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Desulfonation and Degradation of the Disulfodiphenylethercarboxylates from Linear Alkyldiphenyletherdisulfonate Surfactants

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Earlier work showed that the biodegradation of a commercial linear monoalkyldiphenyletherdisulfonate surfactant as a carbon source for microbial growth leads to the quantitative formation of corresponding disulfodiphenylether carboxylates (DSDPECs), which were not degraded. α-Proteobacterium strain DS-1 (DSM 13023) catalyzes these reactions. These DSDPECs have now been characterized by high-pressure liquid chromatography coupled via an electrospray interface to a mass spectrometer. DSDPECs were a complex mixture of compounds which indicated catabolism via ω-oxygenation and β-oxidation. DSDPECs were subject to quantitative desulfonation in bacterial cultures in which they served as sole sulfur sources for bacterial growth. On average, one sulfonate group per DSDPEC species was removed, and the organism responsible for this desulfonation was isolated and identified as *Rhodococcus opacus* ISO-5. The products were largely monosulfodiphenylether carboxylate-phenols (MSDPEC-phenols). MSDPEC-phenols were subject to extensive dissimilation by bacteria from activated sludge.

The linear monoalkyldiphenyletherdisulfonate surfactants (LADPEDS) (Fig. 1) have been in use for some 40 years in industrial processes (22), which include the production of synthetic latex and its use in carpet production, paints, and paper coatings (13), as well as in subsurface remediation (22, 25, 27). Despite this widespread use of LADPEDS and their classification in the United States as biodegradable (25), little has been published on their metabolism. It was shown recently, however, that their initial metabolism in pure culture is analogous to that of the linear alkylbenzenesulfonate (LAS) surfactants, namely, ω-oxygenation and oxidation of the side chain followed by β-oxidation, which results in the release of the correspondingly smaller disulfodiphenylether carboxylate (DSDPEC) (Fig. 1), which was not further degraded as a source of carbon for growth (28).

The formation of DSDPECs in pure culture was in a company report on work with a typical *ring*-^{14}C-labeled congener of LADPEDS in activated sludge (22). Whereas no further metabolism of DSDPECs was detected in the laboratory (28), the company report showed slow release of $^{14}CO_2$ from the *ring*-^{14}C-labeled DSDPECs incubated in soil, which indicates ring cleavage reactions in soil (22).

It is easy to rationalize the slow dissimilation of the DSDPECs. LADPEDS comprise some 210 racemic compounds (see the legend to Fig. 1). The largest LAS homologue, with a C_{13} side chain, represents six compounds, five of which are racemic; though they are fully degradable (32), we suspect the microbiology involved to be fairly complex and to involve enzymes of relatively high specificity (28, 30). Correspondingly, degradation of the numerous DSDPECs would require orders of magnitude more organisms.

An alternative to large numbers of specific enzymes is a nonspecific attack. This is especially useful with organosulfonates, because the general desulfonation of aromatic compounds yields the corresponding phenol (8), and the hydroxy analogue of a benzenesulfonate (i.e., the phenol) is generally degradable (35). Recent work indicates that a desulfonation of "recalcitrant" xenobiotic compounds could lead to their further degradation or binding to soil components (15, 16, 18, 24, 26).

The present paper shows that DSDPECs can indeed be desulfonated and that the products are available for the further biological attack already indicated in soil systems (22).

MATERIALS AND METHODS

Materials. LADPEDS, as DOWFAX 8390, was supplied by Dow Chemical Company, Midland, Mich. (28). DSDPECs were generated as catabolic products from LADPEDS in liquid cultures (1 mM LADPEDS; the molecular weight of the disodium salt is 598 [28]) and separated from the growth medium by solid-phase extraction (see below). DSDPECs have the same UV spectrum as the parent LADPEDS (28), and DSDPECs were quantified as having the same molar absorption coefficient as LADPEDS (28). MSDPEC-phenols were generated as desulfonation products from DSDPEC in liquid cultures and separated from the growth medium by semipreparative high-pressure liquid chromatography (HPLC) (see below). Commercial LAS surfactant (Marlon A 350; Hüls, Marl, Germany) was converted to the corresponding sulfophenylcarboxylates (SPCs) described for the conversion of LADPEDS to DSDPEC.

