Metabolite Production during the Biodegradation of the Surfactant Sodium Dodecyltriethoxy Sulphate under Mixed-culture Die-away Conditions

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(Received 12 August 1985; revised 14 November 1985)

Sodium dodecyltriethoxy sulphate (SDTES), either pure or as a component of commercial surfactant mixtures, underwent rapid primary biodegradation by mixed bacterial cultures in OECD screen and river-water die-away tests. Inoculation of [35S]SDTES-containing solutions with OECD screen test media acclimatized to surfactants or their degradation products led to production of various 35S-labelled glycol sulphates and their oxidation products, all known to occur during degradation of [35S]SDTES by pure bacterial isolates. Triethylene glycol monosulphate was the major catabolite together with smaller amounts of di- and monoethylene glycol monosulphates implying, by analogy with pure cultures, that ether-cleavage was the major primary biodegradation step. The oxidation product (carboxylate derivative) of each glycol sulphate was also detected together with metabolites tentatively identified as αβ-oxidation products of the dodecyl chain. Relatively little SO4\(^{2-}\) was liberated directly from SDTES but mixed cultures derived from sewage could metabolize the sulphated glycols to SO4\(^{2-}\).

The environmental relevance of these degradation routes was established by following metabolite production from [35S]SDTES in full-scale river-water die-away tests. Triethylene glycol sulphate was formed first, then rapidly oxidized to acetic acid 2-(diethoxy sulphate) which persisted as the major metabolite for 2–3 weeks. Small amounts of sulphated derivatives of di- and monoethylene glycols were also detected during the same period. Very little SO4\(^{2-}\) was formed directly from SDTES but large amounts accompanied the eventual disappearance of glycol sulphate derivatives. None of the 35S-labelled organic metabolites was persistent and, whenever [35S]SDTES was a component of a commercial mixture, all ester sulphate was completely mineralized to 35SO4\(^{2-}\) within 28 d.

INTRODUCTION

Detergent formulations for personal and domestic use (e.g. hair shampoos, washing-up liquids) are designed to combine good foaming properties with low skin irritancy (Shore & Berger, 1976). Important components in such products are alkylethoxy sulphate surfactants. These surfactants undergo rapid primary biodegradation in activated sewage sludge and river-water die-away tests (Itoh et al., 1979; Swisher, 1970; Yoshimura & Masuda 1982), but there is evidence that mineralization of the ethoxylate carbon in these (Vashon & Schwab, 1982) and the...
related non-ionic surfactants (Tobin et al., 1976) is considerably slower than that of the alkyl chain. The slower degradation of the ethoxylate moiety is also consistent with recent observations (Hales et al., 1982, 1986) on the biodegradation of sodium dodecyltriethoxy sulphate (SDTES; see Hales et al., 1986, Table 1 for compound names and structures), a typical component of commercial alkylethoxy sulphate surfactant mixtures. These studies using pure bacterial isolates showed that primary biodegradation of SDTES was accomplished mainly by ether-cleaving (etherase) enzyme systems that led to accumulation of several short-chain ethylene glycol sulphates as metabolic end-products. Some isolates cleaved SDTES specifically at the alkyl–ether bond to produce triethylene glycol monosulphate (EO3). Other strains were less specific and liberated mono-, di- and triethylene glycol monosulphates (EO1, EO2 and EO3 respectively). The compounds EO2 and EO3 underwent oxidation of the free hydroxyl group to the corresponding carboxylic acid derivatives (EO2' and EO3'). Direct liberation of sulphate from SDTES was a minor route in all isolates tested. One organism also appeared to use an ω-/β-oxidation pathway to produce compounds still containing residues of the alkyl chain. Thus, biodegradation of SDTES in pure cultures proceeded along three distinct pathways (etherase, sulphatase and ω-/β-oxidations) with accumulation of a number of sulphated glycols and related compounds.

Biodegradability tests for assessing the environmental acceptability of synthetic compounds usually employ mixed cultures of various origins. The tests fall into two groups (Gilbert & Watson 1977; Gilbert, 1979): biodegradability potential tests (e.g. OECD screen tests), which indicate the susceptibility of the chemical to microbial biodegradation; and simulation tests (e.g. river-water die-away test), which provide information about rates of biodegradation under relevant environmental conditions. Under these test conditions, many features of the biodegradation pathways (including rates, metabolites formed, accumulations of intermediates) may be quite different from those observed in pure cultures. In the present study, the analytical methods developed for the pure culture work were used to broaden the environmental relevance of the earlier experiments in two ways: first by establishing whether or not the metabolites formed in pure cultures were also present during SDTES degradation by mixed cultures used in OECD screen tests; second, by determining quantitatively the fate of SDTES (pure, and as a component of a commercial mixture) and its metabolites in full-scale river-water die-away experiments.

