wie im Algenwachstumshemmungstest (OECD Guideline 201) und im akuten Toxizitätstest für Daphnien (EEC Guideline 79/831) gezeigt werden konnte.

1 Introduction

Ethoxylated fatty amines and amides have at least one long alkyl chain and 2 to 50 EO groups (polyoxyethylene) covalently linked to a nitrogen atom. These chemicals are used in a wide range of applications such as agro-adjuvants, wetting agents and emulsifiers. Due to these uses it is reasonable to suppose that ethoxylated fatty amines and amides end up in the environment. Therefore, knowledge about their biodegradability is essential to assess the impact on the environment.

Sawyer et al. (1956) showed that ethoxylated amides are susceptible to microbial oxidation. Recently, the biodegradability of ethoxylated fatty amines has been determined in screening tests and simulation tests of activated sludge plants. The biodegradability of the ethoxylated fatty amines decreases with increasing numbers of polyoxyethylene groups (*Schöberl* et al., 1988). Ethoxylated fatty alcohols have been studied extensively (*Swisher*, 1987). The linear versions of these alcohol polyglycol ethers are highly biodegradable under aerobic conditions (*Larson* and *Games*, 1981; *Vashon* and *Schwab*, 1981; *Steber* and *Wierich*, 1983). Unlike the total mineralization of these alcohol ethoxylates, alkylphenol ethoxylates are converted into carbon dioxide, water and moderately stable intermediates (*Rudling* and *Solyom*, 1974; *Giger* et al., 1981; *Brunner* et al., 1988). These intermediates, low nonylphenol ethoxylates, are formed particularly at low temperatures in activated sludge plants (*Kravetz* et al., 1984). However, after appropriate acclimatization alkylphenol ethoxylates do undergo complete mineralization.

The purpose of our study was to determine the biodegradability of various ethoxylated fatty amines and amides in the closed bottle test and to assess the toxicity of intermediates formed during biodegradation processes. Closed bottle test results were compiled and compared in order to formulate a biodegradation route and general rules on the biodegradability of these compounds.

2 Materials and methods

2.1 Chemicals

The ethoxylated fatty amines and amides (trade names: Ethomeen S/12, Ethomeen T/12, Ethomeen HT/12, Ethomeen

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Table 1. Biodegradation in closed bottle tests of various ethoxylated fatty amines on day 28 and at the end of the prolonged tests

Test compound	% at day 28	% at end of the test; last day between brackets
Tallowbis(2-hdroxyethyl)amine[a]	60[b]	
Tallowbis(2-hydroxyethyl)amine	52	64 (42)
Oleylbis(2-hydroxyethyl)amine	63[b]	74 (42)
Oleylbis(2-hydroxyethyl)amine	13	54 (126)
Polyoxyethylene(15)tallow amine	42	61 (214)
Polyoxyethylene(15)tallow amine[a]	28	67 (214)
Polyoxyethylene(15)oleyl amine	23	64 (126)

a Hydrogenated alkyl chain
b Silica gel added

T/25, Ethomeen HT/25, Ethomeen S/25, Ethomeen HT/60 and Ethomid HT/15) were obtained from Akzo Chemicals BV, Deventer, The Netherlands. All other chemicals used (reagent grade) were purchased from Janssen Chimica, Beerse, Belgium.

2.2 *Inoculum and settled sewage*

Secondary activated sludge and primary settled sewage were obtained from an activated sludge plant treating predominantly domestic sewage.

2.3 *Test organisms and maintenance*

The algal growth inhibition test was carried out with the unicellular green algae, Selenastrum capricornutum, ATCC 22662. This strain was maintained on mineral salts agar slants in light at room temperature. The acute toxicity test for Daphnia was carried out with Daphnia magna maintained in a continuous culture on synthetic medium. The animals used were less than 24 hours old at the start of the test.

2.4 *Test methods*

The closed bottle tests were carried out according to an OECD test guideline (1982). The introduction of a few minor deviations and the prolongation of the closed bottle test have been described by *van Ginkel* and *Stroo* (1992).

The SCAS test was carried out according to an OECD test guideline (1981). The test unit was fed with settled sewage and 50 mg/L polyoxyethylene(15)tallow amine (hydrogenated tallow), whereas the control unit was fed only with settled sewage. The incubation temperature was 20 °C.

The algal growth inhibition test was carried out according to the OECD Test Guideline (1984). One deviation from the protocol of the algal growth inhibition test was introduced. The algae were not inoculated in the OECD medium but directly in filtered effluent from the control unit.