Activated sludge was obtained from the urban sewage treatment plants in Konstanz and Radolfzell, Germany, and from the BASF industrial plant in Ludwigshafen, Germany. The sources of routine chemicals have been given elsewhere (14, 16).

Growth media. Two different growth media were used. Carbon-limited phosphate-buffered minimal-salts medium was used in the generation of DSDPECs from LADPEDS (28, 33). Sulfur-limited Tris-buffered salts solution with three sources of carbon in excess (16) was used to enrich for organisms able to

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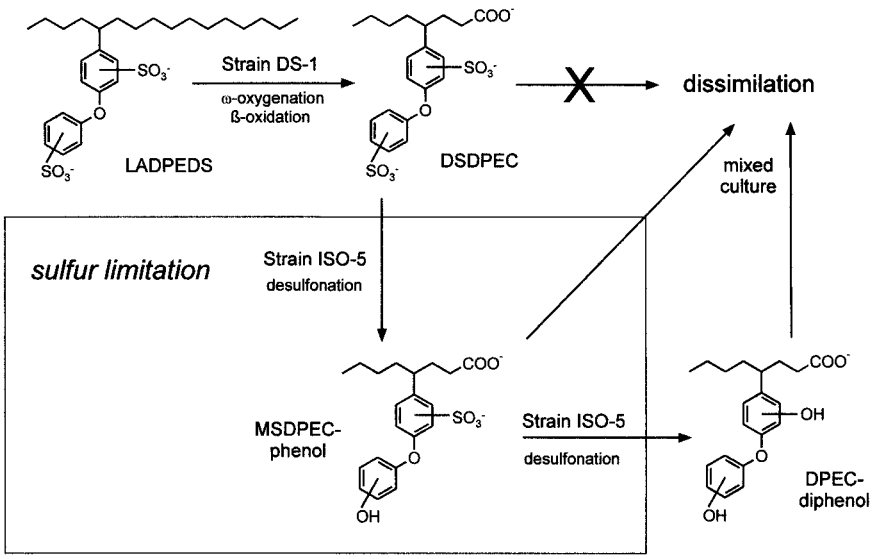

FIG. 1. LADPEDS, its dissimilation to a corresponding DSDPEC by strain DS-1, the product of its subsequent desulfonation by *R. opacus* ISO-5, the product of a subsequent desulfonation, and putative fate of the residual carbon skeleton. Commercial LADPEDS is nominally a set of compounds with a C_{16} alkane subterminally substituted with a disulfodiphenylether: there are thus seven positional isomers of the substituent on the chain, each with a chiral center; 10 possible combinations of alkyl, sulfono, and ether substituents on the first ring; and three positional isomers of sulfonation on the second ring. LADPEDS thus represents up to 210 racemic compounds; *meta* substitution of the sulfono substituent relative to the alkyl chain is unlikely. Dialkylated species and monosulfonated ethers also occur (22), and several homologues were found in the preparation (see the text). The commercial product is thus extremely complex. Strain DS-1 produces several intermediates per substrate with LAS (W. Dong, D. Schleheck, and A. M. Cook, unpublished data), and we have now confirmed that this also occurs with LADPEDS. Strain ISO-5 has limited capacity to carry out a second desulfonation, and a mixed culture catalyzes extensive dissimilation of MSDPEC-phenols and DPEC-diphenols (see the text). The first row represents published data (28); the other reactions were observed in this study (22).

desulfonate DSDPECs. This medium was optimized for work with strain ISO-5: the phosphate concentration was raised to 4 mM, and only one carbon source was present, 25 mM glucose.

Enrichment cultures, growth under sulfur-limited conditions, and bacteria used. Enrichment cultures to utilize commercial DSDPECs (or LADPEDS) as the sole source of sulfur for the growth of microorganisms were done with a 1% inoculum in 3-ml cultures in 30-ml screw-cap tubes, which were aerated at 30°C in a roller (16, 36). Cultures were considered positive when both growth and substrate disappearance (HPLC) occurred.

Molar growth yields, on the 3-ml scale (to economize on the amount of DSDPECs to be purified), were obtained by inoculating a set of tubes with different concentrations (0 to 50 μM sulfur) of sulfate or DSDPECs and quantifying the protein synthesized. The molar growth yield was the slope of the line obtained by plotting the protein formed against the initial substrate concentration (2).