METHODS

Chemicals. Reagents of Analar grade were obtained from BDH. Sources and compositions of commercial mixtures of alkylethoxy sulphate surfactants, Empicol ESB/3S and Dobanol 25/3EO sulphate (i.e. sulphated Dobanol 25/3EO), have been described previously (Hales et al., 1982, 1986).

Unlabelled SDTES, [35S]SDTES, triethylene glycol mono[35S]sulphate and other 35S-labelled glycol sulphates and oxidized glycol sulphates (for use as TLC standards) were prepared as described previously (Hales et al., 1982, 1986).

Methylene-blue-active substance (MBAS) assay. Anionic surfactants associate with the intensely coloured methylene blue cation to give a chloroform-extractable complex, but the unassociated dye has a very low solubility in chloroform. Consequently extraction of the surfactant–dye complex into chloroform and measurement of its absorption at 650 nm affords a convenient assay for anionic surfactants. Any compound containing a strongly anionic group and a hydrophobic moiety may give a response, so the assay is not necessarily specific for the surfactant under test, especially where environmental samples (e.g. river-water) are involved. For this reason, results of such tests are usually expressed as MBAS rather than amount of a specific surfactant.

The manual method (Abbott, 1962) for quantification of anionic surfactants in solution was automated using standard automatic analyser equipment. Sample solution flowing at 1.2 ml min⁻¹ in plastic tubing was mixed with an alkaline methylene blue reagent (approx. 30 mg l⁻¹, 1.0 ml min⁻¹) and the mixture was segmented with chloroform (3.28 ml min⁻¹). The phases were mixed by passage through a PTFE coil and then separated in a phase separator. The retrieved chloroform layer, containing the surfactant–methylene blue complex, was mixed (2.48 ml min⁻¹) in further coils with an acidic solution of methylene blue (approx. 8 mg l⁻¹, 2.0 ml min⁻¹). The chloroform layer was separated again and its absorption at 650 nm was determined using a flow-cell in a colorimeter. The amount of MBAS in the sample was determined by comparison with standard solutions.
Primary biodegradation in OECD screen tests. Standard mineral medium used in the test was prepared from four stock solutions of the following composition: (i) 0.25 g FeCl₃, 6H₂O 1⁻¹; (ii) 27.5 g anhydrous CaCl₂ 1⁻¹; (iii) 22.5 g MgSO₄, 7H₂O 1⁻¹; (iv) 8.5 g KH₂PO₄ 1⁻¹; 21.75 g K₂HPO₄ 1⁻¹; 17.7 g NaH₂PO₄ 1⁻¹; 1.7 g NH₄Cl 1⁻¹. To prepare the mineral medium, 1 ml portions of each stock solution were mixed and made up to 11 with deionized water. For the screen test, three 2 l Erlenmeyer flasks each containing 11 standard mineral medium were supplemented separately with 10 mg l⁻¹ of either SDTES, Empicol ESB/3S or Dobanol 25/3EO sulphate. Each flask was inoculated with 0.5 ml effluent from an activated sewage plant and was then shaken (100 r.p.m.) at ambient temperature for 20 d. Samples (10 ml) were removed daily and assayed for MBAS (Fig. 1a).

Primary biodegradation in river-water die-away tests. Water was obtained from the mid-stream of the Shropshire Union Canal in Hargrave, Cheshire, UK (Ordnance Survey National Grid reference SJ 481 618). One-litre quantities in 2 l Erlenmeyer flasks were supplemented separately with 10 mg of a surfactant (SDTES, Empicol ESB/3S or Dobanol 25/3EO sulphate). Organisms present in the river-water served as inoculum. Flasks were shaken (100 r.p.m.) and samples removed and assayed as before (Fig. 1b).

