Biodegradation **11:** 377–383, 2000.

Degradation of organic contaminants found in organic waste

I. Angelidaki[1]*, A.S. Mogensen[1] & B.K. Ahring[1,2]
[1] *Department of Biotechnology, Building 227, The Technical University of Denmark, 2800 Lyngby, Denmark;* [2]*School of Engineering and Applied Science, Department of Civil and Environmental Engineering, University of California, USA (* author for correspondence; e-mail: ria@ibt.dtu.dk)*

Accepted 21 November 2000

Key words: anaerobic degradation, LAS, nonylphenol ethoxylates, PAH, phthalates, screening

Abstract

In recent years, great interest has arisen in recycling of the waste created by modern society. A common way of recycling the organic fraction is amendment on farmland. However, these wastes may contain possible hazardous components in small amounts, which may prevent their use in farming. The objective of our study has been to develop biological methods by which selected organic xenobiotic compounds can be biotransformed by anaerobic or aerobic treatment. Screening tests assessed the capability of various inocula to degrade two phthalates di-*n*-butylphthalate, and di(2-ethylhexyl)phthalate, five polycyclic aromatic hydrocarbons, linear alkylbenzene sulfonates and three nonylphenol ethoxylates under aerobic and anaerobic conditions. Under aerobic conditions, by selecting the appropriate inoculum most of the selected xenobiotics could be degraded. Aerobic degradation of di(2-ethylhexyl)phthalate was only possible with leachate from a landfill as inoculum. Anaerobic degradation of some of the compounds was also detected. Leachate showed capability of degrading phthalates, and anaerobic sludge showed potential for degrading, polycyclic aromatic hydrocarbons, linear alkylbenzene sulfonates and nonyl phenol ethoxylates. The results are promising as they indicate that a great potential for biological degradation is present, though the inoculum containing the microorganisms capable of transforming the recalcitrant xenobiotics has to be chosen carefully.

Introduction

Within the European Union the total amount of produced sludge is about 6.5 million tons per year (Smith 1996). There are several disposal routes for sludge, including ocean dumping, incineration, spreading on agricultural land, land spreading in forestry or landfilling. At present the disposal of sludge on landfills is with 40% the most important outlet in the EU while 37% of the sewage sludge produced within the EU was used for agricultural purposes in 1994 (Smith 1996). The amount of sewage sludge requiring disposal is expected to increase significantly in the future due to recent environmental developments. The Helsinki agreement called for the banning of ocean sludge dumping by the year 1987 and the EU Environmental Directive on Urban Wastewater required waste water treatment plants with secondary treatment and nutrient removal in sensitive areas (Kiely 1997). With increasing sludge production in the EU larger amounts of sewage sludge will be recycled for agricultural purposes (Smith 1996). This approach seems to be reasonable since agricultural land can become nutrient deficient due to intensive cultivation.

In addition to sewage sludge other wastes, such as organic industrial wastes, manure, and organic household waste, can with great advantage be recycled and used in farmland as fertilizers and as soil improving components.

However, this type of waste may contain hazardous components in small amounts, which might show adverse effects on the ecosystem, e.g., farmland amended with sewage sludge. Indeed, linear alkylbenzene sulfonates (LAS) such as those used in household detergents, nonylphenols (NP), nonylphenol ethoxylates (NPEO), polycyclic aromatic hydrocarbons

(PAH), and phthalates have recently been identified as major anthropogenic organic contaminants in sewage sludge. There is a special public concern about organic components, which may have a potential for acute toxicity, mutagenesis, carcinogenesis or teratogenesis or posses estrogenic effects.

The concentrations of LAS in raw wastewater have been reported to range from 3 mg/L to 21 mg/L (Brunner et al. 1988; De Henau et al. 1989; Holt et al. 1995; Ruiz Bevia et al. 1989). Although LAS and other common surfactants have been reported to be readily biodegradable by aerobic processes, much of the surfactant load into a sewage treatment facility (reportedly 20–50%) is associated with suspended solids (Greiner & Six 1997; McAvoy et al. 1998) and thus escapes aerobic treatment processes. LAS is reported not to be biodegraded by anaerobic biological processes usually employed in sludge stabilization (McEvoy & Giger 1985; Swisher 1987) and it may be found in the gram per kilogram range in anaerobic sludge.

