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Metabolic pathway for the biodegradation of octadecylbis(2-hydroxyethyl)amine

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Abstract

The biodegradation curve of octadecylbis(2-hydroxyethyl)amine determined in a Closed Bottle test suggested an initial oxidation of the alkyl chain and a subsequent degradation of the diethanolamine formed. Using the sludge from the test as inoculum, a bacterium capable of utilizing octadecylbis(2-hydroxyethyl)amine as sole source of carbon and energy was isolated. This bacterium also utilized various other alkylbis(2-hydroxyethyl)amines and octadecylpolyoxyethylene(5)amide. Respirometric studies and the formation of diethanolamine by a washed cell suspension of the pure culture showed that the bacterium only oxidized the alkyl chain. Furthermore, in cell-free extracts a dehydrogenase activity catalysing the oxidation of octadecylbis(2-hydroxyethyl)amine was detected.

Introduction

Ethoxylated fatty amines are used as surfactants in many applications such as agrochemical adjuvants, emulsifiers and wetting agents. As a consequence these chemicals are discharged into the environment. Since some of the ethoxylated fatty amines are toxic to aquatic organisms (Schöberl et al. 1988), it is conceivable that the discharge of these surfactants could cause hazards. Biodegradation of organic compounds is the major process to reduce environmental concentrations, thus preventing the accumulation of toxic substances. Therefore, the biodegradability of ethoxylated fatty amines has to be known to assess the ecological impact of these chemicals.

The biodegradability of some ethoxylated fatty amines has been tested in various biodegradability tests (Swisher 1988). Some authors reported biodegradation percentages up to 100 per cent (Baleux & Caumette 1974; Ruiz Cruz & Dobarganes Garcia 1978). However, biodegradability tests, especially those with high solids levels and specific analysis may give misleading results due to adsorption onto solids. Using oxygen consumption as a measure Berth et al. (1984), Fischer (1972, 1981) and Schöberl et al. (1988) clearly showed the susceptibility of ethoxylated amines to biodegradation. However, total mineralization of ethoxylated amines has been poorly demonstrated until now.

The aim of this study was to establish clearly the mineralization of ethoxylated amines by isolation of a pure culture capable of utilizing alkylbis(2-hydroxyethyl)amines as carbon and energy source. The biodegradation route of octadecylbis(2-hydroxyethyl)amine employed by this bacterium is shown.

Materials and methods

Chemicals

Ethoxylated fatty amines and an ethoxylated fatty amide were obtained from Akzo Chemicals BV, Deventer, The Netherlands. All other chemicals were purchased and of reagent grade.

Media

Mineral salts medium contained per litre: 1.55g K_2HPO_4; 0.85g NaH_2PO_4; 0.5g NH_4Cl; 0.01g $MgSO_4(H_2O)_7$ and 0.1ml trace solution of Visniac & Santer (1957). This medium was supplemented with 15g agar and 1g octadecylbis(2-hydroxyethyl) amine to prepare selective plates. The yeast/glucose plates contained per litre of deionized water: 1g yeast extract; 5g glucose and 15 agar.

Closed Bottle test

The Closed Bottle test was performed according to an OECD test guideline (1982). The inoculum used was secondary activated sludge obtained from an activated sludge plant treating predominantly domestic waste water (Nieuwgraaf, Duiven, The Netherlands). A few minor deviations of the test procedures were introduced:

- The inoculum was preconditioned to reduce the endogenous respiration rates. To this end, the sludge (200mg Dry Weight (DW)/litre) was aerated for a period of seven days. The sludge was diluted to a concentration in the BOD bottles of 2mg DW/litre.
- Ammonium chloride was omitted from the medium to prevent nitrification. The inoculum used contained sufficient nitrogen to support complete biodegradation.
- The biological oxygen demand was determined in one control and one test bottle using a special funnel to prevent spillage of the medium during the determination of the oxygen concentration.
- 2g of silica gel was added to the bottles to reduce the concentration of octadecylbis(2-hydroxyethyl)amine in the water phase and thus preventing substrate inhibition.

The initial concentration of the test compounds was 2mg/l. Biodegradation was calculated as the ratio of the biochemical oxygen demand (BOD) to the theoretical oxygen demand (ThOD).

Determination of the oxygen concentration

The dissolved oxygen concentrations in the closed bottles were periodically determined electrochemically using an oxygen electrode (WTW Trioxmatic EO 200) and meter (WTW OXI 530) (Retsch BV, Kesteren, The Netherlands).