[³⁵S]SDTES degradation by acclimatized mixed cultures. Three sets of media were prepared in Universal bottles stopped with cotton-wool plugs. Each bottle contained 4 ml OECD standard mineral medium and 10 mg l⁻¹ of SDTES, Empicol ESB/3S or Dobanol 25/3EO sulphate. Each bottle was also supplemented with approx. 8 kBq [³⁵S]SDTES. The media were sterilized by autoclaving at 0.1 MPa for 15 min and were stored frozen until required.

Bottles containing either SDTES or Empicol ESB/3S or Dobanol 25/3EO sulphate were inoculated with 1 ml samples from OECD screen test cultures originally containing the corresponding surfactant, at times when primary biodegradation of surfactant was complete according to the MBAS test (5 d for Empicol ESB/3S and 6 d for the others; see Fig. 1a). These inocula were considered to contain organisms acclimatized to degrade the various surfactants. Bottles were agitated at 20 °C and samples (0.2 ml) removed aseptically from each bottle every few days. After incubation for 12 or 13 d, bottles were reincubated with a further 1 ml of the corresponding original OECD screen test culture, now 18 d old and acclimatized to the degradation intermediates of alkylethoxy sulphate surfactants. The Universal bottles were incubated and sampled over a further 7 d period.

Samples (1 ml) of the 18 d-old OECD screen test culture were also used to inoculate three Universal bottles containing fresh surfactant solutions prepared as described above. Bottles were incubated at 20 °C and samples removed occasionally over a period of 30 d.

All samples were analysed by TLC methods established previously (Hales et al., 1982, 1986). Samples (5 µl) were applied to cellulose plates, which were developed in chloroform/methanol/water (8:5:1, by vol.), and radioactive areas located by autoradiography. On the basis of this preliminary analysis, a smaller number of samples was selected and 10 µl of each selected sample was applied to cellulose plates for further analysis by two-dimensional TLC. Plates were developed first in propan-2-ol/water (7:3, v/v), followed by isobutyric acid/0.5 m-aqueous ammonia (5:3, v/v). Radioactive areas were detected by autoradiography with exposure up to 6 weeks. This system separated mono-, di- and triethylene glycol monosulphates and their oxidized counterparts from each other, and from SO₄²⁻ and residual SDTES (Hales et al., 1982).

Radioisotope river-water die-away experiments. River-water (see above) was filtered through a coarse filter (no. 4 ashless, Whatman). One-litre portions of the filtered river-water in 2 l Erlenmeyer flasks were supplemented with standard mineral medium (see above) containing either 5 mg [³⁵S]SDTES plus 5 mg unlabelled SDTES, or 5 mg [³⁵S]SDTES plus 5 mg Empicol ESB/3S. Flasks were stoppered loosely with non-absorbent cotton-wool plugs and shaken at 100 r.p.m. at ambient temperature (21-26 °C). Samples (1 ml) were removed daily and stored frozen until required for analysis. A preliminary analysis was made of samples taken at 3 d intervals from day 1 to day 28. Aliquots (15 µl, approx. 50 Bq) of each sample were applied to cellulose TLC plates and developed in one dimension using chloroform/methanol/water (8:5:1, by vol.). Autoradiography for 7 d was sufficient to establish which samples were suitable for further analysis; two-dimensional TLC on cellulose plates was done on these samples using propan-2-ol/water (7:3, v/v) as solvent system in the first direction and isobutyric acid/0.5 m-aqueous ammonia (5:3, v/v) in the second direction. After autoradiography for 21 d, spots corresponding to the individual metabolites were scraped from the plate and counted as described previously (Hales et al., 1982).

The same method was used for the triethylene glycol mono[³⁵S]sulphate die-away experiments, except that the scale was reduced 20-fold, because the availability of this ester was limited. Filtered river-water (50 ml) in 250 ml Erlenmeyer flasks was supplemented with triethylene glycol mono[³⁵S]sulphate (0.13 mg, 55 kBq) plus unlabelled ester (0.37 mg) to give a total concentration of 10 mg l⁻¹. Flasks were incubated and samples removed and analysed as before.