According to Mackay et al. (1996) contamination of soil environments with LAS is possible due to sludge application on agricultural soil and landfilling. The presence of surfactants in sludge may have undesirable environmental effects since the surfactant molecules may leach to groundwater contributing to groundwater contamination.

Alkylphenol ethoxylates such as NPEO is a group of non-ionic surfactants with world-wide application and are evidently less biodegradable than LAS (Swisher 1987; Steinle et al. 1964; Pitter 1968) and a wide range of removals from 0–90% based on specific analyses such as UV and IR spectroscopy has been reported (Swisher 1987). This suggests that only partial degradation occur, such as conversion from polyethoxylates to nonylphenol diethoxylate (NP2EO), nonylphenol monoethoxylate (NP1EO), and NP. The latter is the most recalcitrant of the intermediates formed during conversion of the NPEO molecules. Mass balances done on treatment plants in Switzerland (Brunner et al. 1988) support these findings.

Due to the low water solubility and lipophilic properties of PAH, these compounds are removed from sewage partly by biodegradation and partly by sorption to sludge. According to Bodzek et al. (1997) PAH are found in significant amounts in sludge (up to 2000 mg/kg sludge dry mass. The PAH mostly originate from fossil fuel combustion and industrial processes (Shuttleworth & Cerniglia 1995). Generally the half-lives of PAH are increasing with an increasing number of aromatic rings, though the degradation rates are dependent upon the test system. Due to the great adsorption abilities of PAH they will precipitate with the particular material in the pre-clearing tank. The sludge from this step is digested in the stabilisation tank. However, PAH are poorly degradable anaerobically and they tend to accumulate in the digested sludge.

Sewage sludge and compost contain relatively high concentrations of di(2-ethylhexyl) phthalate (DEHP) and di-*n*-butyl phthalate (DBP) (Danish Environmental Agency, 1996). It was reported that these compounds probably originate from both households and industries. DEHP accounts for 90% of the phthalate production worldwide. At least 95% of the DEHP produced is used as an additive in PVC plastics which are made into various products such as waterproof clothing, footwear, toys, bloodbags and heat-seal coatings on metal foils. Plastisizers are poorly soluble substances and will partly be removed in the wastewater treatment plant by biodegradation, though also adsorption on sewage sludge is significant. Hence, plastisizers will be transferred to soil following application of sludge on agricultural land or landfilling of sludge.

Biological treatment of wastes containing toxic compounds may constitute an effective and cheap method for detoxifying the wastes. For this to happen, microorganisms that can degrade the compounds are needed. In the present study we report results from a screening of microorganisms capable of degrading LAS, NPEO, PAH, and phthalates.

Materials and methods

Description of the experimental set-up

The tests were performed in batch serum vials where pressure and/or substrate concentrations were followed over time. 118 ml serum vials were used with 40 ml liquid volume. Two different media were used, one for the anaerobic (Angelidaki et al. 1990) and one for the aerobic inocula, containing per liter of water (Milli-Q) 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.05 g $CaSO_4^*2H_2O$, 0.5 g $MgSO_4^*7H_2O$, 0.01 g $FeSO_4^*7H_2O$, 1.0 G $(NH_4)SO_4$.

Both media contained a phosphate buffer, the macro nutrients N, P, K, Ca, Mg, Fe, S and trace metals. With the exception of ethanol the samples did not obtain a carbon source additional to the compounds and the inocula.

The vials were filled with the appropriate medium and autoclaved at 140 °C for 30 min. All vials were

inoculated 5 to 10% of the respective inocula. Anaerobic and aerobic serum vials were eventually flushed with oxygen-free nitrogen:carbon dioxide (80:20) or ambient air respectively. To the aerobic vials ambient air was added at an overpressure of 1 atmosphere. The serum vials were closed with butyl rubber stoppers and aluminum crimps, and were placed in a 37 °C incubator. The incubation time was two months. The pH of the medium was 7 ($\pm$ 0.1).

Water soluble contaminants were dissolved in the medium whereas contaminants of low solubility were either dissolved in ethanol prior to batch amendment or added as fine crystals. When the contaminant was added from stock solutions in ethanol, corresponding amount of ethanol was added in control vials.

Three sets of control vials (sterile, substrate-unamended, and uninoculated) were made in triplicates.