Analytical methods

Protein in the crude cell-free extracts was determined by the bicinchoninic method (Pierce, Rockford, USA) based on the biuret reaction.

Ammonium was determined colourimetrically by forming indophenol blue with hypochlorite and salicylate in the presence of sodium nitroferricyanide as catalyst (Verdouw et al. 1978).

Diethanolamine was measured with a Varian 370 gas chromatograph fitted with a DB-1 (diameter 5μm; length 30m) column (Interscience, Breda, The Netherlands). The column temperature was 160–260°C (10°C/min) and the carrier gas was N_2.

Octadecylbis(2-hydroxyethyl)amine forms a coloured complex with methyl orange. This complex was extracted from the buffered water solution (pH = 2.0) into a 1,2-dichloroethane layer. The yellow colour of the 1,2-dichloroethane layer was measured photometrically at 420nm (Waters & Kupfer 1976).

The biochemical features (API 20NE) were examined according to the instructions of the manufactures (API system, Montalieu-Vercieu, France).

Isolation, maintenance, growth and cultivation

The bacteria used in this study were enriched from

secondary activated sludge (Closed Bottle test) in a mineral salts medium containing 1 g l^{-1} octadecylbis (2-hydroxyethyl)amine. After growth was observed in the enrichment culture, approximately 0.1 ml of this culture was transferred to fresh mineral salts medium with the same carbon source. Bacteria grown in this culture were subsequently streaked to purity on agar plates containing octadecylbis(2-hydroxyethyl)amine. One strain (SK) used in further studies was checked for purity by streaking onto yeast/glucose plates. Strain SK was maintained on yeast extract/glucose agar plates.

Growth experiments were performed in 100 ml erlenmeyers with 20 ml of mineral salts medium and the respective carbon sources. The final concentration of the growth substrates was 1 g l^{-1}.

The octadecylbis(2-hydroxyethyl)amine utilizing bacterium was cultivated in a continuous culture at a dilution rate of 0.06 h^{-1}. This culture consisted of a vessel (working volume 1 litre) with an air inlet at the bottom. The culture was mechanically stirred. The influent used contained 1 g octadecylbis (2-hydroxyethyl)amine per litre mineral salt medium. The influent was mixed continuously to ensure a homogenuous suspension. The pH and the temperature of the culture were 7 and 30 °C, respectively.

Preparation of washed cell suspensions and cell-free extracts

Cells from the continous culture were harvested by centrifugation (10,000 g) for 10 min. The resulting pellet was washed twice with a 100 mM phosphate buffer (pH= 7) sedimented at 10,000 g for 10 min and suspended in the same buffer.

Cell-free extracts were prepared from washed cell suspensions by sonification at 375 W with an ultrasonic processor (Sonics & materials Inc, Danbury, USA). Samples of the washed cell suspensions (10 ml) were kept in an ice-cooled vessel and sonified for 4 times 15 s, the periods of sonification being separated by intervals of 1 min. The disintegrated suspensions were clarified by centrifugation at 30,000 g for 30 min at 4 °C.

Respiration experiments

Oxygen uptake was measured with a Biological Oxygen Monitor (Yellow Springs Instruments) which consisted of an electrode and a water jacketed vessel (5 ml). A washed cell suspension was incubated in the vessel at 30 °C for at least five min to allow the determination of the endogenous respiration rate. Subsequently, 0.1 ml of a substrate solution (1 g l^{-1}) was injected and the increase in the respiration rate was determined.

Formation of diethanolamine and ammonium

Washed cell suspensions of strain SK were incubated at 30 °C in a shaking bath with various alkylbis(2-hydroxyethyl)amines (350 mg/litre). Samples were withdrawn from the culture at various time intervals for analyses. Prior to analyses, the bacteria in the samples were removed from the water phase by centrifugation.

Enzyme assays

Spectrophotometric enzyme assays were carried out in a Shimadzu UV 160A spectrophotometer in 1 cm light-path cuvettes. All assays were performed at 30 °C.

(a) Tertiary amine dehydrogenase (EC 1.5.99.-). DCPIP (2,6-dichlorophenolindophenol)-dependent tertiary amine dehydrogenase was determined by measuring the decrease in absorbance at 600 nm. The reaction mixture (3 ml) contained 0.12 µmol DCPIP, 7 µmol phosphate buffer (pH 7) and 2 ml cell-free extract. The reaction was started by adding 10 µmol octadecylbis(2-hydroxyethyl)amine.