It was not always possible to repeat die-away experiments because of the large volumes involved and limited availability of radiolabelled substrates. However, when experiments were repeated they agreed to within 10%. In addition, the results from similar experiments (e.g. those with [³⁵S]-labelled SDTES and EO3) were comparable and consistent with each other (see below).
RESULTS

OECD screen and river-water die-away tests

The concentrations of MBAS decreased rapidly after 2–4 d in both the OECD screen test (Fig. 1a) and the river-water die-away test (Fig. 1b), and the time-courses of MBAS removal for different surfactant compositions were very similar in both tests. Although the rates of disappearance of MBAS were similar for each surfactant (mixture), degradation of Empicol ESB/3S began first, followed closely by Dobanol 25/3EO sulphate, and about 1 d later by SDTES. In both die-away experiments containing Dobanol 25/3EO sulphate, 75% of the MBAS was removed much faster than the remainder; the latter probably corresponds to the fraction of Dobanol 25/3EO sulphate that contains methyl or ethyl branches in the alkyl chain at C-2 (Dias & Alexander, 1971; Kravetz et al., 1982; Patterson et al., 1967).

The main difference between the two tests was that acclimatization was faster in the river-water die-away medium than in the OECD medium. Two factors that might account for this observation are the difference in diversity (and possibly size) of bacterial populations in river-water compared with sewage effluent, and a more varied nutrient composition in river-water compared with the OECD medium. In any event, primary biodegradation of SDTES and the commercial mixtures was complete in both tests within a few days.

Radiotracer die-away experiments with inocula acclimatized in OECD screen tests

The mixed cultures present in OECD screen tests were assessed, at different stages of acclimatization, for patterns of metabolite production from [35S]SDTES alone, or in commercial mixtures. Two states of acclimatization were examined: first, acclimatization to the surfactants; second, acclimatization to intermediates of surfactant degradation, this being important in view of the accumulation of various glycol sulphate derivatives that occurs during SDTES degradation in pure cultures. The opportunity for further adaptation of the cultures during the radiotracer experiments themselves was minimized by the use of small inocula.

Tubes containing [35S]SDTES and either unlabelled SDTES or Empicol ESB/3S or Dobanol 25/3EO sulphate in OECD screen test medium were inoculated with samples taken at two time points from OECD screen test media containing, initially, SDTES or Empicol ESB/3S or Dobanol 25/3EO sulphate at 10 mg l⁻¹ (Fig. 1a). The first inocula were taken at days 5 to 6 of the tests, immediately after removal of the parent surfactant (determined as MBAS) was complete; these inocula contained bacteria acclimatized to degrade the parent surfactant. The second inocula were taken at day 18 of the tests and contained bacteria that had been exposed to the intermediates of alkylethoxy sulphate biodegradation. Samples from the incubation vessels were examined for radiolabelled metabolites of [35S]SDTES by TLC/autoradiography, and relative amounts of each metabolite were assessed visually.

Incubations with surfactant-acclimatized bacteria. In the incubations containing pure SDTES or Dobanol 25/3EO sulphate, the [35S]SDTES disappeared within 24 h (Table 1). In contrast, in the incubation containing Empicol ESB/3S, primary biodegradation took 2–4 d. Triethylene glycol monosulphate (EO3) was the main intermediate formed initially, with smaller amounts of diethylene glycol monosulphate (EO2) and monoethylene glycol monosulphate (EO1) present. In the early stages little or no inorganic sulphate was produced, indicating that direct desulphation of SDTES was not a major primary degradation route. In the incubation bottle containing Empicol ESB/3S, during the latter stages of SDTES degradation, the proportion of radioactivity appearing as EO2 and EO1 increased considerably, as did the amount converted to 35SO₄²⁻. Subsequently, in all incubations, acetic acid 2-(diethoxy sulphate) (EO3') was formed, followed by acetic acid 2-(ethoxy sulphate) (EO2'). By analogy with pure culture studies (Hales et al., 1982, 1986) both compounds were probably derived from oxidation of their respective glycol sulphates. During the first week a small amount of EO1 was also formed but this quickly disappeared. At the same time, the amounts of SO₄²⁻ increased and by the end of the second week it was the major 35S-labelled metabolite.

Two further important observations were made on the fate of [35S]SDTES in the presence of Empicol ESB/3S. First, acetic acid 2-sulphate (EO1') accumulated, which was the first time that
this material had been identified as an intermediate of SDTES biodegradation in pure or mixed cultures. Whether it was produced by oxidation of EO1 or by chain shortening of longer intermediates (e.g. EO2' and/or EO3') is not known. Second, some metabolites, tentatively identified as $\omega$-/$\beta$-oxidation intermediates (Hales et al., 1986), also appeared in this experiment; they were probably butanoate 4-(triethoxy sulphate), hexanoate 6-(triethoxy sulphate) and octanoate 8-(triethoxy sulphate).

