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Complete degradation of xenobiotic surfactants by consortia of aerobic microorganisms

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Abstract

Linear alkylbenzene sulphonates are primarily attacked *via* a hydroxylation of the alkyl chain from the methyl group followed by β-oxidation. The alkyl chain is metabolized by pure cultures to give sulphophenyl carboxylates which accumulate in the medium. In mixed culture, other microorganisms are capable of degrading sulphophenyl carboxylates. Formation of ethylene glycol monosulphates as major products of alkyl ethoxy sulphates demonstrates that the ether bonds are cleaved. The bacteria involved in growing on the alkyl chain are unable to utilize the hydrophilic moiety. This hydrophilic moiety, in turn, is degraded by other microorganisms.

The degradation of alkylphenol ethoxylates and highly branched alcohol ethoxylates proceeds by shortening the polyoxyethylene chain leaving the hydrophobic part of the molecule. The biodegradation of linear alcohol ethoxylates and ethoxylated fatty amines is initiated by a central cleavage or ω-oxidation. Subsequent oxidation of the alkyl chains results in the production of polyethylene glycols and secondary ethoxylated amines. Both polar moieties are metabolized by other microorganisms. Degradation of alkyltrimethylammonium salts and alkylamines is initiated by a cleavage of the C_{alkyl}-N bond. The central fission leads to the formation of alkanals which are readily converted by β-oxidation. The alkyl chain-utilizing bacteria are not able to degrade the methylamines. The methylamines, in turn, are subject to biodegradation by methylotrophs.

The limited metabolic capacities of pure cultures of microorganisms utilizing surfactants point to the requirement of consortia to degrade surfactants completely. Complete degradation of surfactants is accomplished by mixed cultures of microorganisms constructed on the basis of synergistic and commensalistic relationships. However, degradation of a surfactant by one member of a commensalistic consortium may lead to the production of toxic or non-toxic metabolites. Waste water treatment without the build up of such metabolites can be achieved in plants operated with sludge retention times that are suitable for maintaining all microorganisms of the consortium. In contrast, in natural ecosystems the introduction of a surfactant may result in a transient formation of a metabolite.

Introduction

Surfactants (surface-active agents) are materials which accumulate at gas/liquid, liquid/liquid and liquid/solid interfaces. To achieve this accumulation, all surfactants include two distinct components in their molecular structure. A hydrocarbon part which on its own would be poorly soluble in water is called the hydrophobic or lipophilic group. The hydrophobic moiety is usually a C_8 to C_{22} alkyl chain or an alkylaryl group. The alkyl chain may be linear or branched. The other part of the molecule, which is water-soluble, is known as the hydrophilic group. Surfactants are classified according to the nature of the charge of this polar moiety. Anionic surfactants are negatively charged usually due to a sulphonate or sulphate group. Nonionic surfactants contain no ionic constituent and the majority of all nonionics are polymerisation products of 1,2-epoxyethane. Cationic surfactants are characterised by a quaternary ammonium group which is positively charged. Finally, amphoteric surfactants have both positively and negatively charged moieties in the same molecule.

Surfactants are especially noted for their wetting qualities and their effectiveness as emulsifiers. Moreover some surfactants readily adsorb onto surfaces which leads to surface modification. These properties account for the exploitation of surfactants in many product areas as detergents and household cleaners and to a lesser extent as textile softener, other textile aids, antistatic agents, additives in paints, metal processing, shampoos, cosmetics, and oil drilling operations. Some surfactants have antimicrobial properties which provide the basis for their utility as biocides.

Due to these uses most surfactants appear in natural ecosystems either through a discharge of waste water (e.g. detergents) or through direct application (e.g. biocides). Surfactants can be toxic to bacteria, algae, daphnids and fish at concentrations as low as 1 mg/l. (Lewis 1990; Schöberl et al. 1988). To prevent harmful effects to ecosystems, biodegradation of surfactants is very important. Biodegradation of surfactants ensures low environmental concentrations. Thus studies on the biodegradation of surfactants are often carried out to predict their level in ecosystems and effluents of waste water purification plants. The degradative pathways of anionic, nonionic and cationic surfactants were recently reviewed by White & Russell (1993), White (1993) and van Ginkel (1995). The biodegradation of surfactants in ready and inherent biodegradability tests and simulation tests of waste water treatment plants and natural environments was extensively reviewed by Swisher (1987). These tests clearly prove the susceptibility to biodegradation of surfactants by mixed cultures of microorganisms.

Mixed cultures of microorganisms are established where complex carbon and energy sources are degraded (Slater & Lovatt 1984). Surfactants are complex organic chemicals in which hydrophobic and hydrophilic groups are joined together in the same molecule. It is therefore expected that consortia will play an important role in the biodegradation of these compounds. Biodegradation in both biological waste water treatment plants and natural ecosystems depends on communities of microorganisms. However, the activity of one member of a community degrading a surfactant through concerted metabolic attack may result in the accumulation of metabolites (Slater & Lovatt 1984). Therefore, not only the biodegradation of surfactants, but also the fate of metabolites formed needs to be understood. To this end metabolic studies with pure cultures are assessed in this paper to provide data to understand the fate of all classes of surfactants and their breakdown products in the environment. This knowledge of the metabolism of surfactants gives clues to the interdependence within microbial mixed cultures effecting degradation. Finally, implications of the biodegradation by consortia in waste water treatment systems and ecosystems are discussed.

Anionic surfactants

Alkylbenzene sulphonates

The biological degradation of alkylbenzene sulphonates varies greatly (Cain 1981; Gleddhill 1975). Whereas linear alkylbenzene sulphonates (LAS) are readily biodegradable, branched alkylbenzene sulphonates are degraded at a lower rate. Similarly, methyl branching and quaternary groupings of alkanes increase the resistance to biodegradation (McKenna & Kallio 1965; Britton 1984). LAS is primarily degraded by bacteria, although one fungus also is known to biodegrade LAS. Most knowledge on the metabolism of LAS has been gained through the investigation of the pathways of dodecylbenzene sulphonate.

The pathways for dodecylbenzene sulphonate biodegradation have been studied in a *Bacillus* sp. and *Chladosporium resnae*. Both strains oxidize the side chain and the aromatic ring of dodecylbenzene sulphonate. In these microorganisms the complete mineralization has been shown to follow different pathways. The biodegradation by a *Bacillus* sp. isolated by Willets & Cain (1972[a]) is initiated by the release of the sulphonate group as sulphite and by utilization of acetyl-CoA units cleaved from the alkyl chain by an unknown sequence. These reactions result in the transient formation of *p*-hydroxyphenylalkanoates, which suggests that the rate limiting step of dodecylbenzene sulphonate catabolism by the *Bacillus* sp. is the degradation of the aromatic moiety (Willets 1974). A fungus , *Chladosporium resnae*, isolated from sludge in airplane fuel tanks, is also capable of degrading both the alkyl chain and the polar moiety by similar sequence. However, the fungus cleaves the aromatic C-S bond releasing sulphate so that phenylalkanoates rather than *p*-hydroxyphenylalkanoates are formed (Willets 1973).

However, an overwhelming body of evidence suggests that LAS is predominantly biodegraded by microorganisms which are not able to degrade the aromatic moiety (Divo & Cardini 1980; Swisher 1987). For instance, a *Vibrio* sp. is capable of metabolizing the alkyl chain of dodecylbenzene sulphonate as the sole source of carbon and energy (Bird & Cain 1974). The route for the degradation of the alkyl chain of dodecylbenzene sulphonate in this microorganism is initiated by a hydroxylation to sulphobenzene dodecanol. This reaction is probably carried out by a monooxygenase (Bird & Cain 1974). The alcohol formed is then oxidized via an alkanal to the alkanoic acid. The step before β-oxidation begins with the synthesis of the acyl-CoA thioester from the alkanoic acid. Subsequently, β-oxidation yields a succession of acetyl-CoA units. This sequence of enzyme activities leads to benzylsulphonate butyric acid and benzylsulphonate acetic acid during growth of microorganism on dodecylbenzene sulphonate. Detection of these compounds in the culture supernatant demonstrates that only the alkyl chain of dodecylbenzene sulphonate is utilized as carbon and energy source. This biodegradation pathway is shown schematically in Fig. 1. Oxidation of odd chain undecylbenzene sulphonate produces sulphobenzyl propionic acid and other LAS isomers give rise to other sulphophenyl alkanoates (Bird & Cain 1972; Cain et al. 1972; Divo & Cardini 1980; Willets & Cain 1972^b). Summarizing, sulphophenyl alkanoates (SPC) are excreted due to the inability of most LAS utilizing microorganisms to metabolize the aromatic nucleus.