(b) Tertiary amine monooxygenase. Tertiary amine monooxygenase activity was assayed spectrophotometrically by following the oxidation of NADH in cell-free extract in the presence of octadecylbis(2-hydroxyethyl)amine at 340 nm. The reaction mixture (3 ml) contained 0.5 µmol NADH, 3 µmol octadecylbis(2-hydroxyethyl)

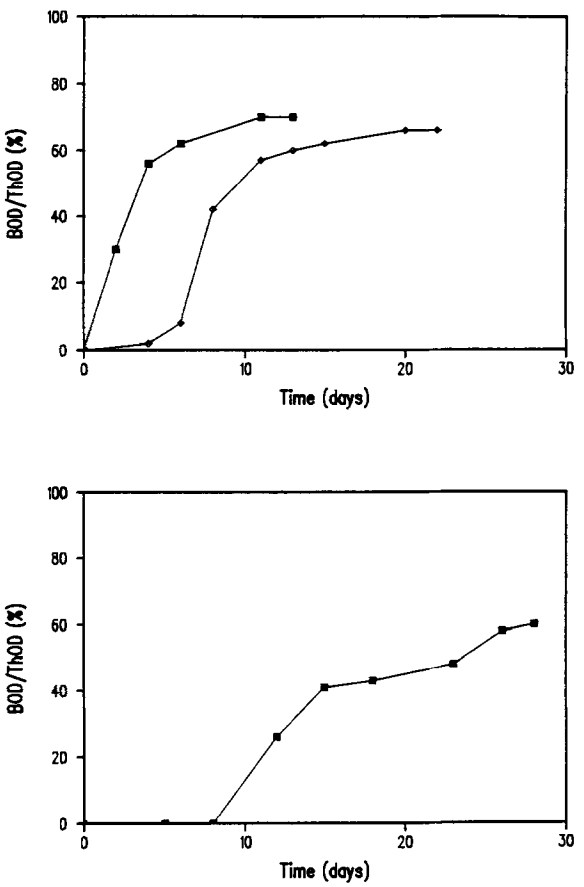

Fig. 1. Biodegradation expressed as the ratio of the BOD to the ThOD of octadecanoate (above, ■), diethanolamine (above, ◇) and octadecylbis(2-hydroxyethyl)amine (below, ■) in the Closed Bottle test.

amine, 15 µmol phosphate buffer (pH= 7) and 1.5 ml cell-free extract.

(c) Octadecanal dehydrogenase (EC 1.1.1.-). Octadecanal dehydrogenase activity was assayed spectrophotometrically by measuring the formation of NADH at 340 nm. The reaction mixture (3 ml) contained 1.8 µmol NAD^+, 2.3 µmol tetradecanal, 14 µmol phosphate buffer (pH 7) and 0.2 ml cell-free extract.

(d) Acyl-CoA synthetase (EC 6.2.1.3). This enzyme was assayed by following the formation of a hydroxamic acid complex which was measured by the increase in A_{540}. The reaction mixture (3 ml) contained 30 µmol $MgCl_2$, 7.5 µmol ATP, 1.5 µmol CoA, 600 µmol hydroxylamine, 43 µmol hexanoic acid and 21 µmol phosphate buffer (pH= 7.0) and 0.5 ml cell-free extract. The reaction was stopped by adding 333 µl 2.5% (w/v) $FeCl_3$ in 2.0 N-HCl and 333 µl 10% (v/v) TCA. The absorption coefficient used was 6411 mol^{-1} cm^{-1} (Beinert et al. 1953).

(e) Acyl-CoA dehydrogenase (EC 1.3.99.3). Acyl-CoA dehydrogenase was assayed by incubating 50 µmol potassium phosphate buffer (pH= 7.6), 0.3 µmol Na_2EDTA, 0.06 µmol 2,6-dichlorophenol indophenol (DCPIP), 0.07 µmol hexadecanoyl-CoA and 0.2 ml cell-free extract. The oxidation of DCPIP was followed spectrofotometrically at 600 nm against a control containing no hexadecyl-CoA (Wheeler et al. 1991).