The acute toxicity test with Daphnia magna was carried out in accordance with an EEC directive (1989), the only deviation being that 5 animals were used instead of 20.

2.5 *Determination of oxygen, NPOC and cell density*

The dissolved oxygen concentrations were determined electrochemically using an oxygen electrode (WTW Trioxmatic EO 200) and meter (WTW OXI 530).

Cell densities of the algae cultures were determined spectrophotometrically using a Shimadzu, UV/VIS Spectrophotometer UV-160A. These determinations were carried out at 436 nm in a cuvette with a light path of 4 cm.

To determine the non-purgeable organic carbon (NPOC) in the effluent of the SCAS units, samples were filtered using Schleicher and Schüll filters (8 μm) to remove any sludge particles. The filtered samples were acidified prior to injecting in a Dohrmann DC-190 TOC apparatus.

3 Results and discussion

3.1 *Closed bottle test results*

Table 1 shows closed bottle test results of some ethoxylated fatty amines. When discussing these results the following definitions are used: "ready biodegradability" is a legislative conception for compounds reaching 60 per cent biodegradation within 28 days, an organic compound is "rapidly" or "slowly" degraded when the biodegradation curve obtained is steep or flat, respectively, and "completely mineralized" compounds reach 60 per cent biodegradation or more and are probably converted completely to carbon dioxide, water and biomass.

The top part of Table 1 shows that all alkylbis(2-hydroxyethyl)amines tested were completely mineralized within 28 days. These results permit a ready biodegradability classification. *Schöberl* et al. (1988) reported 85 % biodegradation of an alkylbis(2-hydroxyethyl)amine in the *Sturm* test. This percentage also demonstrates the ready biodegradability of the alkylbis(2-hydroxyethyl)amines. The alkylbis(2-hydroxyethyl)amines were toxic to microorganisms present in the inoculum of the closed bottle test. Hence, alkylbis(2-hydroxyethyl)amines were tested in the presence of silica gel to reduce the concentration of the test compound in the water phase. This addition provides optimum conditions in the closed bottle test so that the alkylbis(2-hydroxyethyl)amines may achieve > 60 % biodegradation within 28 days. As an example, closed bottle test results of oleylbis(2-hydroxyethyl)amine in the presence and absence of silica gel are shown (Table 1).

Other ethoxylated fatty amines tested were biodegraded 10–42 % on day 28 (Table 1), which is comparable to the results of *Schöberl* et al. (1988) and *Fischer* (1972). Therefore, amines with polyoxyethylene chains are not readily biodegradable. However, in the prolonged closed bottle tests most of these compounds reached about 60% biodegradation, indicating slow but complete mineralization.

3.2 *Biodegradation route of ethoxylated amides and amines*

Many results available suggest a "two phase" biodegradation which is illustrated in a prolonged closed bottle test with polyoxyethylene(15)tallow amine (hydrogenated tallow) (Fig. 1). After two weeks in the closed bottle test a biodegradation percentage of about 25 was reached and subsequently the test compound was completely mineralized at a slower rate. This "two-phase" biodegradation was also found in duplicate tests with polyoxyethylene(5)tallow amide showing an interesting discrepancy (Fig. 2). In one test the biodegradation curve of this amide levelled off at about 30 %, whereas in a replicate test polyoxyethylene(5)tallow amide (hydrogenated tallow) was completely mineralized. The reason for this discrepancy between the test results is probably the limited inoculum in the closed bottle. The inoculum of the closed bottle test contained only 2 mg dry weight/L and therefore cannot represent the full potential of activated sludge plants and nature. The biodegradation curves suggest that the alkyl chains are oxidized in the first phase and that the ethoxylated secondary amines formed are slowly degraded in the second phase of the prolonged closed bottle tests.

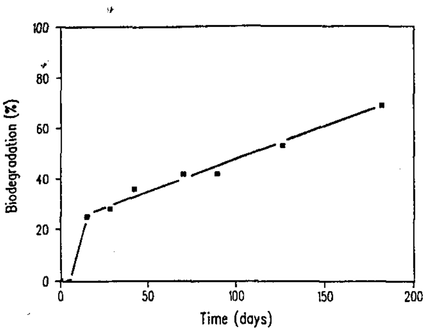


Fig. 1. Percentage of biodegradation of polyoxyethylene(15)tallow amine (hydrogenated tallow) versus time in a prolonged closed bottle test