The degradation of SPC by other microorganisms probably proceeds through two main reactions, namely desulphonation and ring opening. These reactions have been studied with a structural analogue, 4-sulphobenzoate, an intermediate in the biodegradation pathway of toluene sulphonic acid, which undergoes a dioxygenase reaction (Locher et al. 1989). The introduction of two oxygen atoms on the 3 and 4 position of the aromatic nucleus results in a spontaneous removal of sulphite (Locher et al. 1991). The conversion by a dioxygenase reaction is followed by a cleavage at the *ortho* or *para* position. The ring opening leads to formation of products that enter the central metabolism of microorganisms (Cain & Farr 1968; Gledhill 1975).

Alkane sulphonates

Pseudomonas AJ2 is capable of growing on C_8–C_{12} *n*-alkane sulphonates (Thijsse & Wanders 1972). The initial attack on alkane sulphonates depends on the presence of molecular oxygen and NADH indicating monooxygenase activity. A hydroxylation of the α carbon of alkane sulphonates by this monooxygenase yields the labile 1-hydroxy-*n*-alkane-1-sulphonate which hydrolyses to give the corresponding alkanals and sulphite (Thijsse & Wanders 1974). The oxidation of the alkanals leads to the production of homologous alkanoic acids. The alkanoic acids, in turn, are metabolized by β-oxidation.

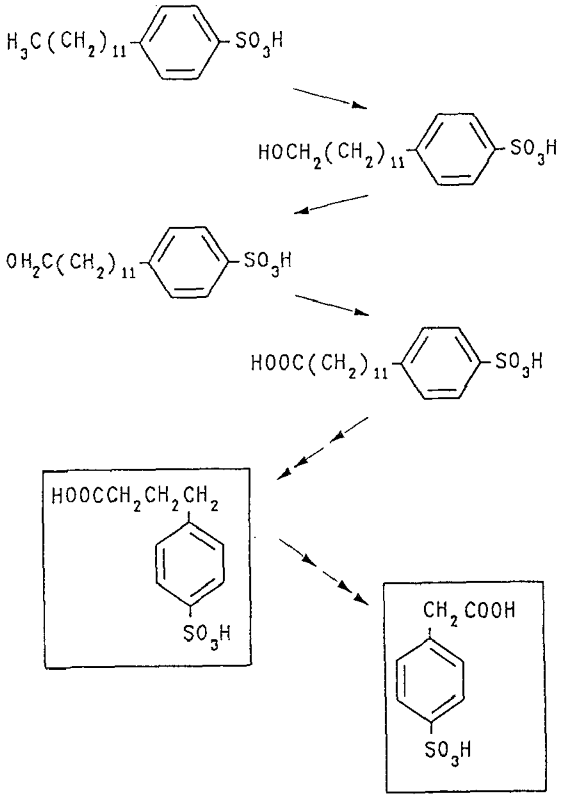

Fig. 1. Major metabolic pathway for the degradation of dodecylbenzene sulphonate with the generation of SPC by pure cultures of microorganisms. Boxes indicate products which are degraded by other microorganisms.

The anionic surfactant dialkyl sulphosuccinate recently has been investigated. The initial transformations of this compound include hydrolysis of both ester bonds by mixed cultures yielding alcohols, monoalkyl sulphosuccinate and sulphosuccinate. A transient production of sulphosuccinate was observed (Hales 1993). The product sulphosuccinate is converted by *Pseudomonas* sp. BS1 through an oxidative desulphonation to form oxaloacetate and sulphite (Quick et al. 1994). Oxaloacetate is subsequently used in the citric acid cycle by *Pseudomonas* sp. BS1 for energy production and biomass formation. Sulphite is rapidly oxidized to sulphate.

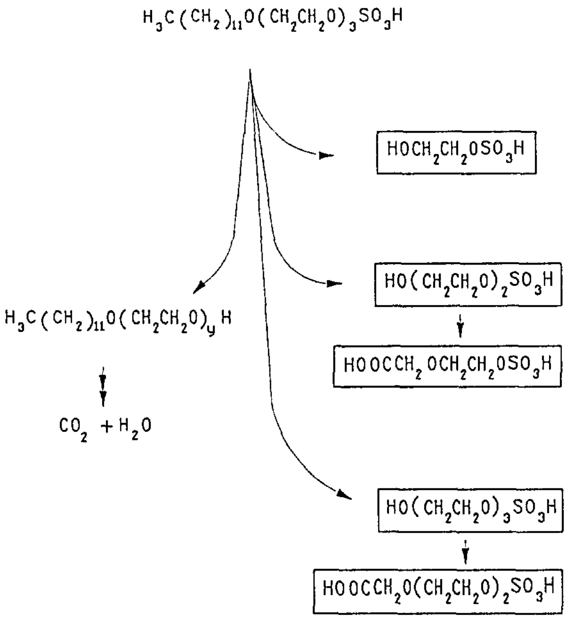

Fig. 2. The postulated pathway for the degradation of dodecyl triethoxy sulphate by *Pseudomonas* DES1 *via* ether and ester scissions. Boxes indicate products which are degraded by other microorganisms.

Alkyl sulphates

Microorganisms grow at the expense of primary and secondary alkyl sulphates (Payne 1963; Payne & Feisal 1963; Lijmbach & Brinkhuis 1973). These alkyl sulphates are initially degraded by the removal of the sulphate group (Payne et al. 1965; Dodgson & White 1983). Primary alkyl sulphates are hydrolysed to the alcohol and sulphate. The alcohol, in turn, is oxidized *via* an alkanal into the corresponding alkanoic acid. Next, alkanoic acid is converted into acyl-CoA which is further degraded by β-oxidation. Evidence for degradation by the β-oxidation pathway of alkyl sulphates was obtained by measuring enzyme activities (Dodgson et al. 1974). The pathway for degradation of secondary alkyl sulphates has also been established. This is by production of a secondary alcohol, which is then converted by a dehydrogenation reaction to an alkanone (Payne et al. 1967). The alkanone undergoes hydroxylation at the carbon adjacent to the carbonyl group, followed by oxidation to the dione. Hydrolysis of the dione generates an alkanoic acid and alkanal suitable for β-oxidation (Lijmbach & Brinkhuis 1973).

Alkyl ethoxy sulphates

Pseudomonas DES 1 was isolated from activated sludge by enrichment culture containing dodecyl triethoxy sulphate as the sole carbon and energy source (Hales et al. 1982). Whilst the alkyl sulphate-degrading microorganisms release sulphate, alkyl triethoxy sulphate-degrading *Pseudomonas* DES1 initially acts at one of the ether bonds. Cleavage of ether bonds results in the production of mono-, di-, and triethylene glycol sulphates in the proportions 35%, 45% and 20%, respectively. Acetic-2-(ethoxy sulphate) and acetic acid-2(diethoxy sulphate) are minor degradation products (Hales et al. 1982). Fig. 2 indicates the major biodegradation pathways of alkyl triethoxy sulphate. Degradation of the hydrophobic group alone is sufficient to support growth of *Pseudomonas* DES 1. Dodecanol, mono- and diethylene glycol dodecylether were identified as metabolites which demonstrates the involvement of ether cleaving enzymes. Another metabolite, triethylene glycol dodecyl ether, is formed through the action of a sulphatase. The hydroxy groups of the metabolites are oxidized by *Pseudomonas* DES1 (Griffith et al. 1987). Although the biodegradation route of ethylene glycol sulphates has not been elucidated, studies with mixed cultures demonstrate that these compounds are easily metabolized (Vashon & Schwab 1982; Griffith et al. 1986).

Nonionic surfactants

Alcohol ethoxylates

The metabolism of alcohol ethoxylates can be initiated by attack on the terminal alcohol group, terminal oxidation of the alkyl chain or by a central fission of the ether bond adjacent to the hydrophobic group. These patterns of initial attack are described for branched alcohol ethoxylates, secondary and primary linear alcohol ethoxylates.