(f) Isocitrate lyase. (EC 4.1.3.1). Isocitrate lyase was assayed as described by Dixon & Kornberg (1959). The reaction mixture (2 ml) contained 100 µmol Tris/HCl buffer (pH= 8.0), 100 µmol $MgCl_2$, 10 µmol phenylhydrazine hydrochloride and 0.1 ml cell-free extract. The reaction was started by the addition of 20 µmol isocitrate and hydrazone formation was recorded at 324 nm. Activities were calculated using an absorption coefficient for phenylhydrazone of $1.7\,10^4$ $l\,mol^{-1}$ cm^{-1}.

Results

Closed Bottle tests with octadecylbis(2-hydroxyethyl)amine, octadecanoate and diethanolamine

Mineralization of octadecylbis(2-hydroxyethyl) amine was measured in the Closed Bottle test described by the OECD (1982). The inoculum used was taken from an activated sludge plant treating predominantly domestic sewage. Figure 1 shows a 'two-phase' degradation of the ethoxylated amine in the Closed Bottle test. This 'two-phase' biodegration was not found for two possible intermediates of the octadecylbis(2-hydroxyethyl)amine degradation. Octadecanoate was completely biodegraded within a week without a lag phase, whereas the biodegradation of diethanolamine started at day 5 was finished at day 15 (Fig. 1).

Isolation and characterization

Octadecylbis(2-hydroxyethyl)amine degrading microorganisms were enriched from activated sludge in a mineral medium containing 1 g l^{-1} octadecylbis (2-hydroxyethyl)amine at 30 °C. After growth was observed in the subculture, one strain (SK) was isolated using agar plates containing 1 g l^{-1} octadecylbis (2-hydroxyethyl)amine. Strain SK was a gram-negative, rod-shaped, white and mobile organism. The organism was able to assimilate gluconate, caprate, malate, maltose, citrate and adipate. Furthermore, the strain was oxidase-negative and capable of utilizing nitrate as electron acceptor. Tallowpolyoxyethylene(5)amide and alkylbis(2-hydroxyethyl) amines with tallow, oleyl and coco alkyl chains supported growth of the bacterium. Strain SK did not use octadecylpolyoxyethylene(15)amine and octadecylpolyoxyethylene(50)-amine. The growth rate (μ) of strain SK on octadecylbis(2-hydroxyethyl) amine was 0.13 h^{-1}.

Table 1. Oxidation of potential intermediates of octadecylbis(2-hydroxyethyl)amine (ODHA) by octadecylbis(2-hydroxyethyl) amine- and glucose-grown washed cell suspensions.
Activities are expressed as percentages of increase of the respiration relative to the endogenous respiration. The endogenous respiration rates of the strain grown on octadecylbis(2-hydroxyethyl)amine and glucose were 7 and 5 nmol O_2 min^{-1} mg protein^{-1}, respectively.

Substrate	Growth substrate	
	ODHA	Glucose
Octadecylbis(2-hydroxyethyl)amine	510	0
Octadecylbis(2-hydroxyethyl)amine oxide	0	0
Octadecenal	420	100
Tetradecanal	580	150
Octadecanoate	510	20
Acetate	320	100
Octadecylamine	0	0
Diethanolamine	5	0
Ethanolamine	50	0
Glycolate	0	ND
Glucose	15	480

ND = Not determined.

Growth and whole cell studies

To obtain information about the biodegradation route of octadecylbis(2-hydroxyethyl)amine by the bacterium, the growth on various substrates and the oxygen uptake by washed cell suspensions of octadecylbis(2-hydroxyethyl)amine- and glucose-grown cells supplied with 20 mg/litre of possible intermediates were determined. Strain SK utilized octadecylbis(2-hydroxyethyl)amineoxide, octadecanoate, glycine and acetate as sole carbon and energy sources. Glycolate, ethanolamine, diethanolamine and octadecylamine did not support growth.

Results of the oxidation experiments are shown in Table 1. Octadecylbis(2-hydroxyethyl)amine, octadecanal and octadecanoate were oxidized by the octadecylbis(2-hydroxyethyl)amine-grown cells. Glucose-grown cells were not able to oxidize these substrates. Possible intermediates such as octadecylbis(2-hydroxyethyl)amineoxide, octadecylamine, diethanolamine and glycolate were not oxidized by octadecylbis(2-hydroxyethyl)amine-grown cells.