Compounds tested

Of the PAH naphthalene, 1-methylnapthalene, fluoranthene, phenanthrene and pyrene were chosen. The PAH were added either as a mixture of the five PAH mixed in equal amounts (40 mg/l each i.e., 200 mg/l total PAH concentration in the vial) or as individual compounds. Among the phthalates, DBP, and DEHP were selected. LAS was used as a mixture of LAS with an alkyl chain length of 9 to 13 units and among the nonylphenols 4-nonylphenol and NP1EO and NP2EO were used. The compounds were tested at three concentrations of 20, 100 and 200 mg/l. For the mixture of PAH the concentration was counted as the sum of the five compounds. PAH at large concentrations were directly weighted in the vials, while at the smaller concentrations PAH were added from stock solutions in ethanol. Corresponding amount of ethanol (80 μl per vial) was added in all the vials of the series also in the controls.

Inocula

The inocula originated from several different Danish natural environments and from anaerobic reactors. Environments tested were; primary and activated sludge from wastewater treatment plants, sediments from lakes and streams. Furthermore, several contaminated soils were tested such as from old gas works, sludge amended soils, soils located close to benzin stations, soils close to detergents factory. In addition, compost from a composting plant, granular sludge from an anaerobic sludge blanket reactor, and digested manure from an anaerobic reactor were tested. Finally leachate from landfills was tested. The inocula chosen along with redox conditions, and xenobiotics used during the screening test are shown in Table 1.

Test of degradation and inhibition

Indirect method

Different methods for testing degradation of the xenobiotic compounds were used. Due to the high number of samples, an indirect method was applied. The indirect degradation test method was based on pressure measurements in the headspace of the vials, with a pressure transmitter attached through a needle and a tube to the headspace of the vials. In aerobic vials there was one atmosphere overpressure at the beginning of the experiment, and a pressure decrease is expected when degradation occurs in the vials, due to the consumption of oxygen. For the degradation of naphthalene, twelve moles oxygen is depleted per mole of naphthalene degraded, whereas only ten mole carbon dioxide are released. Hence, the gas consumption is higher than the gas production and the gas pressure in the head space is decreased. As the carbon dioxide might also partly be dissolved in the aqueous phase, the pressure decrease should be even more significant. An equivalent pattern can be shown for the other compounds.

In the anaerobic vials atmospheric pressure was applied at the beginning of the test. A pressure increase was expected due to CO_2 and CH_4 production. During naphthalene degradation four moles of carbon dioxide and six moles of methane are released per mole naphthalene mineralized. Even though a certain amount of CO_2 is kept in the liquid phase, an increase of the pressure is found in the head-space of the vials upon degradation of the compounds.

Pressure in headspace of the vials with the xenobiotic compounds were compared with substrate unamended vials.

Direct method

In the direct method, degradation of the compound was established by quantifying the concentration of the xenobiotic compound at the beginning and at the end of an experimental period of approx. 2 months. Direct measurement of the degradation of the compounds was performed only in selected samples.

Table 1. Samples used as inoculum together with the redox conditions used for the biotransformation studies, and the contaminants tested

Origin of inoculum	Redox conditions	Contaminant tested
Anaerobic sludge Lundtofte	Anaerobic, aerobic	LAS[1,2], PAH[3], naphthalene, methylnaphthalene, fluoranthene, pyrene, phenanthrene, NPE, DBP, DEHP
Anaerobic sludge Damhuså	Anaerobic, aerobic	NPE, LAS, PAH[3], DBP, DEHP
Aerobic sludge Damhuså	Anaerobic	NPE, LAS
Aerobic sludge Lundtofte	Anaerobic, aerobic	NPE, LAS
Sediment Damhuså stream	Anaerobic	NPE, LAS
Sediment Lake Arresø	Anaerobic, aerobic	LAS, DBP, DEHP, naphthalene, methylnaphthalene, fluoranthene, pyrene, phenanthrene, NPE
Soil Nr. Herlev, Hillerød	Anaerobic, aerobic	NPE, LAS, PAH[3], DBP, DEHP
Soil Møllehøj, Arresø	Anaerobic, aerobic	NPE, LAS, DBP, DEHP
Soil Colgate-Palmolive	Anaerobic, aerobic	NPE, LAS
Soil Oil contaminated, Jutland		PAH[3]
Soil Hjørring Gaswork		PAH[3],
Compost AFAV, Hillerød	Anaerobic, aerobic	NPE, LAS, DBP, DEHP
Granular sludge UASB reactor, Eerbeek	Anaerobic, aerobic	NPE, LAS
Manure Mesophilic CSTR	Anaerobic, aerobic	NPE, LAS
Landfill leachate		DBP, DEHP

[1] C_{12}-LAS.
[2] Commercial mixture of LAS homologs.
[3] Mixture of naphthalene, 1-methylnapthalene, fluoranthene, phenanthrene, and pyrene.