For experiments to identify products, cells were removed from 100-ml cultures by centrifugation (10,000 × *g*; 30 min; 4°C). The supernatant fluid was brought to pH 2 with 1 M HCl and passed through a membrane filter with a 0.2-μm pore diameter. The acidified medium involved in the generation of DSDPEC was diluted to ~0.1 mM DSDPEC for analysis by liquid chromatography-mass spectrometry (LC-MS); that for the generation of MSDPEC-phenols was concentrated by evaporation to about 60 μM MSDPEC-phenol.

Strain ISO-5 was grown in 300 ml of medium with 50 μM DSDPEC as a sulfur source to generate MSDPEC-phenols for the degradation test, and the cells were removed by centrifugation (10,000 × *g*; 30 min; 4°C). The supernatant fluid was acidified to pH 2 with 1 M HCl, and a 50-ml portion was loaded onto a semipreparative, reversed-phase column (Beckman ULTRASPHERE ODS; 10 by 250 mm; 5-μm particle size) that was equilibrated with 4 mM phosphate buffer, pH 2 (buffer A), at 5 ml/min. The column was then washed for 10 min with buffer A. Bound material eluted in one broad peak after the methanol concentration was ramped to 100% in 2 min; this material was collected in a 15-ml fraction. Two collected fractions were pooled and evaporated to dryness at 40°C in a rotary evaporator, and the dry material was dissolved in 10 ml of carbon-limited salts medium. This MSDPEC-phenol salts medium was inoculated with 0.2 ml of sludge derived from a communal sewage treatment plant (Konstanz, Germany); prior to inoculation, the activated sludge was repeatedly washed and resuspended in carbon-limited salts medium. The culture was incubated at 30°C on an orbital shaker, and samples for analysis by HPLC and for the quantification of protein were taken at intervals.

α-Proteobacterium strain DS-1 (DSM 13023) (28) was grown under carbon-limiting conditions throughout the work. *Pseudomonas putida* S-313 (DSM 6884) was grown under sulfur-limited conditions with orange II as the sole added source of sulfur (10).

Analytical methods. Commercial LADPEDS, DSDPECs, and MSDPEC-phenols and the compounds tested as sulfur sources for strain ISO-5 were routinely determined by gradient elution with a perchlorate-containing eluent in reversed-phase HPLC with 125- by 3-mm columns of Nucleosil-5-C18 (28) (gradient system I). Ion chromatography with suppression was done as described elsewhere (16).

The HPLC used for LC-MS was a Series 1100 (Hewlett-Packard Schweiz AG, Urdorf, Switzerland) with a variable-wavelength UV detector set to 220 nm. The HPLC column (150 by 1 mm) used was a Phenomenex Ultracarb 5 ODS 30 (Brechbuehler AG, Schlieren, Switzerland). Eluent A was aqueous 5 mM acetic acid and 5 mM triethylamine, and eluent B was acetonitrile-water (80:20) containing 5 mM acetic acid and 5 mM triethylamine (6) (gradient system II). The flow rate was 100 μl/min, and the column was maintained at 30°C. The column was equilibrated with eluent containing 95% eluent A and 5% eluent B. Samples (100 μl) were injected, and a linear gradient to 100% eluent B in 35 min was applied: after another 5 min, the initial conditions were regenerated in 5 min, with an equilibration phase of 5 min, giving a total run time of 50 min. All mass spectra were acquired on a Platform LC single quadrupole mass spectrometer, using electrospray ionization (Micromass UK Ltd., Manchester, United Kingdom). Full scan spectra were acquired in negative ion mode, scanning from *m/z* 50 to 800 at 1 s/scan. The mass range was calibrated, and the sensitivity of the instrument was tested using 2 mM $NaNO_3$ infused at a flow rate of 70 μl/min. The electrospray interface temperature was set to 150°C, and the flow of nitrogen gas was set to 500 liters/h. The needle and cone voltages were set to 4 kV and 60 V, respectively.