Formation of ammonium and diethanolamine

Complete degradation of octadecylbis(2-hydroxyethyl)amine by non-growing, washed cell suspensions of strain SK has to lead to the formation of ammonium. Therefore, a washed cell suspension of octadecylbis(2-hydroxyethyl)amine grown cells was incubated with octadecylbis(2-hydroxyethyl) amine. The ethoxylated amine in the medium disappeared within four hours. During this incubation period *no* accumulation of ammonium was detected. This result proves that the bacterium formed a nitrogen-containing product. The two-phase degradation in the Closed Bottle test and respiration studies indicate that during the degradation of octadecylbis(2-hydroxyethyl)amine, diethanolamine was excreted. Indeed, Fig. 2 shows the stoichiometric formation of diethanolamine from octadecylbis (2-hydroxyethyl)amine by a washed cell suspension of strain SK. Furthermore, after growth of the bacterium in a mineral salts medium containing 1 g/litre

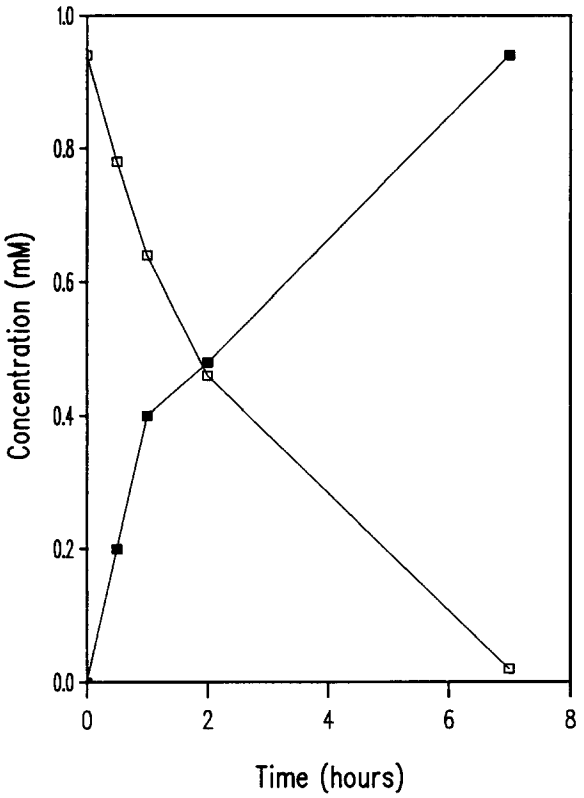

Fig. 2. Formation of diethanolamine (■) during the degradation of octadecylbis(2-hydroxyethyl)amine (□) by a washed cell suspension of strain SK.

octadecylbis(2-hydroxyethyl)amine, diethanolamine was almost quantitatively recovered. Diethanolamine also accumulated when octadecylbis(2-hydroxyethyl)amine-grown cells were incubated with alkylbis(2-hydroxyethyl)amines with tallow, oleyl and coco alkyl chains.

Enzyme assays

To investigate the biodegradation route of octadecylbis(2-hydroxyethyl)amine a number of enzymes were assayed. The enzyme responsible for the initial attack of octadecylbis(2-hydroxyethyl)amine, a tertiary amine dehydrogenase, utilized DCPIP (2,6-dichlorophenolindophenol) as an artificial electron acceptor (Table 2). NAD^+ or $NADP^+$ could not replace DCPIP. The DCPIP-dependent oxidation of octadecylbis(2-hydroxyethyl)amine may result in the formation of diethanolamine and octadecanal. Accumulation of diethanolamine in cell-free extracts was detected in the presence of DCPIP. Tertiary amine monooxygenase activity was not detected in cell-free extracts of the strain grown on octadecylbis(2-hydroxyethyl)amine. The subsequent conversion of octadecanal was likely to proceed via octadecanoic acid and octadecanoyl-CoA. Tetradecanal instead of octadecanal was used in the enzyme assay to determine the aldehyde dehydrogenase activity because an octadecanal 'solution' is turbid. Cell-free extracts prepared from octadecylbis(2-hydroxyethyl)amine grown cells contained an NAD^+-dependent alkanal dehydrogenase. Finally, acyl-CoA synthetase and acyl-CoA dehydrogenase were detected in cell-free extracts (Table 2). The activities of tertiary amine dehydrogenase and alkanal dehydrogenase in cell-free extracts of octadecylbis(2-hydroxyethyl)amine- and glucose-grown cells were comparable (Table 2).

Isocitrate lyase activity was induced in octadecylbis(2-hydroxyethyl)amine-grown cells suggesting a possible role for acetate in the metabolism. The activity of octadecylbis(2-hydroxyethyl)amine-grown cells was 460 nmol min^{-1} (mg protein)$^{-1}$ compared with 9 nmol min^{-1} (mg protein)$^{-1}$ in glucose-grown and 240 nmol min^{-1} (mg protein)$^{-1}$ in acetate-grown cells.