Analysis

The pressure in the batch vials inoculated under aerobic conditions was determined with a digital pressure gauge.

LAS C12 samples were dried at 60 °C, dissolved in methanol, and then filtered in order to avoid impurities from the inocula. LAS C12 was quantified by HPLC using UV-detection (λ = 225 nm) and an eluent consisting of MeOH:Milli-Q water (1:0.28) with 9.2 g/l of $NaClO_4$. Samples with PAH, phthalate esters, and NPEO were subjected to liquid-liquid extraction of homogenous sample using dichloromethane. Samples were shaken with dichloromethane. The dichloromethane was separated with centrifugation, and the procedure was repeated three times. Gas chromatographic separations were performed with a Hewlett Packard Model 6890 GC equipped with a HP-5 column. The mass selective detection was performed using a Hewlett Packard Model 5973 MSD. Deuterium labeled DEHP and pyrene were used as internal standards. All the samples were frozen and were analyzed at the end of experiment.

Results and discussion

Vials incubated under aerobic conditions showed a decrease of the initial pressure when degradation was present. In figure1 the degradation of PAH is shown in the vials inoculated with sludge amended soil. When PAH was added in a concentration of 20, 100 or 200 mg/l the pressure in the vials decreased, indicating degradation of the added compounds (Figure 1a). In the PAH unamended controls the pressure slightly decreased relative to the sterile control due to digestion of ethanol, though the pressure remained higher than the PAH amended vials. In figure 1b the concentrations (in area counts) of the individual PAH are shown at the start and end of the experiment. There was a significant reduction of the concentrations of the measured PAH at the end of the experiments compared to the initial concentrations, confirming that the PAH were biodegraded.

In Figure 2 degradation of DBP under anaerobic conditions is shown. When degradation occurred, the pressure in the vials increased due to production of methane and carbon dioxide. The pressure of the controls without DBP addition also increased due to mineralization of organics contained in the inoculum and the ethanol which was added in a concentration corresponding to the DBP amended vials. However,

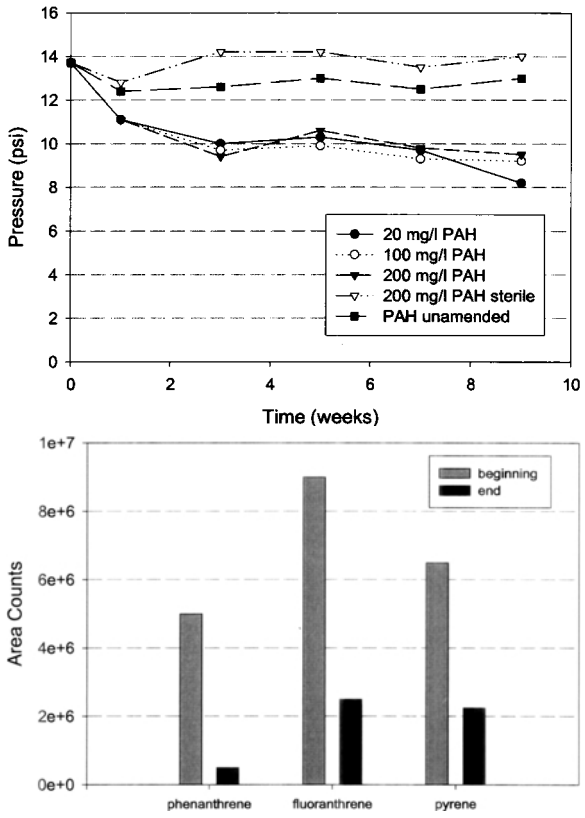

Figure 1. Pressure depletion in PAHs amended aerobic vials and relative concentration in vials at the beginning and end of the experiment.