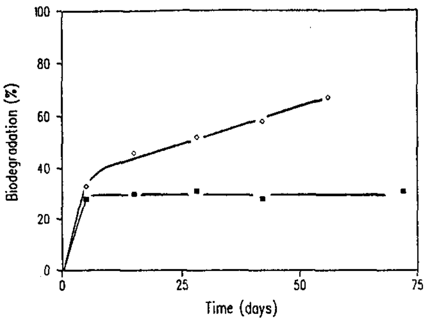


Fig. 2. Separate prolonged closed bottle test results with polyoxyethylene(5)tallow amide (hydrogenated tallow) showing a discrepancy

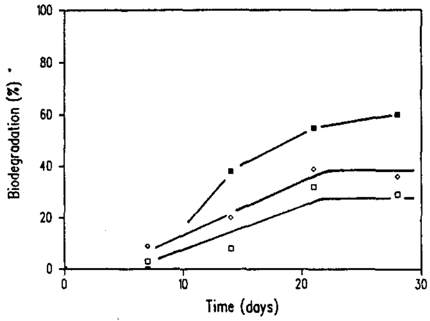


Fig. 3. The biodegradation of ethoxylated tallow amines with 2 (■), 15 (◇) and 50 (□) EO groups in closed bottle tests

This supposition is confirmed with closed bottle test results of fatty amines with various numbers of EO groups. The biodegradation curves of ethoxylated tallow amines (hydrogenated tallow) with 2, 15 and 50 oxyethylene groups reached different percentages on day 28 (Fig. 3). These percentages correspond to the oxidation of the alkyl chain in the respective ethoxylated fatty amines. For tallowbis(2-hydroxyethyl)amine largely consists of hydrocarbon whereas, polyoxyethylene(50)tallow amine contains only a small percentage of hydrocarbon. The resistance to biodegradation by an increased degree of ethoxylation was first pointed out by *Bogan* and *Sawyer* (1955). These authors, however, attributed the reduced biodegradability of the higher ethoxylates to transport problems through the cell membranes.

From a comparison of the biodegradation curves of ethoxylated amines with the curves of the amides it is clear that the first phase of the biodegradation in the closed bottle test of the amides was completed on day 5 whereas the alkyl chain of the amines was biodegraded on day 15 (Figs. 1 and 2). This difference may be explained by a rapid enzymatic hydrolysis of the amide bond. The C-N bond of amines, on the other hand, has to be split by an oxidative reaction.

On the basis of the evaluation of the results a biodegradation route of ethoxylated fatty amines and amides is proposed (Fig. 4). The central fission of the ethoxylated fatty amines and amides is in line with the alcohol ethoxylate biodegradation route found by *Ichikawa* et al. (1978). On the other hand, *Rudling* and *Salyom* (1974) indicated that alkylphenol ethoxylate biodegradation results in the formation of a relatively bioresistant alkylphenoxy moiety. Using HPLC/MS *Stephanou* and *Giger* (1982) positively identified an enrichment in shorter chain nonylphenol ethoxylates, which was due to the monomer-wise shortening of the EO chain.

The following general rules have been formulated on the basis of closed bottle test results and the proposed biodegradation route. Alkylbis(2-hydroxyethyl)amines are readily biodegradable. All polyoxyethylenealkyl amines are "rapidly" biodegradable via an initial oxidation of the alkyl chain. The intermediates formed, i.e. secondary ethoxylated amines, are slowly biodegradable. The alkyl chain of the ethoxylated fatty amines does not substantially affect the biodegradation rate.

3.3 Coupling of the SCAS test and aquatic toxicity tests

The rapid initial oxidation of the alkyl chain of the ethoxylated fatty amines and amides results in loss of surfactant properties of these compounds and probably forms a non-toxic organic compound. To prove this assumption, the SCAS test was coupled to aquatic toxicity tests. The SCAS test was fed with 50 mg/L of polyoxyethylene(15)tallow amine (hydrogenated tallow). Prior to the addition of the test compound the effluent NPOC (non-purgeable organic carbon) values obtained from the test unit and the control unit were comparable and constant. Both the calculated and the measured NPOC of the test compound in the influent of the SCAS test were 32 mg/L. This value was used to calculate the removal percentages. After the first additions, a removal of approximately 55% was immediately achieved (Fig. 5). This removal confirms the biodegradation mechanism proposed because the secondary amine with 15 oxyethylene groups could account for the NPOC found in the effluent. Between day 20 and day 40 the removal percentages increased to 90–95% (Fig. 5). The increased removal of the NPOC shows the susceptibility of the intermediate to biodegradation. The NPOC detected in the effluent (days 0–20) did not cause foaming in the test unit at the end of each cycle, which demonstrates loss of surfactant properties.