Table 2. Specific enzyme activities in cell-free extracts of the strain after growth on octadecylbis(2-hydroxyethyl)amine (ODHA) and glucose. Activities are expressed in nmol min^{-1} (mg protein)$^{-1}$.

Enzyme	Growth substrate	
	ODHA	Glucose
Tertiary amine dehydrogenase	35	15
Tertiary amine monooxygenase	0	0
Alkanal dehydrogenase	17	23
Acyl-CoA synthetase	3	ND
Acyl-CoA dehydrogenase	25	ND

ND = Not determined.

Discussion

The biodegradation curve of octadecylbis(2-hydroxyethyl)amine obtained in the Closed Bottle test is characterized by two growth phases. Comparing the extent of the first and second growth phases, this 'two-phase' growth may correspond with the oxidation of the alkyl chain and diethanolamine, respectively. At day 28 a biodegradation percentage of 60 was achieved (Fig. 1); according to the OECD/EEC guidelines octadecylbis(2-hydroxyethyl)amine should therefore be classified as 'readily' biodegradable.

The 'ready' biodegradability of octadecylbis(2-hydroxyethyl)amine is in accordance with the ease with which an organism capable of utilizing octadecylbis(2-hydroxyethyl)amine as sole carbon and energy source was isolated. The isolated bacterium is an aerobic gram-negative mobile rod-shaped organism. All ethoxylated fatty amines with two polyoxyethylene groups support growth. Strain SK is not able to grow on fatty amines with 15 and 50 polyoxyethylene groups.

The strain isolated is capable of utilizing octadecanal, octadecanoate and acetate as sole carbon and energy source. Other possible intermediates such as diethanolamine, ethanolamine and glycolate, however, do not support growth. Additional evidence from respirometric studies also suggests that diethanolamine and potential intermediates of this compound, viz. ethanolamine and glycolate are not involved in the biodegradation of octadecylbis(2-hydroxyethyl)amine by strain SK (Table 1). This assumption is confirmed by the finding that the degradation of octadecylbis(2-hydroxyethyl)amine by a washed cell suspension of the bacterium results in the stoichiometric excretion of diethanolamine (Fig. 2). Based on these results a degradative pathway of octadecylbis(2-hydroxyethyl)amine in the bacterium is proposed (fig. 3).

The feasibility of such a route has been further investigated by measuring the specific enzyme activities in extracts of cells grown on octadecylbis(2-hydroxyethyl)amine and glucose (Table 2). The presence in cell-free extracts of a DCPIP-dependent octadecylbis(2-hydroxyethyl)amine dehydrogenase that catalyses the conversion of octadecylbis

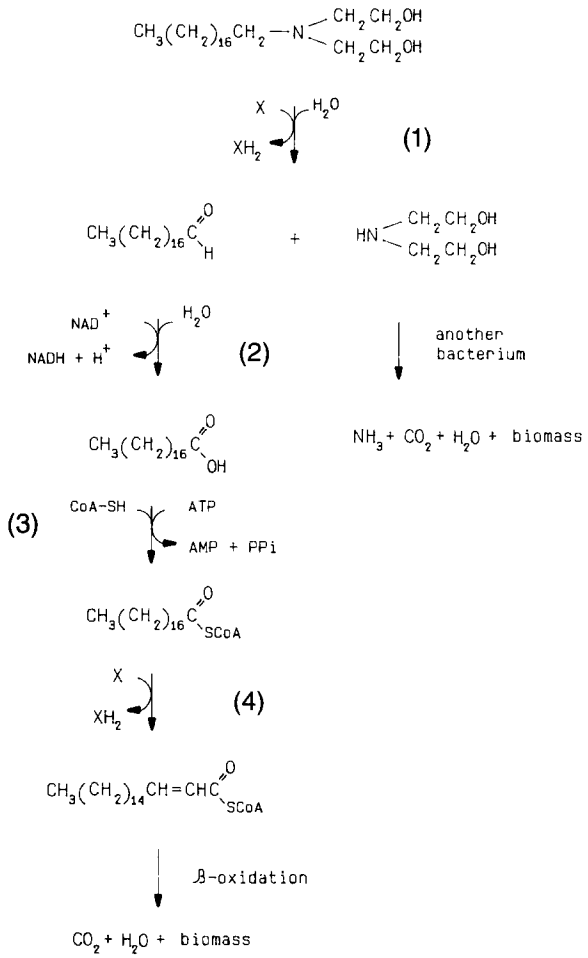

Fig. 3. Proposed pathway for the metabolism of octadecylbis(2-hydroxyethyl)amine by strain SK *(1)* tertiary amine dehydrogenase *(2)* alkanal dehydrogenase *(3)* acyl-CoA synthetase *(4)* acyl-CoA dehydrogenase.