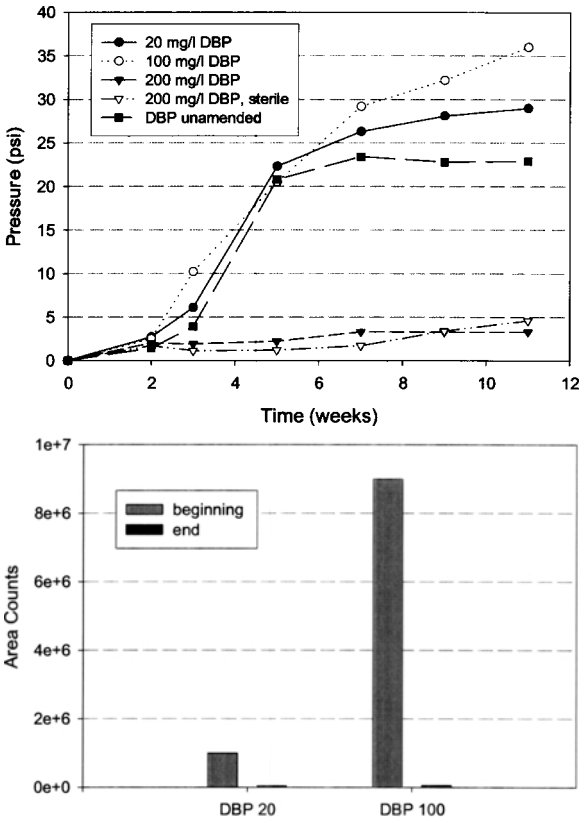

Figure 2. Pressure increment in DBP amended anaerobic vials and relative concentration in vials at the beginning and end of the experiment.

the pressure increase in the vials with 20 and 100 mg/l DBP was higher than in the controls, which was due corresponding to biodegradation of DBP. Vials with 200 mg/l DBP showed no increase of the pressure indicating that this concentration was toxic to bacteria preventing both degradation of DBP and of the organics contained in the inoculum. In figure 2b the concentration of DBP (20 mg/l and 100 mg/l initial amended concentrations) is shown at the beginning and at the end of the experiment. DBP was almost gone at the end of the experiment, which provides evidence of DBP biodegradation.

The results from the all screening test are summarized in Table 2. Most compounds were degraded both under aerobic and anaerobic conditions. Some compounds were easily degradable by most inocula. Such compounds were naphthalene, DBP, while the remaining compounds showed only degradation with a few inocula. DEHP is a compound that has been reported as recalcitrant under anaerobic conditions. Ejlertson (1997) has reported that DEHP was unaffected, during anaerobic incubation, throughout an experimental period of 330 days. However, in our screenings experiments we found indications of degradation of DEHP by an inoculum originating from lecheate from a landfill. It is possible that deposition of plastics, and other materials containing DEHP has favored the selection of organisms capable of degradation of DEHP. The landfill percolate did not show capability of aerobic DEHP degradation.

LAS is known to be easily degraded aerobically, e.g., in activated sludge reactors during waste water treatment, but is not degraded in anaerobic wastewater treatment systems (McEvoy & Giger 1985). In our screenings experiments we found inocula showing capability for conversion of LAS under anaerobic conditions, namely by an inoculum obtained from sediments of a lake. In addition, inocula that were found in aerobic environments such as compost and activated sludge from a wastewater treatment plant, showed capability of anaerobic degradation of LAS.

Table 2. Results from the screenings test for degradation of xenobiotic compounds

Group	Tested xenobiotic compound	Aerobic	Anaerobic
LAS	Linear Alcylbenzene Sulfonates	+	+
NPE	4-Nonylphenol	−	−
	Nonylphenolmonoethoxylate	+	+
	Nonylphenoldietoxylate	+	+
PAH	Naphthalene	+	+
	Phenanthrene	+	+
	Fluoranthene	+	−
	Pyrene	+	+
Phthalate	DEHP	+	+
	DBP	+	+

+: Indicates degradation; −: indicates not degradation.

PAH were found to be degradable under both aerobic and anaerobic conditions, though a higher number of inocula contained relevant microorganisms for aerobic PAH degradation.

However, it should be kept in mind that the compounds investigated in this study were added in the vials and were therefore in a readily bioavailable form. The toxicants contained in sludge could be more bound to the particulate matter and thus have reduced biovailability (Cerniglia 1992).