Biodegradation of tetradecanol nonaoxyethylene primarily labelled with ^3H at the α and γ carbon atom resulted in rapid formation of ^3H_2O with little carbon dioxide evolution (Kravetz 1990). This implicates a central fission of alcohol ethoxylates. Different mechanisms have been proposed for the attack on the ether bond adjacent to the alkyl chain. Kravetz (1990) postulated that the stable ether linkage is transformed into an ester bond through a hydroxylation of the α carbon. However, Schöberl et al. (1982) found in respiratory

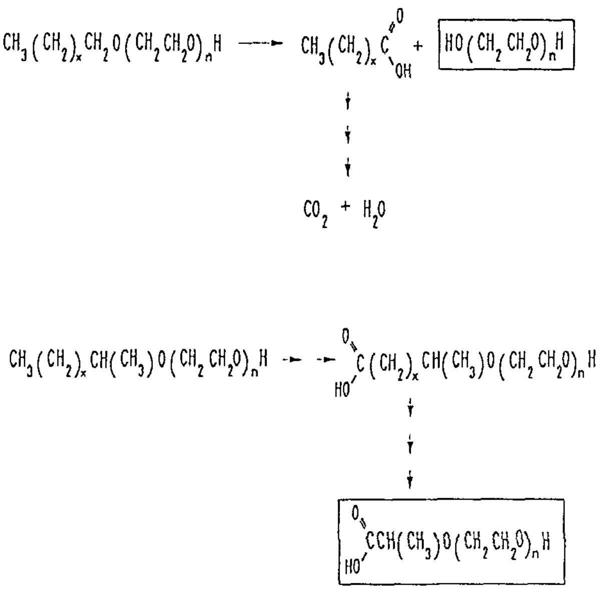

Fig. 3. Proposed biodegradation routes of primary linear alcohol ethoxylates *via* a central fission (above) and of secondary linear alcohol ethoxylates *via* an oxidation of the far end of the alkyl chain (below). Boxes indicate products which are degraded by other microorganisms.

studies that an alcohol ethoxylate-grown *Alcaligenes* sp. utilizes dodecanol and dodecanoic acid without lag. This result strongly indicates that the metabolic pathway of linear primary alcohol ethoxylates is initiated *via* a hydrolysis of the ether bond. Dodecanol is then converted into dodecanoic acid prior to β-oxidation. The *Alcaligenes* sp. is unable to metabolize polyethylene glycol. Previously a *Pseudomonas* sp. was isolated that is capable of degrading alcohol ethoxylates. This *Pseudomonas* sp. stoichiometrically excretes polyethylene glycol when grown on a nonanol ethoxylate (Ichikawa et al. 1978). Metabolic attack on secondary linear alcohol ethoxylates is probably initiated by an oxidation of the far end of the alkyl chain to give carboxy alkyl ethoxylates (Watson & Jones 1979). The ω-oxidation is followed by β-oxidation. This sequence leads to the production of carboxylated polyethylene glycols. The bonding of the secondary alcohol to the polyoxyethylene chain prevents a central fission. The pathways of linear alcohol ethoxylates are shown in Fig. 3.

Clearly, the pathway for the dissimilation of linear alcohol ethoxylates leads to the formation of polyethylene glycols (PEGs) and carboxylated PEGs. The EO chain of the majority of linear alcohol ethoxylates is shorter than thirty. PEG is, therefore, composed of one to thirty units of 1,2-epoxyethane (EO) attached by ether linkages and terminated with hydroxyl groups (MW < 1300). Low molecular weight PEGs are readily biodegraded by a variety of microorganisms, whereas high molecular weight PEGs (MW > 1000) were found to be less efficiently utilized (Watson & Jones 1977). The primary reaction in the biodegradation of PEG is the oxidation of a terminal primary alcohol group by membrane-bound dehydrogenase. Subsequently, the alkanal is oxidized to carboxylated PEG. Carboxylated PEGs have been identified in the culture filtrates of bacteria grown on PEG. Subsequently the ether bond of carboxylated PEG is cleaved, resulting in a PEG molecule that is reduced by one glycol unit and glyoxylic acid. Glyoxylic acid liberated by the ether cleavage is metabolized through the tartronic semialdehyde. Tartronic semialdehyde is further metabolized via the glycerate pathway to pyruvate (Kawai 1987).

Branched alcohol ethoxylates are not initially metabolized through a central fission or a ω/β-oxidation of the alkyl chain because no PEGs are produced. A shortening of the polyether moiety of branched alcohol ethoxylates by the elimination of EO groups was suggested by Kravetz (1990). The elimination of EO groups leads to the production of branched alcohol ethoxylates with only a few EO groups. The ethoxylate moiety is first degraded because the high degree of branching causes an impeded attack on the alkyl chain of the molecule by aerobic microorganisms.

Alkylphenol ethoxylates

The alkylphenol ethoxylates are probably degraded by the mechanism described for branched alcohol ethoxylates. The pathway for alkylphenol ethoxylate metabolism has not been fully elucidated. The metabolic activities of a pure culture of butylphenol hexaoxyethylene-degrading bacteria give rise to the formation of butylphenol dioxyethylene and butylphenol monooxyethylene. The identification of these products demonstrates that butylphenol ethoxylates are metabolized by bacteria by stepwise removal of oxyethylene groups (Baggi et al. 1977). Recently, a *Pseudomonas* sp. was isolated using a nonylphenol ethoxylate with 10 EO units as the sole carbon and energy source (Maki et al. 1994). The *Pseudomonas* sp. only degrades the polyether moiety confirming the results of Baggi et al. (1977). The dominant metabolite resulting from the degradation of this moiety was

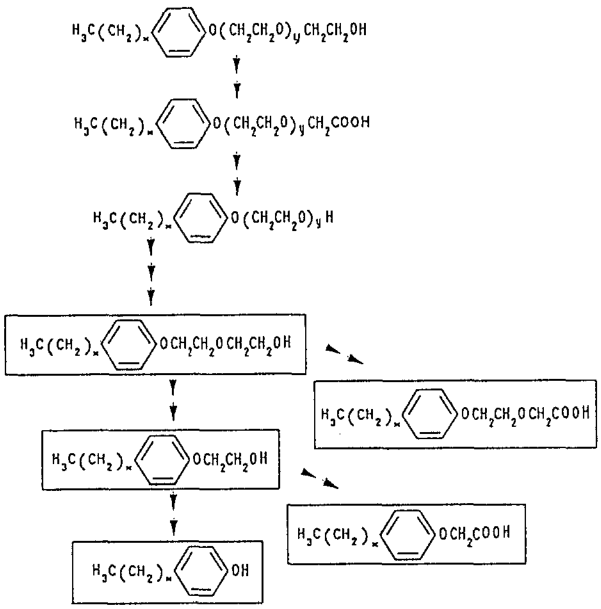

Fig. 4. Proposed initial reactions in the microbial degradation of alkylphenol ethoxylates by pure cultures. Boxes indicate products which are degraded by other microorganisms.

a nonylphenol ethoxylate with 2 EO groups (Maki et al. 1994).

An attack on the oxyethylene chain at the terminal position by microorganisms in mixed cultures is apparent from the identification of several metabolites. Metabolites which have been identified in sewage effluents and sludge include nonylphenol, nonylphenol monooxyethylene nonylphenol dioxyethylene, nonylphenol acetic acid and nonylphenol ethoxyacetic acid (Brunner et al. 1988). Alkylphenol ethoxylates with less than three EO units are efficiently transformed by mixed cultures and the predominant metabolites are the corresponding alkylphenoxy carboxylic acids (Ahel et al. 1994[a]). A general pathway for the initial degradation of alkylphenol ethoxylates is given in Fig. 4.

Subsequently the alkylphenol part is degraded by other microorganisms. Biodegradation of nonylphenol by pure cultures has not been reported. However, seawater and sediment were found to liberate $^{14}CO_2$ from radiolabelled nonylphenol, demonstrating the susceptibility of this compound to biodegradation (Ekelund et al. 1993). Nonylbenzene, a structurally related compound, is attacked via ω/β-oxidation of the side chain (Sariaslani et al. 1974). Similarly, the degradation of nonylphenol probably proceeds through the breakdown of the alkyl chain. ω/β-Oxidation of the alkyl chain only occurs when it has few or no branches (Osburn & Benedict 1966). However, most alkylphenols have highly branched alkyl chains which prevent the β-oxidation and retard the biodegradation of alkylphenols. Due to extensive branching in the alkyl chain, microorganisms preferentially grow on the EO chain of alkylphenol ethoxylates.