(2-hydroxyethyl)amine into diethanolamine is in agreement with the proposed pathway. Monooxygenase activity has not been detected, which is not surprising in view of a direct conversion of octadecylbis(2-hydroxyethyl)amine into octadecanal and diethanolamine by the dehydrogenase measured. The presence of a alkanal dehydrogenase, acyl-CoA synthetase and acyl-CoA dehydrogenase further supports the proposed biodegradation route (Table 2). Finally, the presence of isocitrate lyase in octadecylbis(2-hydroxyethyl)amine-grown cells suggests a role of acetate being the end product of β-oxidation.

Whole cells of glucose-grown strain SK are not

able to oxidize octadecylbis(2-hydroxyethyl)amine. However, the enzymes responsible for the initial steps in octadecylbis(2-hydroxyethyl)amine catabolism are present in glucose-grown cells (Table 2). This indicates that the enzymes involved in the degradation of octadecylbis(2-hydroxyethyl)amine are constitutive. The inability of whole-cells of glucose-grown microorganisms to oxidize octadecylbis(2-hydroxyethyl)amine is probably caused by the absence of an uptake system.

There have been a number of studies on the microbial degradation of tertiary amines, which in all cases involve the initial cleavage of one of the C-N bonds (Colby & Zatman 1971; Cripps & Noble 1973; Meiberg & Harder 1978; Williams & Callely 1982; Uetz et al. 1992). Microorganisms capable of growing on nitrilotriacetic acid as sole source of carbon and energy have been isolated and primarily identified as *Pseudomonas* spp. Results of simultaneous respiration experiments support a pathway of degradation involving an initial oxidative cleavage of a C-N bond of nitrilotriacetic acid (Tiedje et al. 1973). Cripps & Noble (1973) detected an NADH- and O_2-dependent enzyme that catalyses the conversion of nitrilotriacetic acid into iminodiacetic acid and glyoxylate. Recently, a two-component monooxygenase that hydroxylates nitrilotriacetate was purified and characterized (Uetz et al. 1992). Aerobic triethanolamine metabolism has also been investigated in some detail (Williams & Callely 1982) and the key enzyme was found to be an NADH/O_2-dependent monooxygenase. This metabolic pathway proceeds via de dealkylation of triethanolamine to yield diethanolamine and glycolaldehyde. The oxidation of trimethylamine is also initiated by an NAD(P)H dependent monooxygenase by many microorganisms (Colby & Zatman 1973; Large et al 1972). The product trimethylamine-N-oxide is subsequently dimethylated to form demethylamine and methanal (Large 1972; Large et al. 1972; Colby & Zatman 1973). Amine monooxygenase activity has not been detected in cell-free extracts of strain SK grown on octadecylbis(2-hydroxyethyl)amine. Furthermore, the bacterium is not capable of oxidizing octadecylbis(2-hydroxyethyl)amineoxide. These results indicate that the octadecylbis(2-hydroxyethyl)amine degradation in strain SK is not initiated by a monooxygenase.

Other trimethylamine-utilizing microorganisms employ a more direct route. In this case, trimethylamine is metabolised by a direct cleavage of the amine. The enzyme responsible, trimethylamine dehydrogenase, yields dimethylamine and formaldehyde (Colby & Zatman 1971; Meiberg & Harder 1978). The direct cleavage of octadecylbis(2-hydroxyethyl)amine by a dehydrogenase is comparable with the enzymes found in *Pseudomonas sp. and Hyphomicrobium* sp. grown on trimethylamine. Both amine dehydrogenases are conveniently assayed using an artificial electron acceptor.