Conclusions

The screenings test showed that a range of inocula have a capacity towards degradation of recalcitrant xenobiotic compounds. Especially the degradation of DEHP and LAS under anaerobic conditions is promising considering the importance of eliminating these compounds during waste water treatment. New bacteria with the capability of degrading organic toxicants will give the possibility of bioprocessing waste containing toxic compounds by introducing the appropriate bacteria to, for instance, in a biogas reactor system, where the organic matter of the waste will be converted into biogas with simultaneous degradation of organic contaminants. The effluent from such a process could then be applied on agricultural soil as fertilizers and soil improving component.

Acknowledgment

This work was supported by grants from The Strategic Environmental Research programme 1997-2000 (Subprogramme on sustainable land use).

References

Angelidaki I, Petersen SP & Ahring BK (1990) Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite. Appl. Microbiol. Biotechnol. 33: 469–472

Bodzek D, Janoszka B & Dobosz C (1997) Determination of polycyclic aromatic compounds and heavy metals in sludge from biological sewage treatment plants. J. Chromatography A 774: 177–192

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

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3: 351–368

Ejlertsson J (1997) Anaerobic degradation of phthalic acid esters during digestion of municipal solid waste under landfilling conditions. Ph.D. Thesis, Linköbing studies in arts and science. Sweden.

Danish Environmental Protection Agency (1998) Indsamling og anvendelse af organisk dagrenovation i biogasanlæg. Miljøprojekt 386 (in danish)

Greiner, MS & Six E (1997) Evaluation of the results of the LASA-monitoring in Germany. Tenside Surfactants Detergents 34: 250–255

Henau H De, Matthijs, E & Namkung E (1989) Trace analysis of linear alkylbenzene sulfonate (LAS) by HPLC. Detailed results from two sewage treatment plants. In: Quaghebeur D, Temmerman I & Angeletti G (Eds) Organic Contaminants in Waste Water, Sludge and Sediment. Elsevier Applied Science, London

Holt MS, Waters J & Comber MHI (1995) AIS/CESIO environmental surfactant monitoring programme. SDIA sewage treatment pilot study on linear alkylbenzene sulphonate (LAS). Water Res. 29: 2063–2070

Kiely G (1997) Environmental engineering. McGraw-Hill (ed). pp. 574–583, 605–611

Mackay D, di Guardo A & Paterson S (1996) Assessment of chemical fate in the environment using evaluative regional and local-scale models: illustrative application to Chlorobenzene and Linear Alkylbenzene sulfonates. Environm. Toxicol. and Chem. 15: 1638–1648

McAvoy DC, Dyer SD & Fendinger NJ (1998) Removal of alcohol ethoxylates, alkyl ethoxylate sulfates, and linear alkylbenzene sulphonates in wastewater treatment. Environm. Toxicol. and Chem. 17: 1705–1711

McEvoy J & Giger W (1986) Determination of linear alkylbenzenesulfonates in sewage sludge by high-resolution gas chromatography/mass spectrometry. Environm. Sci. Technol. 20: 376–383

Quaghebeur D, Temmerman I & Angeletti G (Eds) Elsevier Applied Science, London

Pitter P (1968) Relation between degradability and chemical structure of nonionic polyethylene oxide compounds. Surf. Cong. 1: 115–123

Ruiz Bevia F, Prats D & Rico C (1989) Elimination of L.A.S. (linear alkylbenzene sulfonate) during sewage treatment, drying and compostage of sludge and soil amending processes. In: Shuttleworth KL & Cerniglia CE (Eds) Organic Contaminants in Waste Water, Sludge and Sediment. Environmental aspects of PAH biodegradation. Appl. Bioch. and Biotechnol. 54: 291–302

Smith SR (1996) Agricultural recycling of sewage sludge and the environment

Wallingford Steinle EC, Myerly RC & Vath CA (1964) Surfactants containing ethylene oxide: Relationship of structure to biodegradability. J. Amer. Oil Chemists Soc. 41: 804–807

Swisher RD (1987) Surfactant Biodegradation. Marcel Dekker, New York

Volkering F, Breure AM & Andel JG (1993) Effect of microorganisms on the bioavailability and biodegradation of crystalline naphthalene. Appl. Microbiol. Biotechnol. 40: 535–540