Solid-phase extraction was done on a column of Chromabond HR-P (2 g; Macherey & Nagel, Düren, Germany) (18); extraction and recovery were quantitative, and the contamination by sulfate was undetectable, as observed previously (18). Protein in bacterial cells was assayed by a Lowry-type method (3). A

TABLE 1. Products from the dissimilation of commercial LADPEDS by α-proteobacterium strain DS-1 detected by LC-MS[a]

Length of side chain[b]	Monocarboxylated disulfonate		Monocarboxylated monosulfonate	
	$(M\text{-}H)^{-}$[c]	Intensity[d]	$(M\text{-}H)^{-}$	Intensity
4	415	ND	335	+
5	429	TR	349	TR
6	443	+++	363	++
7	457	+	377	+
8	471	+++	391	++
9	485	TR	405	TR
10	499	+	419	TR
11	513	TR	433	ND
12	527	ND	447	ND
13	541	ND	461	ND

[a] The abundant compounds were recognized from the pattern of peaks for $(M\text{-}H)^{-}$, $(M\text{-}2H)^{2-}$, $(M+Na\text{-}2H)^{-}$, and $[M+(H_5C_2)_3N\text{-}H]^{-}$, together with the desulfonated fragment $(M\text{-}H\text{-}SO_3)^{-}$; the regularity in the pattern of elution of homologues allowed traces to be identified. Gradient system II was used for the separation.

[b] Number of C atoms.

[c] Data (m/z) from the peaks for $(M\text{-}H)^{-}$ are given: the signals were spread over several minutes, presumably reflecting the complexity of the parent surfactant (see the legend to Fig. 1).

[d] ND, not detected; TR, trace detected; +, ++, and +++, signal intensities.

partial 16S rRNA gene sequence (450 bp) of strain ISO-5 was determined by the German Culture Collection (Braunschweig, Germany), where the sequence data were aligned and compared as described elsewhere (17, 23).

RESULTS

Generation of DSDPECs from commercial LADPEDS. LADPEDS was previously detected in dried solutions by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) MS (28), but new separation techniques (6) allowed LC-MS to be used to examine the starting material for these experiments. LADPEDS was separated in gradient system II, and the major signals obtained, from 25 to 35 min, were C_{16}-LADPEDS. They were recognized as follows: m/z = 553 $(M\text{-}H)^{-}$, m/z = 276 $(M\text{-}2H)^{2-}$, m/z = 575 $(M+Na\text{-}2H)^{-}$, and m/z = 654 $[M+(H_5C_2)_3N\text{-}H]^{-}$, together with the desulfonated fragment m/z = 473 $(M\text{-}H\text{-}SO_3)^{-}$. There were significant amounts of LADPEDS with chain lengths of C_{10}, C_{11}, and C_{12} and traces with chain lengths of C_{13}, C_{14}, C_{18}, and C_{20}. The second major signal was near 34 min, where m/z was 473 $(M\text{-}H)^{-}$, and this was presumed to be the monosulfonated diphenylether referred to in the manufacturer's description (see the legend to Fig. 1). The dialkyl compounds (see the legend to Fig. 1) would have much longer retention times and were not observed under these conditions.

LADPEDS was subject to partial dissimilation of the alkyl chain by strain DS-1. When this phenomenon was first described, only partial infrared spectra were available with which to deduce the identity of DSDPEC (Fig. 1), together with MALDI-TOF MS identifications of the analogous product(s) from LAS (28). We then realized that we had underestimated the number of products from each LAS congener (D. Schleheck, unpublished data; 6). Correspondingly, the LC-MS analyses of putative DSDPEC showed a wide range of compounds (Table 1).

The major compound formed from LADSPEDS was the C_8-DSDPEC, the chain length anticipated from physiological data (28); the second major compound was C_6-DSDPEC. These "compounds," however, were really large groups of compounds which eluted over some 15 min, analogous to the educt being nominally 210 racemic compounds (Fig. 1, legend). Smaller amounts of C_{10}-DSDPEC were also detected, as well as readily measurable amounts of C_7-DSDPEC and traces of C_5-, C_9-, and C_{11}-DSDPECs. The mean chain length (about C_8) thus corresponds to that predicted earlier (28). The mass spectral identifications also confirm the prediction of the identity of the family of products, i.e., DSDPECs. The monosulfonated components of commercial LADPEDS were also subject to dissimilation to products with a similar range of chain lengths (Table 1). No unsaturated species (6; W. Dong and A. M. Cook, unpublished data), and no significant amounts of dicarboxylated LADPEDS (5) were detected. For convenience, this whole group of products is called DSDPEC.

Enrichment cultures to degrade 100 μM commercial LADPEDS as the sole added source of sulfur with inocula from sewage works gave slight growth. Commercial LADPEDS was found by ion chromatography to contain inorganic sulfate, giving 10 μM sulfate in the growth medium. LADPEDS, which had been purified of this contamination by solid-phase extraction supported no growth.