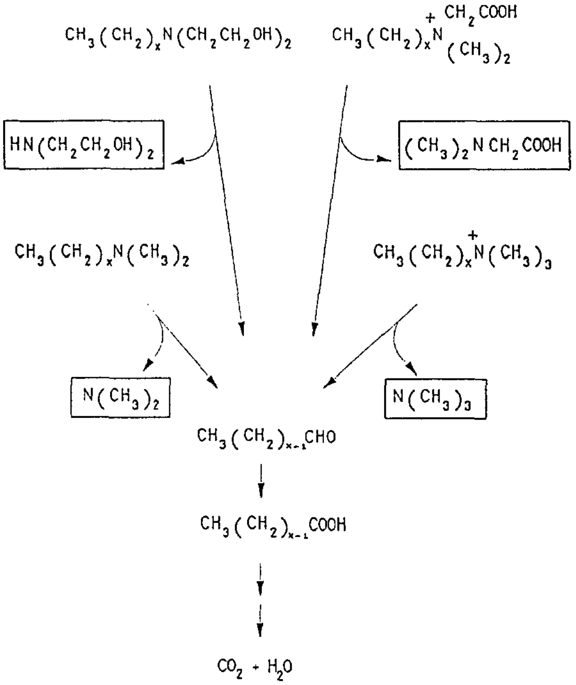

Fig. 5. Pathways for the degradation of fatty amine derivatives by pure cultures of microorganisms. Boxes indicate products which are degraded by other microorganisms.

Ethoxylated fatty amines

The pathway for the catabolism of alkylbis(2-hydroxyethyl)amine illustrates a general strategy used by microorganisms to deal with tertiary amines (Fig. 5). The C_{alkyl}-N bond is attacked by a bacterium through a dehydrogenation reaction, forming diethanolamine as a breakdown product. The dehydrogenase involved requires *in vitro* DCPIP as an artificial acceptor. The dehydrogenase activity probably generates octadecanal which is utilized as the carbon and energy source. In this bacterium, isocitrate lyase was induced after growth on octadecylbis(2-hydroxyethyl)amine and acetate, indicating that the alkyl chain of this ethoxylated fatty amine is metabolized via β-oxidation (van Ginkel & Kroon 1993).

Although diethanolamine persists in a pure culture, this breakdown product is known to be biodegradable. A yellow Gram negative rod is able to split diethanolamine, yielding glycolaldehyde and ethanolamine. The ethanolamine is converted into ethanal *via* ethanolamine-*O*-phosphate (Williams & Callely 1982).

Cationic and amphoteric surfactants

Quaternary ammonium salts

Two mechanisms for the initial attack of alkyltrimethylammonium salts by microorganisms have been distinguished. Firstly, a hydroxylation of the far end of the alkyl chain (Dean-Raymond & Alexander 1977), and secondly a central fission of the C_{alkyl}-N bond (van Ginkel et al. 1992).

Evidence for the cleavage of the C_{alkyl}-N bond of hexadecyltrimethylammonium chloride was presented by Van Ginkel et al., (1992). Gas chromatographic analysis proved stoichiometric production of trimethylamine during growth on hexadecyltrimethylammonium chloride by a *Pseudomonas* sp. The metabolism of trimethylamine will be discussed below. In the *Pseudomonas* sp., the initial enzymatic attack on hexadecyltrimethylammonium chloride was shown to be catalyzed by an oxygen/NADH dependent monooxygenase yielding trimethylamine. Washed cell suspensions of the *Pseudomonas* sp. grown on hexadecyltrimethylammonium chloride oxidize the growth substrate, hexadecanal and hexadecanoic acid at comparable rates. These results indicate that the first intermediate in the biodegradation pathway of hexadecyltrimethylammonium chloride is hexadecanal which is transformed to acyl-CoA *via* hexadecanoic acid. Oxidation of hexadecanal is catalyzed by an alkanal dehydrogenase. The acid formed by the dehydrogenase is further metabolized through β-oxidation (Fig. 5). An alternative route for alkyltrimethylammonium salts was described by Dean-Raymond & Alexander (1977). These authors isolated 9-carboxynonyltrimethylammonium and 7-carboxyheptyltrimethylammonium salts from a phosphate medium when washed cell suspensions of a *Xanthomonas* sp. were incubated with decyltrimethylammonium bromide. These acidic metabolites are associated with the oxidation of the methyl group of the alkyl chain. In spite of this finding there is substantiated evidence which favours a fission of the C_{alkyl}-N bond (Swisher 1987).

Alkylamines

Analogous to the metabolism of hexadecyltrimethylammonium chloride and alkylbis(2-hydroxyethyl)-amines, microorganisms are capable of utilizing the alkyl chain(s) of fatty amines, i.e. alkylamines, dialkylamines, alkyldimethylamines and dialkylmethylamines. Removal of the alkyl chains from the fatty amines by pure cultures generates ammonia, methylamine and dimethylamine. The microorganisms involved in the removal of alkyl chains from fatty amines display a narrow substrate specificity with respect to the number of alkyl chains (van Ginkel et al. 1995).

Specific evidence with respect to the utilization of alkyl chains was obtained with a microorganism grown on dodecyldimethylamine. Substrate uptake experiments indicate that dodecanal and dodecanoic acid are intermediates in the biodegradation pathway (Kroon et al. 1994). Confirmation was obtained with gas chromatographic detection of dodecanal and dodecanoic acid. Detection of these intermediates was hampered by their low concentrations. However, in the presence of decanal and acrylic acid as inhibitors, dodecanal and dodecanoic acid accumulate. A dehydrogenase was detected which converts dodecyldimethylamine into decanal and dimethylamine. The sequence is completed by conversion of dodecanal to dodecanoic acid and finally degradation to carbon dioxide and water through β-oxidation. The pathway for dodecyldimethylamine degradation in a *Pseudomonas sp.* is depicted in Fig. 5 (Kroon et al. 1994). These findings support the view that C_{alkyl}-N fission of alkylamines and alkyltrimethylammonium salts by microorganisms is a general strategy to gain access to the alkyl chains. Although the methylamines are resistant to breakdown in pure cultures of alkylamine- and alkyltrimethylammonium salt-degrading microorganisms, they are degraded to ammonia, water and carbon dioxide. Methylamines are mineralized in nature by methylotrophic microorganisms exhibiting enzymatic activities towards C_1 compounds. Initial degradation steps of trimethylamine may differ. Microorganisms may start with an *N*-oxygenation, followed by cleavage to yield methanal and dimethylamine (Myers & Zatman 1971; Large et al. 1972; Colby & Zatman 1973). On the other hand, the pathway in a *Pseudomonas* sp. involves a direct cleavage of the C-N bond by a dehydrogenase (Colby & Zatman 1971). Next, dimethylamine is demethylated *via* methylamine to ammonia catalyzed by monooxygenases, dehydrogenases and synthetases (Eady et

Table 1. Products formed due to degradation of either the hydrophobic or hydrophilic part of surfactants by pure cultures of microorganisms.

Surfactant	Product	Reference
Dodecylbenzene sulphonate	Benzylsulpho-2-acetic acid	Bird & Cain 1972
Dodecylbenzene sulphonate	Benzylsulpho-4-butanoic acid	Bird & Cain 1972
Undecylbenzene sulphonate	Benzylsulpho-3-propanoic acid	Willets & Cain 1972[b]
2-Decylbenzene sulphonate	Benzylsulpho-3-butanoic acid	Divo & Cardini 1980
2-Undecylbenzene sulphonate	Benzylsulpho-4-pentanoic acid	Divo & Cardini 1980
2-Dodecylbenzene sulphonate	Benzylsulpho-3-butanoic acid	Divo & Cardini 1980
2-Tridecylbenzene sulphonate	Benzylsulpho-4-pentanoic acid	Divo & Cardini 1980
3-Undecylbenzene sulphonate	Benzylsulpho-3-pentanoic acid	Divo & Cardini 1980
3-Dodecylbenzene sulphonate	Benzylsulpho-4-hexanoic acid	Divo & Cardini 1980
3-Tridecylbenzene sulphonate	Benzylsulpho-3-pentanoic acid	Divo & Cardini 1980
4-Tridecylbenzene sulphonate	Benzylsulpho-4-heptanoic acid	Divo & Cardini 1980
Dodecyl triethoxy sulphate	Ethylene glycol sulphate	Hales et al. 1982
Dodecyl triethoxy sulphate	Diethylene glycol sulphate	Hales et al. 1982
Dodecyl triethoxy sulphate	Triethylene glycol sulphate	Hales et al. 1982
Dodecyl triethoxy sulphate	Acetic acid-2(ethoxy sulphate)	Hales et al. 1982
Dodecyl triethoxy sulphate	Acetic acid-2(diethoxy sulphate)	Hales et al. 1982
Dodecanol octaoxyethylene	Polyethylene glycol	Ichikawa et al. 1978
Dodecanol octaoxyethylene	Polyethylene glycol	Schöberl et al. 1982
Dodecanol decaoxythylene	Decaethylene glycol	Watson & Jones 1979
Butylphenol hexaoxyethylene	Butylphenol monooxyethylene	Baggi et al. 1977
Butylphenol hexaoxyethylene	Butylphenol dioxyethylene	Baggi et al. 1977
Decylphenol decaoxyethylene	Decylphenol dioxyethylene	Maki et al. 1994
Alkylbis(2-hydroxyethyl)amine	Diethanolamine	van Ginkel & Kroon 1993
Alkyltrimethylammonium chloride	Trimethylamine	van Ginkel et al. 1992
Dodecyldimethylamine	Dimethylamine	Kroon et al. 1994
Didodecylmethylamine	Methylamine	van Ginkel et al. 1995

al. 1971; Eady & Large 1971; Meiberg & Harder 1979; Hirsch et al. 1971).