After incubation for 12–13 d, the flasks were reinoculated with the OECD screen cultures, now 18 d old and containing bacteria acclimatized to intermediates of surfactant degradation. Within 24 h of this second inoculation, some significant changes were evident (Table 1): EO3' (SDTES incubation) and EO1' (Empicol ESB/3S incubation) disappeared completely and simultaneously with an increase in the concentration of sulphate. On the other hand, EO2', EO3 and EO2 were removed slowly. In the Empicol ESB/3S incubation, the disappearance of EO3 was accompanied by an increase in EO3', and the $\omega$-/$\beta$-oxidation intermediates also underwent fairly rapid removal.

**Incubations with inocula acclimatized to intermediates of surfactant degradation.** The primary biodegradation of the parent surfactant in cultures previously acclimatized to degradation products was relatively slow, requiring 6 d for completion (cf. Fig. 1). In all incubations, EO3 was the main product, with some inorganic sulphate and a little EO2 also being formed. The EO3 was subsequently removed more rapidly than EO2. In contrast to surfactant-acclimatized cultures, oxidized glycol sulphates were detected only transiently in one of the incubations (pure SDTES). In the other two incubations, $\omega$-/$\beta$-oxidation intermediates were also observed transiently. With the exception of these small differences, the metabolite patterns for the three incubations were remarkably similar. No $^{35}$S-labelled organic metabolites were detectable in any flask after 29 d, all the radioactivity appearing as $^{35}$SO$_4^{2-}$.
Table 1. *Metabolite production from* $[^{35}S]$SDTES *in media containing various surfactants and inoculated with OECD test cultures acclimatized to those surfactants*

Approximate amounts of $[^{35}S]$SDTES and its radiolabelled metabolites*

<table>
<thead>
<tr>
<th>Surfactant composition of medium</th>
<th>Incubation period (d)</th>
<th>Glycol sulphates</th>
<th>Oxidized glycol sulphates</th>
<th>Oxidation products</th>
<th>Inorganic sulphate</th>
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<tr>
<td>[35S]SDTES plus pure SDTES</td>
<td>0†</td>
<td>++++</td>
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<td>20</td>
<td>+</td>
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<tr>
<td>[35S]SDTES plus Empicol ESB/3S</td>
<td>0†</td>
<td>++++</td>
<td>+</td>
<td>+</td>
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<td>21</td>
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<tr>
<td>[35S]SDTES plus Dobanol 25/3EO sulphate</td>
<td>0†</td>
<td>++++</td>
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* Amounts assessed by visual inspection of autoradiograms are recorded on a scale of + to ++++. Absence of a compound is indicated by –. See Hales et al. (1986) (preceding paper) for structures of metabolites.
† Inoculated with 5-6-d-old OECD culture (see Fig. 1) grown on the appropriate pure surfactant or commercial mixture.
‡ Cultures reinoculated 24 h previously with the same OECD culture used for the initial inoculation, now 18 d old and acclimatized to surfactant-degradation intermediates.
Biodegradation of SDTES in mixed cultures

Fig. 2. Time-courses for the biodegradation of [35S]SDTES and its 35S-labelled metabolites. Radiolabelled components were separated by TLC and amounts estimated as described in the text. Results are expressed as percentages of the total radiolabel present in each die-away test. ○, SDTES; ▽, EO3; ▼, EO3'; ▲, EO2; □, EO2'; ●, inorganic sulphate. (a) Biodegradation of pure [35S]SDTES in an unacclimatized, river-water die-away test. EO1 (not shown) was also formed transiently (<2%) during day 2, but EO1' was not detected at any time. (b) Biodegradation of triethylene glycol monof[35S]-sulphate and its 35S-labelled metabolites in an unacclimatized river-water die-away test: EO2, EO1 and EO1' were not detected. (c) Biodegradation of [35S]SDTES and its 35S-labelled metabolites in the presence of Empicol ESB/35 in an unacclimatized river-water die-away test; EO1 (not shown) was also formed transiently (<2%) during day 2, but EO1' was not detected at any time.