Enrichment cultures to degrade 60 μM DSDPECs as the sole added source of sulfur for growth were prepared. Of the three inocula tested, only that from the industrial sewage treatment plant was positive. This enrichment culture was largely homogeneous when examined microscopically after three subcultures in fresh medium, and the major morphotype was coryneform. The coryneform strain ISO-5 was isolated from the culture, and it catalyzed the same reactions(s) (see below) as the enrichment culture. The 16S rRNA gene of strain ISO-5 was subjected to partial sequencing, and the sequence was identical with that of *Rhodococcus opacus* (DSM 43205), a typical coryneform organism (31). Our isolate was thus *R. opacus* ISO-5, which was deposited with the German Culture Collection as DSM 14600.

DSDPEC and other compounds as sulfur sources for *R. opacus* ISO-5. Strain ISO-5 utilized sulfate with a molar growth yield of 5 kg of protein/mol of S up to about 40 μM sulfate (Fig. 2), a value which corresponds to complete incorporation into cell material (8). The growth yield with DSDPEC was also 5 kg of protein/mol (Fig. 2), so strain ISO-5 removed an average of one sulfonate group per DSDPEC species. Growth with DSDPEC was concomitant with the disappearance of individual compounds during growth (Fig. 3, inset), with a steady shift of peaks in HPLC determinations to longer retention times (Fig. 3), and the formation of individual products was concomitant with growth (Fig. 3, inset). Each product peak had a UV spectrum different from that of the substrate (not shown), which was the first indication that the aromatic ring was subject to microbial attack. The products were examined by LC-MS (Table 2), and MSDPEC-phenols (Fig. 1) were detected. The major set represented the chain length C_8 (Table 2), and it was spread over several minutes, as were the precursors (cf. Table 1); further, individual peaks with other chain lengths were observed (Table 2). DPEC-diphenols were also detected (Fig. 1 and Table 2), which indicates that some species were subject to a second desulfonation: these products were individual

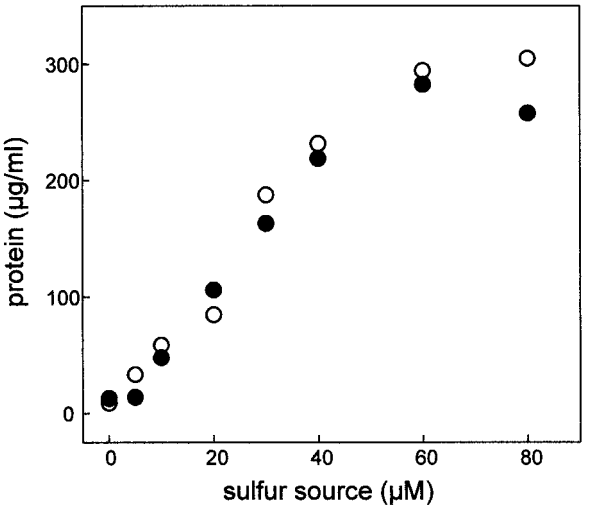

FIG. 2. Growth yield of *R. opacus* ISO-5 in salts medium with different limiting concentrations of sulfate (○) and of DSDPEC (●).

TABLE 2. Products from the desulfonation of DSDPEC by *R. opacus* ISO-5 detected by LC-MS[a]

Length of side chain[b]	MSDPEC-phenol[c]		DPEC-diphenol	
	$(M-H)^-$	Intensity	$(M-H)^-$	Intensity
4	351	+	287	ND
5	365	TR[d]	301	TR[d]
6	379	TR[d]	315	++[d]
7	393	ND	329	+[d]
8	407	++	343	++[d]
9	421	TR	357	ND
10	435	TR	371	ND

[a] Gradient system II was used for the separation.
[b] Number of C atoms.
[c] Data (m/z) from the peaks for $(M-H)^-$ are given; unless otherwise indicated, the signal was spread over several minutes, presumably reflecting the complexity of the parent surfactant (see the legend to Fig. 1). ND, not detected; TR, trace detected; + and ++, signal intensities.
[d] In contrast to the many broad peaks observed, especially those shown in Table 1, this peak is sharp and seemingly represents only one or a few chemical species.

sharp peaks rather than the broad sets of compounds in the precursors (Table 1).