Alkylbetaines

A catabolic pathway for mineralizaton of alkylbetaines, amphoteric surfactants, has been postulated for a consortium of at least three microorganisms in which the first microorganism cleaves the C_{alkyl}-N bond. This is based on the observation that dimethylglycine is formed during the oxidation of dodecylbetaine by a glucose-grown bacterium and on the literature cited with respect to fatty amines and derivatives. The product of the first microorganism, dimethylglycine was proposed to undergo a scission at the $C_{carboxymethyl}$-N bond to form glycolate and dimethylamine. The glycolate is used as carbon and energy source by a second microorganism. Dimethylamine in turn is demethylated by a third organism (unpublished results).

Consortia of microorganisms degrading surfactants

Consortia

Biodegradation of surfactants primarily takes place in waste water treatment plants and in natural ecosystems such as soils, rivers, ditches and seas. In these plants and ecosystems, surfactants encounter mixed cultures of microorganisms. Only a few surfactants, i.e. alkane sulphonates, alkyl sulphates and alkylamines are completely mineralized by one microorganism. Microorganisms have evolved enzyme systems to initiate biodegradation of these surfactants by removal of the mineral moiety. This removal leads to alcohols, alkanals and alkanones which are degraded through β-oxidation. However, due to the amphiphilic nature of the surfactant molecules the involvement of consortia is expected. Indeed, the majority of surfactants is degraded by consortia due to limited metabolic capacities of individual microorganisms (Table 1). Consortia of microorganisms may be constructed on the basis of commensalistic and synergistic relationships.

In the case of commensalism one microorganism benefits, whereas the other is not affected. The most obvious commensalistic interaction is the production and release into the medium of a substance by one organism. This substance is utilized by another microorganism as growth substrate. A *Pseudomonas* sp. capable of oxidizing the alkyl chain of hexadecyltrimethylammonium chloride releases trimethylamine (van Ginkel et al. 1992). A trimethylamine-degrading bacterium grown together with the *Pseudomonas* sp. ensures that the quaternary ammonium salt is totally mineralized. The transient formation of trimethylamine during the degradation of tetradecyltrimethylammonium salt by activated sludge in a batch culture demonstrates the existence of commensalistic relationships (Nishiyama et al. 1995). Another *Pseudomonas* sp. grown on LAS could not degrade SPC but upon the addition of other microorganisms the aromatic products were degraded (Divo & Cardini 1980). Such a commensalistic relationship for LAS-degrading microorganisms is also described by Hrsak et al. (1982). A consortium of at least two bacteria can degrade alcohol ethoxylates through commensalistic activity. One bacterium converts alcohol ethoxylates into polyethylene glycol *via* the utilization of the alkyl chain (Ichikawa et al. 1978). A second bacterium biodegrades polyethylene glycol (Kawai 1987).

Synergistic associations are capable of effecting biodegradation beyond the separate abilities of each species in the community. Examples of synergistic relationships have been found with the degradation of LAS, decyltrimethylammonium chloride and dodecylbetaine. An *Acinetobacter* sp. only utilizes LAS when the growth medium contained yeast extract. This requirement for yeast extract indicates that the degradation of the alkyl chain of LAS depends on substances excreted by other strains present in the mixed culture (Hrsak et al. 1982). A four-member consortium enriched on LAS degrades the surfactant (Jimenez et al. 1991). Three organisms are capable of oxidizing the alkyl chain, which was demonstrated by the loss of surfactancy. Mineralization of the aromatic ring of LAS was not detected when individual strains were incubated with ^{14}C ring labelled LAS. Since all four bacteria were necessary to recover $^{14}CO_2$ from LAS, Jimenez et al. (1991) concluded that synergistic relations among these bacteria are essential. Alkylbetaines are degraded by a mixture of at least three microorganisms having complementary activities. Cultivation of these microorganisms on dodecylbetaine was only possible in mixed cultures due to synergistic interactions (unpublished results). Decyltrimethylammonium chloride is completely mineralized through the combined activity of a *Xanthomonas* sp. and a *Pseudomonas* sp. The *Xanthomonas* sp. requires an unidentified cofactor produced by the other bacterium in the consortium. A pure culture of the *Xanthomonas* sp. is capable of growing on decyltrimethylammonium bromide in the presence of yeast extract (Dean-Raymond & Alexander 1977).

Mixed cultures of microorganisms are needed to completely biodegrade most surfactants. Partial degradation may lead to the formation of products with increased or decreased toxicity compared to the original compound. For instance, the degradation of linear alcohol ethoxylates proceeds *via* an ether link cleavage, giving non toxic PEGs (Ichikawa et al. 1978; Schöberl 1988). Immediate detoxification was also found with ethoxylated fatty amines (van Ginkel et al. 1993). Apparently, the ability to initiate surfactant metabolism by cleaving the molecule into a hydrophobic and a hydrophilic part results in immediate detoxification. In contrast to this mechanism, the stepwise removal of EO groups of branched alcohol ethoxylates and alkylphenol ethoxylates leads to the formation of surfactants which are toxic to fish (Kravetz 1990; Kravetz et al. 1991; Patoczka & Pulliam 1990). To predict the formation of compounds in waste water treatment plants and natural environments, a thorough understanding of the metabolic capacities of microorganisms and behaviour of consortia in these systems is a prerequisite.

Waste water treatment plants

Domestic waste water is primarily treated in activated sludge plants. In activated sludge systems a settling tank is used to remove sludge from the effluent. Most of the settled sludge is recycled to the aeration tank where a high concentration of activated sludge is accomplished through controlled sludge wasting. Competent microorganisms degrading an organic compound will be retained in an activated sludge plant only if its maximum growth rate is greater than the sludge wastage rate (Pirt 1985). Under steady-state conditions the specific growth rate of the settling microorganisms (activated sludge) is equal to the sludge wastage rate. The effect of the sludge wastage rate on the biodegradation of organic compounds in activated sludge plants has been demonstrated by Birch (1991) and Cech & Chudoba (1988).

In three cases formation of detectable levels of intermediate products during the degradation of surfactants in activated sludge plants is impossible: (a) the surfactant is completely degraded by one microorganism; (b) the growth rate of the product-utilizing microorganisms is greater than or equal to the growth rate of the surfactant-degrading microorganisms; or (c) degradation by microorganisms with a synergistic relationship. The growth kinetics of consortia with synergistic relationships resemble that of single species as demonstrated by mathematical modelling (Powell 1985). As a consequence, production of intermediates by such consortia may be excluded.

Nonetheless, degradation products of some surfactants have been detected in the effluents of activated sludge plants (Romano & Ranzani 1992; Ahel et al. 1994^{b}). The formation of a metabolite from a surfactant which is degraded by a commensalistic consortium may occur when the maximum growth rate of the surfactant-utilizing microorganism is greater than the maximum growth rate of the product-utilizing microorganisms. The product accumulates at sludge retention times (SRTs) by which the surfactant-degrading microorganisms are able to maintain themselves and the microorganisms responsible for the product removal are unable to multiply sufficiently fast to avoid their elimination from the activated sludge system. This may explain the common observation of degradation products in the effluent of activated sludge plant.