The growth of Selenastrum capricornutum was determined in the effluents of the test unit and the control unit collected on days 1–7 and on days 15–21. Furthermore, the acute toxicity to Daphnia magna was determined in both effluents collected on days 8–15. Growth of the algae was not inhibited and all Daphnids survived in the effluent of the test unit. Therefore, the effluent of the test unit containing the water-soluble

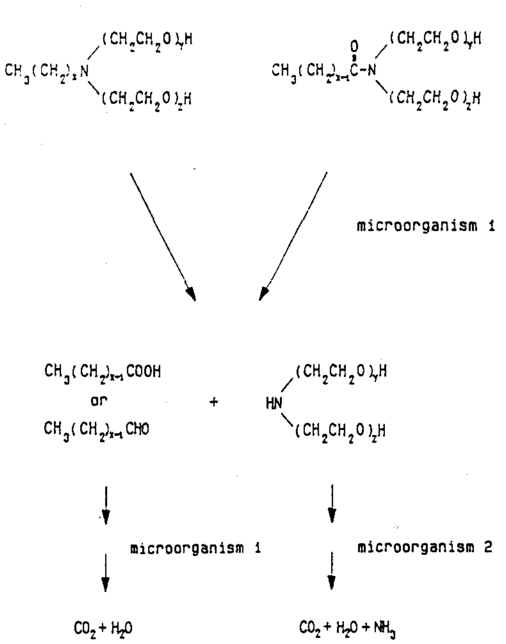
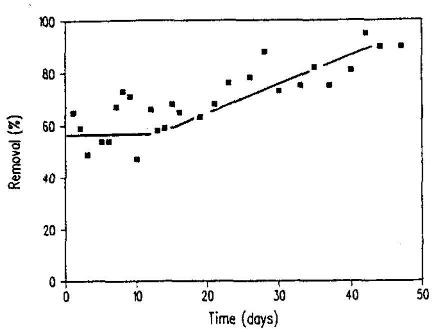


Fig. 4. Proposed biodegradation route of ethoxylated fatty amines and amides



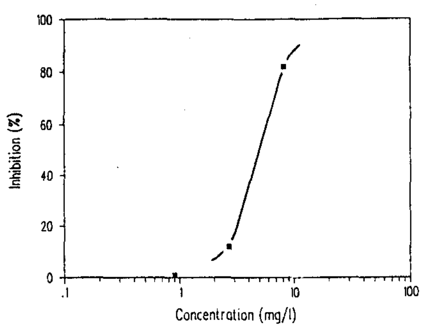


Fig. 5 (left). Removal of polyoxyethylene(15)tallow amine (hydrogenated tallow) from sewage in the SCAS test

Fig. 6 (right). Toxicity of polyoxyethylene(15)tallow amine (hydrogenated tallow) to Selenastrum capricornutum determined in the effluent of the control unit of the SCAS test

intermediate was non-toxic to both algae and Daphnids whereas the parent surfactant compound was toxic.

For instance, the chronic toxicity of polyoxyethylene(15)tallow amine (hydrogenated tallow) to algae is shown by an effect-concentration relationship (Fig. 6). Effluent of the control unit of the SCAS test was used as diluent. From this relationship an EC_{50} of 4.9 mg/L (3.0 mg NPOC/L) was calculated. This effect concentration agrees with the value described by *Schöberl* et al. (1988).

The non-toxicity of the secondary ethoxylated amine shows that loss of surfactant properties and loss of toxicity are correlated. In the case of the alkylphenol ethoxylates, it was established that more toxic metabolites are produced by biodegradation (*Patoczcka* and *Pulliam*, 1990). The conversion of nonylphenol ethoxylates into nonylphenol, nonylphenol mono- and nonylphenol diethoxylates does not result in loss of surfactant properties. The different toxicity of the biodegradation products of various nonionic surfactants can be explained using their biodegradation mechanisms. Alkylphenol ethoxylates are initially attacked by shortening the polyoxyethylene chain, whereas the ethoxylated fatty amines and alcohol ethoxylates are degraded by an initial central fission which immediately results in detoxification.

Summarising, ethoxylated fatty amines and amides are readily converted into carbon dioxide, water, biomass and slowly degradable secondary amines. These intermediates are not toxic to aquatic organisms. Decisive evidence for the central fission of the ethoxylated fatty amines obtained in a study with alkylbis(2-hydroxyethyl)amine degrading bacteria will be reported in a later paper.

Acknowledgments

The authors would like to express their thanks to I. Arends for determining the acute toxicity to Daphnids and to express their appreciation to U. Mark and M. Kolvenbach for their helpful comments.

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