The complexity of the range of DSDPECs desulfonated led us to explore the substrate range of the organism. Commercial LAS was converted by strain DS-1 into SPCs (5), and strain ISO-5 grew with SPCs as the sole source of sulfur: as observed with LADPEDS, the HPLC peaks of desulfonated SPCs shifted to longer retention times after growth, UV spectra different from those of the educts, and mass spectra which showed these compounds to be desulfonated to the corresponding phenols. The chain length was not significantly altered during desulfonation (data not shown). Strain ISO-5 utilized benzenesulfonate; 4-toluenesulfonate; 4-ethylbenzenesulfonate; 4-octylbenzenesulfonate; 4-phenolsulfonate; 4-chlorobenzenesulfonate; 2-, 3-, and 4-aminobenzenesulfonate; 2-, 3-, and 4-nitrobenze-

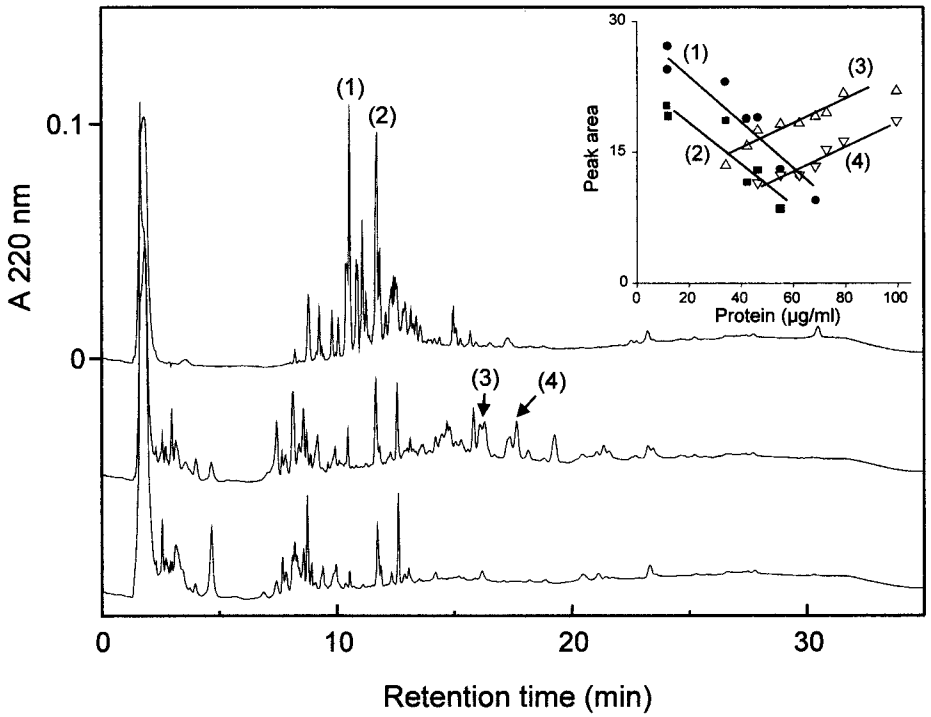

FIG. 3. HPLC chromatograms of DSDPEC in sulfur-limited salts medium (upper chromatogram), of the same medium after the growth of *R. opacus* ISO-5 (central chromatogram), and of outgrown sulfate salts medium (lower chromatogram). Gradient system I was used. The peaks visible in the negative control (lower chromatogram) are natural excretion products from strain ISO-5, so the peaks with retention times of <13 min in the central chromatogram are the same excretion products from strain ISO-5. The inset is a plot of the disappearance of individual peaks as a function of growth and the appearance of products as a function of growth. The numbers are keyed to the numbers in the main figure.