The transient formation of sulphophenyl alkanoates (SPC) in batch cultures probably reflects the different maximum growth rates on the alkyl chain of LAS and SPC (Leidner et al. 1977; Gledhill et al. 1991). Degradation of SPC seems the rate-limiting step for the total mineralization of LAS. Consequently, production of SPC may be observed in activated sludge plants. SPC present in the influents of activated sludge plants analyzed by HPLC indicate partial biodegradation of LAS in sewers (Romano & Ranzani 1992; Cavalli et al. 1993). In a full-scale waste water treatment plant, approximately 20% of the LAS were converted into SPC which were discharged into a river (Romano & Ranzani 1992). Cavalli et al. (1993) also found a production of SPC in a full-scale treatment system.

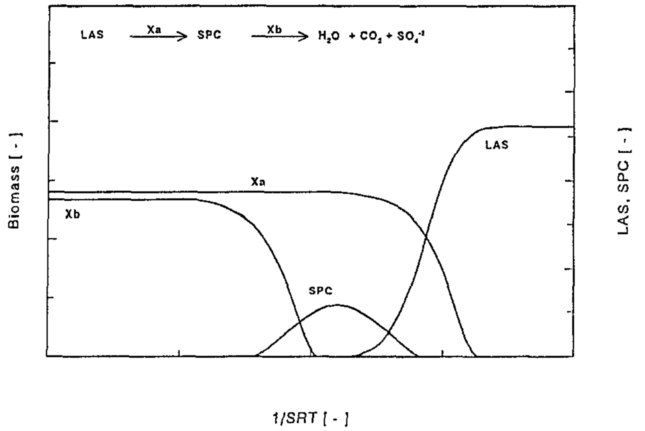

Fig. 6. Steady-state substrate concentration and population of two microorganisms growing on the alkyl chain of LAS (X_a) and SPC (X_b) at various sludge retention times. The model assumes Monod kinetics with different maximum growth rates of the respective microorganisms.

The formation of SPC cannot be related to SRTs due to the absence of relevant data. Fig. 6 shows a simulation of the conversion of LAS into SPC and the subsequent biodegradation of SPC at various SRTs assuming different maximum growth rates of the competent microorganisms. Microorganisms utilize both moieties of LAS simultaneously at high SRTs. At lower SRTs, SPC remain unutilized by the activated sludge and appear in the effluent. Finally, if the SRT is maintained below the maximum generation time of LAS degrading microorganisms, LAS will not be degraded. Pitter & Fuka (1979) have compiled biodegradation studies on LAS and concluded that LAS may be incompletely degraded. In continuous systems, the formation of SPC may be attributed to low SRTs preventing SPC-degrading microorganisms to maintain themselves in the system.

In waste water treatment plants both biodegradation pathways for linear alcohol ethoxylates are operative (Steber & Wierich 1985). PEGs produced by a central fission of the surfactant were detected in the effluent. Carboxylated PEGs present in the effluent arise from a ω/β-oxidation of the alkyl chain of alcohol ethoxylates. These results were obtained by using double labelled alcohol ethoxylates (Steber & Wierich 1985). The presence of the metabolites shows that the hydrophobic moiety of alcohol ethoxylates is consumed faster than the hydrophilic part.

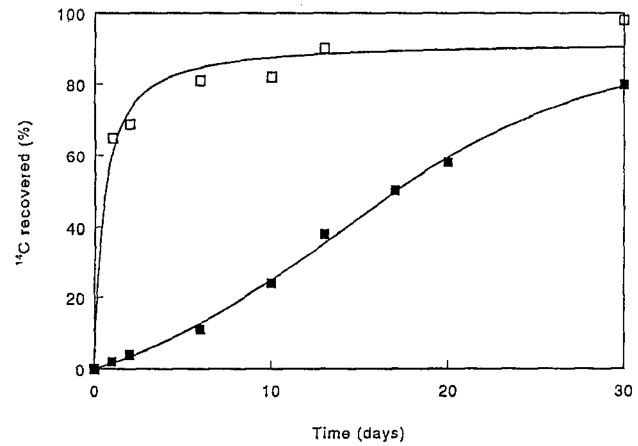

Fig. 7. $^{14}CO_2$ evolution during the biodegradation of alkyl chain-labelled (☐) and ethoxylate chain labelled (■) linear alcohol ethoxy sulphate (redrawn from Vashon & Schwab 1982).

Analyses in many waste water treatment plants have shown that products of the alkylphenol ethoxylate breakdown are ubiquitous (Brunner et al. 1988; Ahel et al. 1994[b]). Alkylphenol ethoxylates with more than six EO groups were completely converted in both high and low loaded full-scale waste water treatment plants. However, alkylphenol ethoxylates with less than three EO units were only removed at high percentages in a treatment plant with low sludge loading (Ahel et al. 1994[b]). The sludge loading usually determines the SRT which is low at high sludge loading. Ahel et al. (1994[b]) found a correlation between the removal of alkylphenol ethoxylates with a few EO units and nitrification. Loss of nitrification and the removal of these nonionics at high SRTs can be attributed to the wash-out of slow-growing microorganisms such as nitrifying bacteria. These results support the premise that metabolites are not formed in waste water treatment systems operated at relatively low SRTs.

In conclusion, the most important parameter for controlling surfactant-degrading microorganisms in waste water treatment plants is the SRT. To ensure complete mineralization of surfactants, a significant population of surfactant-degrading microorganisms must be maintained. This implies that surfactants are only completely degraded when the SRT enables maintenance of all microorganisms involved in the biodegradation of the surfactant. In the case of wash-out of one member of a consortium formation of a metabolite may occur.

Natural ecosystems

Surfactant-degrading microorganisms are present in many ecosystems (Larson & Games 1981; Gledhill et al. 1991; Sigoillot & Nguyen 1992). They usually occur in mixed populations of hydrophobic and hydrophilic moiety-degrading species. In seawater, a consortium of four microorganisms participates in the mineralization of LAS. Three bacteria among this consortium are capable of degrading the alkyl chain. The SPC formed are taken up by another bacterium and converted into carbon dioxide, water, sulphate and biomass. Synergistic relationships among these bacteria exclude transient formation of SPC during the degradation of LAS (Sigoillot & Nguyen 1992). However, transient formation of SPC has been detected in e.g. river water (Gledhill et al. 1991), demonstrating commensalistic relationships. SPC were detected in the fluid, since it is degraded less readily than the alkyl chain of LAS.

Vashon & Schwab (1982) studied the biodegradation of dodecyl decaethoxy sulphate with radiolabelled material in estuarine water. Dodecyl decaethoxy sulphate was uniformly labelled with ^{14}C in the ethoxy moiety and ^{14}C labelled at the α carbon of the alkyl chain. In batch cultures, the alkyl chain was mineralized within 5 days, whereas the degradation of the polar part started after a short lag period and 80% ^{14}C carbon was recovered as carbon dioxide on day 30 (Fig. 7). It is evident from the results that this compound is degraded by a consortium of microorganisms with commensalistic relationships. Similar results were obtained in a river die-away test. Alkyl ethoxy sulphate is readily transformed into ethoxy sulphates. These ethoxy sulphates persisted for a period of approximately two weeks in this river die-away test (Griffith et al. 1986).

The slower degradation of polyethylene glycols relative to the alkyl part of alcohol ethoxylates in natural water has been shown by Vashon & Schwab (1982). Polyethylene glycols may therefore be present in surface water from the partial degradation of alcohol ethoxylates which were recently introduced. Indeed, polyethylene glycol concentrations found in surface water receiving waste water ranged from undetectable levels to 145 μg/L (Leenheer et al. 1991). In contrast to this, the hydrophobic moiety of alkylphenol ethoxylates was detected in river water (Ahel et al. 1994[c]). This confirms the findings that the EO chain is degraded at high rates relative to the branched hydrophobic part of these surfactants. So, after the introduction of a surfactant into an ecosystem, metabolites may be formed transiently due to different growth rates of microorganisms growing on the groupings of a surfactant.

Conclusions

Susceptibility of surfactants to biodegradation has long been known and catabolic pathways have been documented for several surfactants. In general, a central fission of surfactants yields the hydrophobic chain in the form of alcohols, alkanals or alkanoic acids, which are readily assimilated by central metabolic pathways. A second strategy for microorganisms to metabolize the alkyl chain is through ω/β-oxidation. Mineralization of the alkyl chain by a microorganism leads to formation of the hydrophilic moiety. For example, LAS is converted into SPC by pure cultures. In general, the hydrophilic moiety is not toxic to aquatic organisms because it has lost its surfactant properties. The polar moiety may undergo initial attack when the degradation of the alkyl chain is prevented, for instance due to extensive branching. This results in ecotoxic surfactants, i.e. branched nonylphenol ethoxylates with a few EO units. The biodegradation studies with pure cultures indicate that the metabolic capacity of many surfactant-degrading microorganisms is limited.