The 'ready' biodegradation in the Closed Bottle test and the isolation of a strain capable of utilizing octadecylbis(2-hydroxyethyl)amine demonstrate that the potential to mineralize octadecylbis(2-hydroxyethyl)amine is present in nature. The stoichiometric formation of diethanolamine from octadecylbis(2-hydroxyethyl)amine demonstrates that *no recalcitrant* intermediates are formed because, diethanolamine is readily mineralized as shown by the Closed Bottle test result (Fig. 1). Moreover, Williams & Callely (1982) isolated a bacterium capable of utilizing diethanolamine as sole carbon and energy source. A consortium of this bacterium and strain SK is therefore capable of complete degradation of octadecylbis(2-hydroxyethyl)amine.

Conclusions

Octadecylbis(2-hydroxyethyl)amine, a non-ionic surfactant, is readily biodegradable in a Closed Bottle test. A bacterium capable of growing on this non-ionic was isolated. During growth of the bacterium on octadecylbis(2-hydroxyethyl)amine, the non-ionic surfactant is cleaved resulting in diethanolamine and an aldehyde which then undergoes β-oxidation. The central fission of the molecule is catalysed by a DCPIP-dependent dehydrogenase. β-Oxidation proceeds rapidly and results in a total mineralization of the alkyl chain whereas diethanolamine accumulates as an end-product. In mixed cultures diethanolamine is readily biodegraded by other microorganisms.

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References

Baleux B & Caumette P (1974) Biodegradation of nonionic surfactants. Study of a new experimental method and screening test. Rev. Inst. Pasteur Lyon 7: 278–297

Beinert H, Green DE, Hele P, Hift H, Korf von RW & Ramakrishnan CW (1953) The acetate activating enzyme system of heart muscle. J. Biol. Chem. 203: 35–45

Berth P, Gerike P, Gode P & Steber J (1984) Ecological evaluation of important surfactants. Surfactant Congress 8: 1 227–236

Colby J & Zatman LJ (1971) Trimethylamine metabolism in obligate and facultative methylotrophs. Biochem. J. 132: 101–112

Cripps RE & Noble AS (1973) The metabolism of nitriloacetate by a pseudomonad. Biochem. J. 136: 1059–1068

Dixon GH & Kornberg HL (1959) Assay methods for key enzymes of the glyoxylate cycle. Biochem. J. 3: 72

Fischer WK (1972) Correlation between the constitution and biodegradability of nonionic surfactants. Surfactant Congress 6: 73–75

Fischer WK (1981) Important aspects of ecological evaluation of fatty alcohols and their derivatives. In: Fettalkohol Rohstoffe Verfahren und Verwendung. Henkel, Dusseldorf, Englisch Edition (pp 188–222)

Large PJ (1971) The oxidative cleavage of alkyl nitrogen bonds in microorganisms. Xenobiotica 1: 457–467

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: 137–138

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

OECD (1982) Guidelines for testing of chemicals, section 3; Degradation and accumulation No. 301 D: ready biodegradability, Closed Bottle test. Paris, France

Ruiz Cruz J & Dobarganes Garcia MC (1978) Pollution of natural water by synthetic detergents XIII. Biodegradation of non-ionic surfactants in river water and determination of their biodegradability by different test methods. Grasas Aceites 29: 11–17

Schöberl P, Bock KJ & Huber L (1988) Okologisch relevante Daten von Tensiden in Wasch und Reinigungsmitteln. Tenside Detergents 25: 86–98

Swisher RD (1987) Surfactant Biodegradation, 2nd edition. Surfactant Science Series, Vol. 18. Marcel Dekker Inc, New York and Basel

Tiedje JM, Mason BB, Warren CB & Malec EJ (1973) Metabolism of nitrilotriacetate by cells of *Pseudomonas* species. Appl. Microbiol. 25: 811–825

Uetz T, Schneider R, Snozzi M & Egli T (1992) Purification and characterization of a two-component monooxygenase that hydroxylates nitrilotriacetate from *Chelatobacter* strain ATCC 29600. J. Bacteriol. 174(4): 1179–1188

Verdouw H, Echteld CJA van & Dekkers EMJ (1978) Ammonia determination based on indophenol formation with sodium salicylate. Water Res. 12: 399–402

Visniac W & Santer M (1957) The thiobaccilli. Bacteriol. Rev. 21: 195–213

Waters J & Kupfer W (1976) The determination of cationic surfactants in the presence of anionic surfactant in biodegradation test liquors. Anal. Chimica Acta 85: 241–251

Wheeler PR, Bulmer K & Radledge C (1991) Fatty acid oxidation and β-oxidation complex in *Mycobacterium leprae* and two axenically mycobacteria that are pathogens. J. Gen. Microbiol. 137: 885–893

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