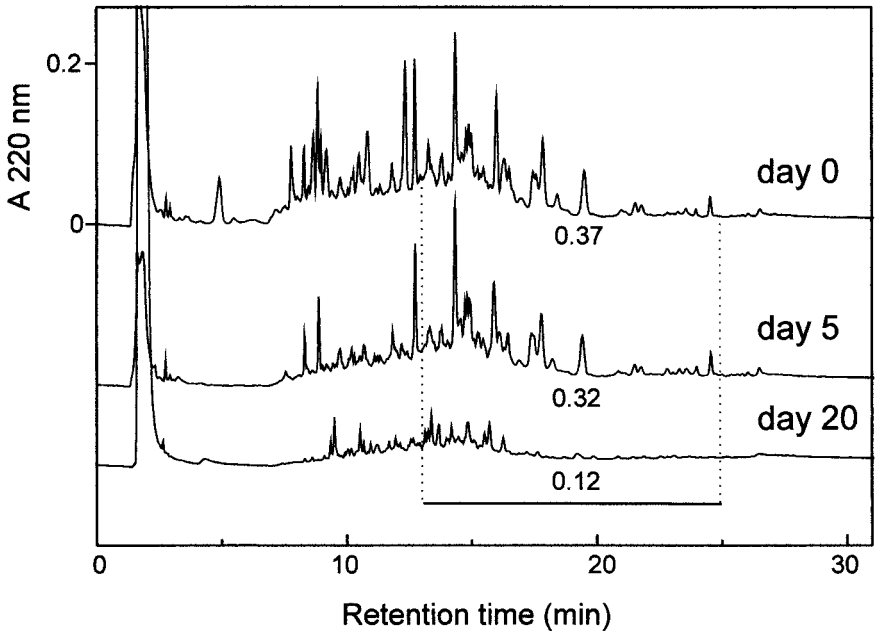

FIG. 4. HPLC chromatograms of the levels of MSDPEC-phenols and DPEC-diphenols during incubation with an inoculum derived from sewage sludge. Gradient system I was used. The total peak area of UV-absorptive material in the interval from 13 to 25 min (bar) is indicated.

nesulfonate; 4- and 3-sulfobenzoate (but not 2-sulfobenzoate), 5-sulfosalicylate; 5-amino-2-chlorotoluene-4-sulfonate; naphthalene-2-sulfonate; anthraquinone-2-sulfonate; 8-amino-1,5-naphthalenedisulfonate; and the dyestuffs orange II, roccelline, Ponceau R, Erichrome red B, azophloxine, and tartrazine but not amaranth, Ponceau S, or Congo red as sole sulfur sources for growth. The product tended to bind to the organism, which was especially visible with the dyestuffs when outgrown cultures were centrifuged. In the case of orange II (and tartrazine), the desulfonation product from the cell pellets was extracted into alcohol and examined by HPLC. The product was indistinguishable from the product from orange II (and tartrazine) generated by *P. putida* S-313 and identified previously (10).

Dissimilation of MSDPEC-phenol. The products of desulfonation of DSDPEC (MSDPEC-phenol [Fig. 3]) were separated from the growth medium by semipreparative HPLC and supplied as sources of carbon in what we regarded as a miniaturized Organization for Economic Cooperation and Development 301 biodegradation test; the natural excretion products from strain ISO-5 were also present in this preparation (Fig. 3).

We observed growth as turbidity, largely due to rod-shaped bacteria, and substrate disappearance (Fig. 4), whereas the negative control, i.e., without MSDPEC-phenol, showed no growth. Within the first 5 days of incubation, the natural excretion products from strain ISO-5 were mostly degraded (retention time, <13 min), but only a little MSDPEC-phenol (retention time, >13 min) was degraded. MSDPEC-phenol disappeared in the following 15 days to ~32% of the initial amount when quantified as the total peak area of UV-absorptive material (A_{220}; retention time, from 13 to 25 min) (Fig. 4); the biomass production over this period was 30 μg of protein/ml. We estimated an initial concentration of ~0.5 mM MSDPEC-phenol in the medium, and we assumed a mean side chain of C_8 (Table 2). Thus, the initial concentration of carbon was 10 mM, of which 7 mM disappeared. The molar growth yield was thus about 4 g of protein/mol of C, a normal value (2).

DISCUSSION

Basic research with commercial surfactants is often made more complex by the nature of the commercial product. This is certainly the case with LADPEDS, where the single structure in Fig. 1 represents some 210 optically active compounds, to say nothing of the homologues and the monosulfonated species that were also detected. There are thus many HPLC peaks for any relevant value of *m/z*. There was good indirect evidence to predict the generation of DSDPEC from LADPEDS (28); these preliminary data have now been confirmed by LC-MS. We initially considered C_8-DSDPEC to be the product (28). This prediction was accurate, in that the mean chain length represented about C_8-DSDPEC. However, a wide range of chain lengths was found as opposed to a single chain length (Table 1). A further prediction (5) foresaw that metabolism is due solely to [omega]-oxygenation and β-oxidation. Detectable amounts of DSDPEC with side chains of uneven lengths (Table 1) were observed, but we assume that they derive solely from the C_{11}- and C_{13}-LADPEDS and not that we have detected the α-oxidation (7) reported for LAS in major reviews (29, 32).