From the above it can be deduced that complete biodegradation of most surfactants has to be achieved by consortia of microorganisms. The metabolic interaction of the microorganisms which mineralize surfactants is either commensalistic or synergistic. The idea of surfactant degradation by consortia of microorganisms offers a conceptual framework within which a number of observations concerning the production of metabolites in waste water treatment plants and natural ecosystems may be rationalized. Insight into the degradation of surfactants by consortia is important to predict the fate of these chemicals in waste water treatment systems and ecosystems. A study of the fate of surfactants in waste water treatment plants should take into account the microbial degradation of surfactants by consortia under different conditions. Complete degradation of surfactants in waste water treatment plants occurs when the wash-out of all members of a surfactant-degrading consortium is excluded. The wash-out of competent microorganisms is primarily determined by the SRT.

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References

Ahel M, Hrsak D & Giger W (1994^{a}) Aerobic transformation of short-chain alkylphenol polyethoxylates by mixed bacterial cultures. Arch. Environ. Contam. Toxicol. 26: 540–548

Ahel M, Giger W & Koch M (1994^{b}) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - I. occurrence and transformation in sewage treatment. Water Res. 28: 1131-1142

Ahel M, Giger W & Schaffner C (1994^{c}) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - II. occurrence and transformation in rivers. Water Res. 28: 1143–1152

Baggi G, Beretta L, Galli E, Scolastico & Treccani V (1977) Biodegradation of alkylphenol polyethoxylates. In: Chater KWA & Somervile HJ (Eds) The Oil Industry and Microbial Ecosystems (pp 129-136). Heyden, London

Birch RR (1991) Prediction of the fate of detergent chemicals during sewage treatment. J. Chem. Tech. Biotechnol. 50: 411–422

Bird JA & Cain RB (1972) Metabolism of linear alkylbenzene sulphonates by a *Vibrio* sp. Biochem. J. 127: 46p

Britton LN (1984) Microbial degradation of aliphatic hydrocarbons. In: Gibson DT (Ed) Microbial Degradation of Organic Compounds (pp 89–129). Marcel Dekker, New York

Brunner PH, Capri A, Marcomini A & Giger W (1988) Occurrence and behaviour of linear alkylbenzene sulphonates, nonylphenol, nonylphenol mono, and nonylphenol diethoxylates in sewage and sewage sludge treatment. Water Res. 22: 1465–1472

Cain RB & Farr DR (1968) Metabolism of arylsulphonates by microorganisms. Biochem. J. 106: 859-877

Cain RB, Willets AJ & Bird JA (1972) Surfactant biodegradation: metabolism and enzymology. In: Walters AH, Hueck EH, van der Plas (Eds) Biodeterioration of Materials, Vol 2 (pp 136–144). Applied Science Publishers Ltd. Barking

Cain RB (1981) Microbial degradation of surfactants and builder components. In: Leisinger T, Cook AM, Hutter R & Nuesch J (Eds) Microbial degradation of xenobiotics and recalcitrant compounds. (pp 325-370). Academic Press Inc. New York

Cech JS & Chudoba J (1988) Effect of the solids retention time on the rate of biodegradation of organic compounds. Acta Hydrochim. Hydrobiol. 16: 313–323

Cavalli L, Gellera A & Landone A (1993) LAS removal and biodegradation in a wastewater treatment plant. Environ. Toxicol. Chem. 12: 1977 1777–1788

Colby J & Zatman LJ (1971) The purification and properties of a bacterial triamine dehydrogenase. Biochem. J. 121: 9p-10p

Colby J & Zatman LJ (1973) Trimethylamine metabolism in obligate and facultative methylotrophs. Biochem. J. 132: 101–112

Dean-Raymond D & Alexander M (1977) Bacterial metabolism of quaternary ammonium compounds. Appl. Environ. Microbiol. 33: 1037–1041

Divo C & Cardini G (1980) Primary and total biodegradation of linear alkylbenzene sulphonates. Tenside Deterg. 17: 30–36

Dodgson KS, Fitzgerald JW & Payne WJ (1974) Chemically defined inducers of alkylsulphatases present in *Pseudomonas* C12B. Biochem. J. 138: 53–62

Dodgson KS & White GF (1983) Some microbial enzymes involved in the biodegradation of sulfated surfactants. In: Wiseman A (Ed) Topics in Enzyme and Fermentation Biotechnology, Vol 7 (pp 90–155). Ellis Horwood, Chichester

Eady RR, Jarman TR & Large PJ (1971) Microbial oxidation of amines. Partial purification of mixed-function secondary-amine oxidase system from *Pseudomonas aminovorans* that contains an enzymatically active cytochrome-P-420-type haemoprotein. Biochem. J. 125: 449–459

Eady RR & Large PJ (1971) Microbial oxidation of amines spectral and kinetic properties of the primary amine dehydrogenase of *Pseudomonas* AM1. Biochem. J. 123: 757–771

Ekelund R, Granmo A, Magnusson K & Berggren M (1993) Biodegradation of 4-nonylphenol in seawater and sediment. Environmental Pollution 79: 59–61

Ginkel CG van, Dijk JB van & Kroon AGM (1992) Metabolism of hexadecyltrimethylammonium chloride in *Pseudomonas* strain B1. Appl. Environ. Microbiol. 58: 3083–3087

Ginkel CG van & Kroon AGM (1993) Metabolic pathway for the biodegradation of octadecylbis(2-hydroxyethyl)amine. Biodegradation 3: 435–443

Ginkel CG van, Stroo CA & Kroon AGM (1993) Biodegradability of ethoxylated fatty amines and amides and the non-toxicity of their biodegradation products. Tenside Surfact. Deterg. 30 213-216

Ginkel CG van (1995) Biodegradation of cationic surfactants. In: Porter MR & Karsa Blackie RD (Eds) Biodegradability of surfactants, (pp 183–203). Blackie Academic & Professional, London

Ginkel CG van, Pomper MA, Stroo CA & Kroon AGM (1995) Biodegradation of fatty amines: utilization of the alkyl chain by isolated microorganisms. Tenside Surfact. Deterg. 32 355–359

Gledhill WE (1975) Linear alkylbenzene sulfonate: biodegradation and aquatic interactions. Appl. Microbiol. 17: 265–293

Gledhill WE, Trehy ML & Carson BD (1991) Comparative biodegradability of anionic surfactants in synthetic and natural test systems. Chemosphere 22: 873–880

Griffiths ET, Hales SG, Russell NJ & White GF (1986) Metabolite production during the biodegradation of the surfactant sodium dodecyltriethoxy sulphate under mixed-culture die-away conditions. J. Gen. Microbiol. 132: 963–972

Griffiths ET, Hales SG, Russell NJ & White GF (1987) Identification of hydrophobic metabolites formed during biodegradation of alkyl ethoxylate and alkyl ethoxy sufate surfactants by *Pseudomonas* sp. DES1. Biotechnol. Appl. Biochem. 9: 217–229.

Hales SG, Dodgson KS, White GF, Jones N & Watson GS (1982) Initial stages in the biodegradation of the surfactant sodium dodecyltriethoxy sulphate by *Pseudomonas* sp. strain DES1. Appl. Environ. Microbiol. 44: 790–800

Hales SG (1993) Biodegradation of the anionic surfactant dialkyl sulphosuccinate. Environ. Toxicol. Chem. 12: 1821–1828

Hirsch LB, Peterson JA & Thompson AA (1971) An *N*-methylglutamate dehydrogenase from *Pseudomonas* MA. Arch. Biochem. Biophys. 145: 115–120

Hrsak D, Bosnjak M & Johanides V (1982) Enrichment of linear alkylbenzenesulphonate (LAS) degrading bacteria in continuous culture. J. Appl. Bacteriol. 53: 413–422

Ichikawa Y, Kitamoto Y & Hosoi N (1978) Degradation of polyethylene glycol dodecyl ethers by a Pseudomonas isolated from activated sludge. J. Ferment. Technol. 56(4): 403–409

Jimenez L, Breen A, Thomas N, Federle TW & Sayler GS (1991) Mineralization of linear alkylbenzene sulfonate by a four-member aerobic bacterial consortium. Appl. Environ. Microbiol. 57: 1566-1569

Kawai F (1987) The biochemistry of degradation of polyethers. CRC Critical Reviews in Biotechnology 6 (3): 273–307