Rudetracer river-water die-away experiments with unacclimatized inocula

Experiments described hitherto had shown that mixed cultures of sewage origin could degrade SDTES using pathways identified previously with pure isolates (Hales et al., 1982, 1986). In order to establish the environmental relevance of those pathways, a quantitative investigation was made of the biodegradation of [35S]SDTES and its primary metabolites in full-scale river-water die-away tests. With SDTES present as the sole surfactant, primary biodegradation in the river-water cultures was completed within 3 d (Fig. 2a). However, some of the sulphated organic metabolites of [35S]SDTES were more persistent, and release of 35S radioactivity as 35SO4^- was not complete until day 36. During the intervening period, several sulphated intermediates were present. EO3 appeared first (day 2), but by day 3 its concentration was very low and it had disappeared totally within the first week. The appearance of EO3 was followed immediately by rapid production of its oxidation product (EO3'), which was the dominant metabolite from day 3 to day 16 (maximum = 88% of total 35S radioactivity at day 8). Two possible explanations exist for this observation. Initially the culture might contain organisms that produce EO3 from SDTES but are soon outgrown by populations that liberate EO3' directly from SDTES. Alternatively, primary biodegradation of SDTES could be accomplished by liberating EO3, which is subsequently oxidized to EO3'. Several features support the latter explanation. First, the early appearance of a small transient peak of EO3, followed by a short lag before the rapid production of large amounts of EO3' is characteristic of a precursor–product relationship. Second, oxidation of EO3 to EO3' is known to occur during degradation of SDTES by Pseudomonas sp. DES1 (Hales et al., 1982). Third, die-away experiments using EO3 as starting substrate (Fig. 2b) showed a rapid disappearance of the ester and a concomitant appearance of EO3' that was very similar to its appearance in the surfactant die-away tests. Evidently, organisms were present that could adapt rapidly to accomplish the oxidation.

Smaller amounts of EO2 (<5%) and EO2' (<8%) were also formed during SDTES die-away experiments (Fig. 2a), and persisted for approximately 3 and 5 weeks respectively. Very low concentrations (<2%) of EO1 (not shown in Fig. 2a) were produced transiently on day 2, but its oxidation product, EO1', was not observed at any stage. Whether chain-shortening of EO3 or EO3' contributed to the formation of the lower homologues is not clear from the SDTES die-
away experiment (Fig. 2a). However, the EO3 die-away experiment did provide evidence that chain-shortening of EO3' to EO2' can occur (Fig. 2b).

Liberation of inorganic sulphate from SDTES occurred in two phases (Fig. 2a). Initially, a small burst of $^{35}$SO$_4^-$ appeared on the second day, reaching $5\%$ of the $^{35}$S radioactivity after 3 d of incubation; this presumably was the result of sulphatase action on residual SDTES. Alkylsulphatases of appropriate specificity have already been identified in bacteria that participate in the biodegradation of SDTES (Hales et al., 1986). Liberation of sulphatase ceased when SDTES became exhausted but resumed on day 8 and increased rapidly, mirroring the disappearance of the various glycol sulphates. Clearly in mixed cultures, most of the inorganic sulphate was released from oxidized glycol sulphate metabolites, and not from the parent surfactant. However, an adaptation period was needed between formation of the oxidized glycol sulphates (day 3) and the onset of their breakdown to inorganic sulphate (day 8). This was also evident in the EO3 die-away experiments (Fig. 2b).

When $[^{35}$S]SDTES was supplemented with Empicol ESB/3S the pattern of SDTES degradation and metabolite production was very similar to that observed when SDTES was the sole surfactant (Fig. 2c). The main difference was that in the presence of the commercial surfactant, the time taken to complete the liberation of inorganic sulphate was half that required in the presence of pure SDTES.

**DISCUSSION**

Because a major aim of this work was to establish the environmental relevance of studies with pure cultures, it was significant that some of the features of SDTES degradation in mixed cultures reported in this paper had been observed previously for some, but not necessarily all, of the pure cultures.

(i) In all cases etherase-cleavage accounted for the primary biodegradation of most of the SDTES.

(ii) The major metabolites in either OECD screen or river-water die-away experiments were based on the triethylene glycol moiety (EO3 and EO3'), indicating that the cleavage of the alkyl-ether bond is the predominant pathway, and reflecting the ether-cleavage specificities of strains such as SC25A and TESS (Hales et al., 1986). It is also worth noting that cleavage of the alkyl-ether bond is the most direct route to making the C$_{12}$ alkyl chain available for assimilation; cleavage at the other ether bonds or at sulphate produces a dodecyl chain still attached to one or more ethylene glycol units, and further round(s) of etherase action are necessary to liberate the alkyl chain.