There was negligible [omega]-oxygenation of the second methyl group of LADPEDS; in contrast, dicarboxylates were abundant, with LAS as the substrate (Dong and Cook, unpublished). No α,β-unsaturated derivatives of DSDPEC were de-

tected, although low levels of such derivatives were found with LAS as the substrate (Dong and Cook, unpublished; 6). Perhaps they were lost in the multiplicity of compounds present.

DSDPECs, incubated under conditions of carbon limitation and excess sulfate, were not subject to microbial attack (Fig. 1); they were also observed to be stable compounds (28). In contrast, when incubated with *R. opacus* ISO-5 in the absence of sulfate and in the presence of an alternate source of carbon, DSDPECs were subject to quantitative biotransformation (Fig. 3). This biotransformation could be deduced to represent monodesulfonation (Fig. 2), which was largely confirmed by LC-MS (Table 2). Many single desulfonations were detected, giving rise to the corresponding MSDPEC-phenol (Fig. 1 and Table 2), as predicted from earlier work (8, 36). Some species of DSDPEC were subject to two desulfonation reactions to give the DPEC-diphenol (Fig. 1 and Table 2). We presume that the initially monosulfonated species (see the legend to Fig. 1) were also desulfonated, but we suspect that the product was sufficiently nonpolar to adsorb to the cell (see below) and was thus not detected in the culture fluid.

Strain ISO-5 demonstrated a broad substrate range for sulfonated compounds (Fig. 3 and Table 2), so we explored some of its limits. Almost all small compounds tested [benzenesulfonate to 4-(1-octyl)benzenesulfonate] and a range of dyestuffs up to molecular weight 468 (tartrazine) were desulfonated; the larger dyestuffs (above molecular weight 538) were not. Very few of the products were found in solution, and work with the dyestuffs indicated that the apolar products bound to the cell, which presumably reflects the presence of apolar mycolic acids in large amounts at the cell surface, typical of *Rhodococcus* (21).

The phenomenon of desulfonation under sulfate-limiting conditions is controlled by a global regulatory network in gram-negative bacteria (8), and an understanding of the monooxygenase system involved in desulfonating arylsulfonates is becoming available at the molecular level (9). The system is widespread in soils (M. Lechner and A. M. Cook, unpublished data). Analogous regulatory and desulfonative systems are presumably present in gram-positive bacteria (34); coryneforms, especially *Rhodococcus* spp., are well known in desulfurization and desulfonation (1, 19, 20).

This phenomenon of desulfonation of apparently recalcitrant compounds has been observed several times (4, 10–12, 16, 18, 24, 26, 36). Indeed, the failure to obtain enrichment, seen above with LADPEDS, is unusual in our experience (A. M. Cook, unpublished data). The desulfonation products, in contrast to the sulfonates, are reactive compounds which can bind to soils, polymerize, or be subjected to biodegradation (24, 26). The rationale is that the conversion of an arylsulfonate to a phenol will make the latter much more reactive and liable to attack than the former (35).

We believe that strain DS-1 is representative of organisms in many pristine and contaminated environments (28; Dong and Cook, unpublished). In sewage works, with their high levels of sulfate ions, LADPEDS will only be converted to DSDPEC (Fig. 1). In soil, with its sulfate limitation (8), the DSDPEC formed by organisms like strain DS-1 will presumably be subject to desulfonation by organisms like *R. opacus* ISO-5. The resulting MSDPEC-phenol and DPEC-diphenol are then available for dissimilation, as indicated above. We feel this to be a rational explanation of the observations in the industrial laboratory (see the introduction) (22).

We have been unable to quantify many of the reactions we have observed, because of the complexity of the substrates involved in the various reactions and because of the lack of authentic standards. Quantification may be possible if a single species of LADPEDS were available, which should allow quantitative identification of the DSDPEC species formed by strain DS-1. Individual compounds, in turn, could be supplied to strain ISO-5 to follow desulfonation, and work with single compounds would allow further desulfonation or dissimilation to be studied in detail.

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