Kravetz L (1990) Biodegradation pathways of nonionic ethoxylates. In: Glass JE & Swift G (Eds) Agricultural and Synthetic Polymers (pp 96–109). American Chemical Society, Washington DC

Kravetz L, Salanitro PB, Dorn PB & Guin KF (1991) Influence of hydrophobe type and extent of branching on environmental response factors of nonionic surfactants. J. Am. Oil Chem. Soc. 68: 610–618

Kroon AGM, Pomper MA & Ginkel CG van (1994) Metabolism of dodecyldimethylamine by *Pseudomonas* MA3. Appl. Microbiol. Biotechnol. 42: 134–139

Large PJ, Boulton CA & Crabbe MJC (1972) The reduced nicotinamide-adenine dinucleotide phosphate- and oxygen-dependent *N*-oxygenation of trimethylamine by *Pseudomonas aminovorans*. Biochem. J. 128: 137p-138p

Larson RJ & Games LM (1981) Biodegradation of linear alcohol ethoxylates in natural waters. Environ. Sci. Technol. 15: 1488–1493

Leenheer JA, Wershaw RL, Brown PA & Noyes TI (1991) Detection of poly(ethylene glycol) residues from non-ionic surfactants in surface water by ^{1}H and ^{13}C nuclear magnetic resonance spectrometry. Environ. Sci. Technol. 25: 161–168

Leidner H, Gloor R & Wuhrmann K (1976) Abbaukinetik linearer Alkylbensolsulfonate. Tenside Detergent 13: 122–130

Lewis MA (1990) Chronic toxicities of surfactants and detergent builders to algae; a review and risk assessment. Ecotox. Environ. Saf. 20: 123–140

Lijmbach GWM & Brinkhuis E (1973) Microbial degradation of secondary *n*-alkyl sulfates and secondary alkanols. Antonie van Leeuwenhoek 39: 415–423.

Locher HH, Leisinger T & Cook AM (1989) Degradation of *p*-toluenesulphonic acid via side chain oxidation, desulphonation and *meta* ring cleavage in *Pseudomonas (Comamonas) testosteroni* T-2. J. Gen. Microbiol. 135: 1969–1978

Locher HH, Leisinger T & Cook AM (1991) 4-Sulphobenzoate-3,4-dioxygenase. Biochem. J. 274: 833-842

Maki H, Masuda N, Fujiwara Y, Ike M & Fujita M (1994) Degradation of alkylphenol ethoxylates by *Pseudomonas* sp. strain TR01. Appl. Environ. Microbiol. 60: 2265–2271

McKenna EJ & Kallio RE (1965) The biology of hydrocarbons. Ann. Rev. Microbiol. 19: 183–208

Meiberg JBM & Harder W (1978) Aerobic and anaerobic metabolism of trimethylamine, dimethylamine and methylamine in *Hyphomicrobium* X. J. Gen. Microbiol. 106: 265–276

Myers PA & Zatman LJ (1971) The metabolism of trimethylamine-*N*-oxyde by *Bacillus* PM6. 121: 10p

Nishiyama N, Toshima Y & Ikeda Y (1995) Bodegradation of alkyltrimethylammonium salts in activated sludge. Chemosphere 30: 593–603

Osburn QW & Benedict JH (1966) Polyethoxylated alkyl phenols: relationship of structure to biodegradation mechanism. J. Am. Oil Chem. Soc. 43: 141–146

Patoczka J & Pulliam GW (1990) Biodegradation and secondary effluent toxicity of ethoxylated surfactants. Water Res. 24: 965–972

Payne WJ (1963) Pure culture studies of the degradation of detergent compounds. Biotechnol. Bioeng. 5: 355–365

Payne WJ & Feisal VE (1963) Bacterial utilization of dodecyl sulfate and dodecylbenzene sulfonate. Appl. Microbiol. 11: 339–344

Payne WJ, Williams JP & Mayberry WR (1965) Primary alcohol sulfatase in a *Pseudomonas* species. Appl. Microbiol. 13: 698–701

Payne WJ, Williams JP & Mayberry WR (1967) Hydrolysis of secondary alcohol sulphate by a bacterial enzyme. Nature 214: 623–624

Pirt SJ (1985) Principles of Microbe and Cell Cultivation. Blackwell Scientific Publications, Oxford

Pitter P & Fuka T (1979) The problem of ultimate biodegradability of linear alkylbenzene sulphonates. Tenside Deterg. 16: 298–302

Powell GE (1985) Stable coexistence of syntrophic associations in continuous culture. J. Chem. Tech. Biotechnol. 35B: 46–56

Quick A, Russell NJ, Hales SG & White GF (1994) Biodegradation of sulphosuccinate: direct desulphonation of a secondary sulphonate. Microbiology 140: 2991–2998

Romano P & Ranzani M (1992) Anionic surfactants removal and biodegradation in a large treatment plant. Wat. Sci. Tech. 26 9–11: 2547–2550.

Sariaslani FS, Harper DB & Higgins IJ (1974) Microbial degradation of hydrocarbons. Catabolism of 1-phenylalkanes by *Nocardia salmonicolor*. Biochem. J. 140: 31–45

Schöberl P (1982) Mikrobieller Abbau eines Kokosfettalkohol-Ethoxylates durch *Acinetobacter lwoffi*, Stamm ML. Tenside Surfact. Deterg. 19: 329–339

Schöberl P (1988) Okologische relevante Daten von Tensiden in Wasch- und Reinigungsmitteln Tenside Surfact. Deterg. 25: 86–98

Sigoillot JC & Nguyen MH (1992) Complete oxidation of linear alkylbenzene sulfonate by bacterial communities selected from coastal seawater. Appl. Environ. Microbiol. 58: 1308–1312

Slater JH & Lovatt D (1984) Biodegradation and the significance of microbial communities. In: Gibson DT (Ed) Microbial Degradation of Organic Compounds (pp 439–485). Marcel Dekker Inc. New York

Steber J, Wierich P (1985) Metabolites and biodegradation pathways of fatty alcohol ethoxylates in microbial biocenoses of sewage treatment plants. Appl. Environ. Microbiol. 49: 530–537

Swisher RD (1987) Surfactant degradation. Marcel Dekker Inc. New York

Thysse GJE & Wanders TH (1974) Initial steps in the degradation of *n*-alkane-1-sulphonates by *Pseudomonas*. Antonie van Leeuwenhoek 40: 25–37.

Thysse GJE & Wanders TH (1972) Degradation of *n*-alkane-1-sulfonates by *Pseudomonas*. Antonie van Leeuwenhoek 38: 53–63

Vashon RD & Schwab BS (1982) Mineralization of linear alcohol ethoxylates and linear alcohol ethoxy sulfates at trace concentration in estuarine water. Environ. Sci. Technol 16: 433–436

Watson GK & Jones N (1977) The biodegradation of polyethylene glycols by sewage bacteria. Water Res. 11: 95–100

Watson GK & Jones N (1979) The microbial degradation of nonionic surfactants. Soc. Gen. Microbiol. Quart. 6: 78–79

White GF & Russell NJ (1993) Biodegradation of anionic surfactants and related molecules. In: Ratledge C (Ed) Biochemistry of Microbial Degradation (pp 143–177). Kluwer Academic Press Dordrecht

White GF (1993) Bacterial biodegradation of ethoxylated surfactants. Pest. Sci. 37: 159–166

Willets AJ & Cain RB (1972^{a}) Microbial metabolism of alkylbenzene sulphonates. Enzyme system of *Bacillus* species responsible for β-oxidation of the alkyl side chain of alkylbenzene sulphonates. Antonie van Leeuwenhoek 38: 543–555.

Willets AJ & Cain RB (1972^{b}) Microbial metabolism of alkylbenzene sulphonates. Bacterial metabolism of undecylbenzene-*p*-sulphonate and dodecylbenzene-*p*-sulphonate. Biochem. J. 129: 389–402

Willets AJ (1973) Microbial metabolism of alkylbenzene sulphonates. Fungal metabolism of 1-phenylundecane-*p*-sulphonate and 1-phenyldodecane-*p*-sulphonate. Antonie van Leeuwenhoek 39: 585-597

Willets AJ (1974) Microbial metabolism of alkylbenzene sulphonates. The oxidation of key aromatic compounds by a Bacillus. Antonie van Leeuwenhoek 40: 547–559

Williams GR & Callely AG (1982) The biodegradation of diethanolamine and triethanolamine in a Gram-negative rod. J. Gen. Microbiol. 128: 1203–1209