(iii) Small amounts (5–10%) of inorganic sulphate were produced in the first few days of the die-away experiments, but its production stopped when the SDTES was exhausted. The same behaviour had been observed for *Pseudomonas* sp. DES1 which was able to liberate sulphate from SDTES but not from its organic metabolites even after prolonged incubation into stationary phase (Hales et al., 1982). In mixed cultures (OECD or river-water), however, further incubation led eventually to the renewed formation of SO$_4^{2-}$ at the expense of the various glycol sulphates formed from SDTES. Presumably under the die-away conditions, bacteria in the mixed culture were able to adapt to the presence of the newly formed glycol sulphate esters. The second phase of SO$_4^{2-}$ production was considerably faster in the Empicol incubations compared with those containing pure SDTES. This may be due either to the ability of the mixed surfactants to support a higher cell-density and/or a more varied bacterial flora at the end of the first phase of degradation, or to the presence of a wider range of compounds able to induce glycol-utilizing organisms (Watson & Jones, 1977) in the second phase of degradation.

(iv) In some of the mixed cultures and in some pure cultures, there was appreciable oxidation of glycol sulphates (particularly EO3) to the corresponding carboxylates. Inoculation with mixed cultures acclimatized either to surfactant or its degradation intermediates invariably produced unoxidized EO3 as a major metabolite, whereas the unacclimatized inocula (river-water) yielded predominantly the oxidized form. Substantial amounts of oxidized glycol sulphates did accumulate eventually in media inoculated with cultures acclimatized to surfactants but not
with those acclimatized to their degradation intermediates. Presumably the surfactant-acclimatized inocula did not provide organisms capable of degrading glycol sulphate intermediates. Under such conditions, glycol sulphates would accumulate and persist long enough to undergo oxidation to their carboxyl derivatives. An analogous situation obtains in the stationary phase of growth of pure organisms that degrade SDTES (Hales et al., 1982, 1986). Hardy any oxidized glycol sulphates accumulated when the inocula were acclimatized to degradation intermediates of SDTES, presumably because, in this situation, glycol sulphates were readily degraded. In cultures in which oxidized glycol sulphates had accumulated, they were degraded far more rapidly than unoxidized glycol sulphates when the cultures were re-inoculated with organisms acclimatized to SDTES degradation intermediates. This may indicate that the responsible organisms were using chain-shortening mechanisms that required a terminal -COO\(^-\) group to be present in the substrate (Kawai et al., 1978).

(v) Intermediates tentatively identified as products of \(\omega-\beta\)-oxidation were observed as a minor contribution in OECD screen tests and in only one of the four pure cultures studied previously (Hales et al., 1986). In contrast, it has been reported, since the completion of the present study, that \(\omega-\beta\)-oxidation is a significant route in the biodegradation of the related non-ionic surfactant octadecyl heptaethoxylate in model continuous-flow sewage treatment plants (Steber & Wierich, 1985). Possibly, the \(\omega-\beta\)-oxidation pathway becomes more important in the degradation of surfactants with longer alkyl chains.

CONCLUSIONS

Despite the persistence of certain glycol-based metabolites for up to 20 d in some of the die-away tests, total mineralization of \([^{35}S]SDTES\) to \(^{35}SO_4^{2-}\) was usually completed within 28 d and always within 35 d. Clearly both river-water and activated sewage contain organisms competent not only in the primary biodegradation of linear alkylethoxy sulphate surfactants but also in the desulphation of the glycol sulphate and oxidized glycol sulphate products. Moreover, ethylene glycols are known to be readily biodegradable in mixed and pure cultures (Payne & Todd, 1966; Evans & David, 1974; Watson & Jones, 1977; Child & Willetts, 1978; Willetts, 1981), and other work from this laboratory has demonstrated the assimilation/mineralization of the alkyl chain of SDTES in pure and mixed cultures (unpublished results). It seems unlikely, therefore, that the use of this class of surfactant will lead to an accumulation of intermediates or residues in the environment.

The award of CASE Studentships to S.G.H. and E.T.G. by the Science and Engineering Research Council is gratefully acknowledged.

REFERENCES


